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REVIEW

Peptide Design for Enhanced Anti-Melanogenesis: Optimizing Molecular Weight, Polarity, and Cyclization

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Abstract: Melanogenesis is a biochemical process that regulates skin pigmentation, which is crucial role in protecting against ultraviolet radiation. It is also associated with hyperpigmentation conditions such as melasma and age spots, which negatively impact aesthetics and self-confidence. Tyrosinase (TYR), a key enzyme in the melanogenesis pathway, catalyzes the biosynthesis of melanin in the skin. Inhibition of tyrosinase particularly by blocking its active site and preventing the binding of natural substrates such as tyrosine, can reduce melanin production, making it a promising therapeutic target for treating hyperpigmentation. Peptides have emerged as promising therapeutics to regulate melanogenesis by minimizing the side effects associated with conventional skin whitening therapeutics. This review is designed to offer a comprehensive analysis of current strategies in peptide design aimed at optimizing anti-melanogenic activity, by focusing on the role of molecular weight, polarity, and cyclization strategies in enhancing peptide efficacy and stability. It was found that optimal peptide size was within the range of 400–600 Da. The balance between hydrophilic and hydrophobic properties in peptides is crucial for effective TYR inhibition, as higher hydrophilicity enhances affinity for the TYR active site and stronger catalytic inhibition, while hydrophobicity can contribute through alternative mechanisms. Cyclization of peptides enhances their structural stability, serum resistance, and binding affinity while reducing toxicity. This process increases resistance to enzymatic degradation and improves target specificity by limiting conformational flexibility. Additionally, the rigidity and internal hydrogen bonding of cyclic peptides can aid in membrane permeability, making them more effective for therapeutic use. Peptide optimizations through size modification, polarity change, and cyclization strategies have been shown to be promising as reliable and safe agents for melanin inhibition. Future studies exploring specific amino acid in peptide chains are required to improve efficacy and potential clinical applications of these anti-melanogenic peptides as a hyperpigmentation treatment.

Keywords: anti-hyperpigmentation, anti-melanogenesis, amino acid, tyrosinase, TYR

Skin Aging Related to Hyperpigmentation

Aging of skin is a multifactorial mechanism influenced by intrinsic elements, like hereditary and lifestyle. The influence of lifestyle includes malnutrition, obesity, alcohol consumption, and smoking.[1] Extrinsic factors predominantly associated with environmental exposures, that significantly influence the acceleration of skin aging, including UV radiation, smoking, environmental pollutants, infrared radiation and heat.[2–4] Among these, UV radiation is particularly highlighted as the primary environmental trigger for photo-induced skin aging, due to the production of free radicals, including reactive oxygen species within skin cells.[5,6] The accumulation of reactive oxygen species, especially due to ultra violet A (UVA) exposure, generates oxidative stress that plays a key role in skin aging, causing harm to proteins, lipids, nucleic acids, and cellular organelles.[7] Additionally, exposure to ultra violet (UV) radiation from sunlight triggers specific signaling pathways in human keratinocytes, which results in inflammation, premature skin aging, and a heightened risk of skin cancer.[8] The increase in macromolecular damage alongside the formation of senescent fibroblasts in the skin's

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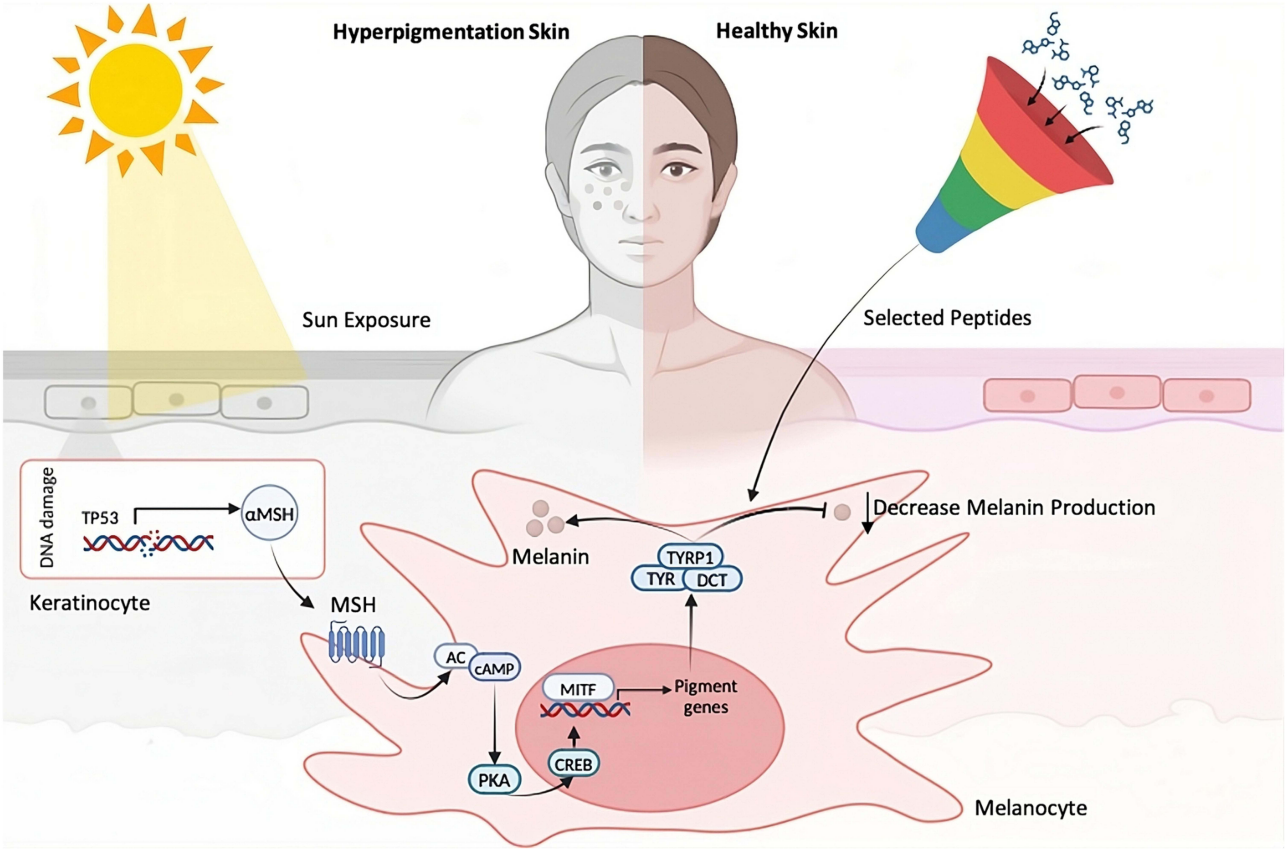
Graphical Abstract



dermal layer are central mechanisms through which environmental factors cause external skin aging.[3] Moreover, the decrease in aquaporin expression in the skin as one ages may contribute to skin dryness associated with aging.[9]

UV radiation, particularly UVB, significantly affects skin health by causing DNA damage, such as thymine dimers and mutations, and generating reactive oxygen species (ROS) that induce oxidative stress. This oxidative stress damages lipids, proteins, and mitochondria, accelerating photoaging and heightening the risk of skin cancer. UV also activates key signaling pathways like Mitogen-Activated Protein Kinase/ Extracellular Signal-Regulated Kinase (MAPK/ERK) and Nuclear Factor kappa-light-chain-enhancer of activated B cells (NF-κB), which regulate inflammation, apoptosis, and the cell cycle, further contributing to immune suppression by impairing skin-resident immune cells. Moreover, UV stimulates melanogenesis via the melanocortin-1 receptor (MC1R)/pro-opiomelanocortin (POMC)/ cyclic adenosine monophosphate (cAMP)/cAMP-response element-binding protein (CREB) pathway, increasing melanin production as protection MC1R/POMC/cAMP/CREB pathway[10] and triggers the secretion of paracrine factors like Stem Cell Factor (SCF) and Alpha-Melanocyte-Stimulating Hormone (α-MSH) from senescent fibroblasts, further influencing hyperpigmentation and tanning.[11]

Oxidative stress plays a central role in photoaging and hyperpigmentation,[12] with the p53 protein linking cytokine receptor-mediated signaling to pigmentation under sun exposure, inflammation, and aging.[13] Combating skin aging involves strategies such as increasing collagen, elastin, hyaluronic acid, and hydroxyproline levels; stimulating fibrillin to strengthen the dermal elastic fiber network; suppressing enzymes like tyrosinase, MMPs, collagenase, hyaluronidase, and elastase; and enhancing antioxidative defenses to neutralize free radicals. Together, these mechanisms underline the multifaceted effects of UV radiation and the complex pathways contributing to skin aging and hyperpigmentation.[14]

Skin aging not only affects physical appearance but also elevates the probability of infections and chronic wounds, including melanoma, pressure sores, venous ulcers, and diabetic foot ulcers.[15] During the aging process, there is a reduction in melanocytes coupled with an irregular rise in melanogenic activity, leading to a mix of hypopigmentation and hyperpigmentation.[16] Hyperpigmentation is a typical feature of aging skin, especially in cases of extrinsic aging caused by long-term sun exposure.[17] This condition can appear as age spots, solar lentigines, melasma, and similar issues.[18] Excessive melanin production can contribute to hyperpigmentation and various skin disorders.[19] Additionally, skin inflammation, burns, wounds, and injuries can also trigger hyperpigmentation.[20]

Various treatments and interventions have been investigated to address hyperpigmentation linked to skin aging. These include the use of topical antioxidants to address signs of aging such as hyperpigmentation and wrinkles,[21] along with energy-based devices designed to stimulate collagen production and reduce hyperpigmentation, which in turn improves skin tone and texture.[22] Recently, a number of compounds like arbutin, glucocorticoids, kojic acid, mercuric chloride and hydroquinone, have been utilized to manage pigmentation disease. Nevertheless, their effectiveness is often compromised by limitations such as poor skin penetration, cytotoxicity risks, decreased effectiveness and low stability, along with the risk of dermatitis and erythema with their extended use.[23] Additionally, research has examined the efficacy of various compounds and treatments, like ursodeoxycholic acid, thiamidol-based regimens[24] and peptides derived from fish scale gelatin[25] in inhibiting hyperpigmentation and improving skin appearance. In summary, hyperpigmentation remains a major issue in skin aging, influenced by factors like UV exposure, oxidative stress, and irregular melanin synthesis. Therefore, it is crucial to understand and investigate various treatment strategies to effectively manage this common skin aging problem.

This review explores the potential of peptides as a novel approach for inhibiting tyrosinase, a key enzyme in melanogenesis, as an alternative to traditional compounds used in hyperpigmentation treatments. Despite the widespread use of compounds like arbutin, kojic acid, and hydroquinone, their clinical efficacy is often limited by issues such as poor skin penetration, cytotoxicity, and low stability. Due to these limitations, peptides—both natural and synthetic—emerge as a more effective, safer, and stable alternative. This review focuses on the molecular properties of peptides, such as molecular weight, polarity, and cyclization, and how these characteristics enhance their ability to act as potent tyrosinase inhibitors, offering a promising strategy for improving skin appearance and treating hyperpigmentation.

Melanogenesis and Key Regulator

Melanogenesis, the process responsible for melanin production, is a complex biological pathway regulated by multiple mechanisms. Melanin serves a vital function in guarding human skin against radiation. However, when melanin accumulates abnormally, it can cause skin aesthetic concerns, leading to development of dark patches, melasma, freckles, and solar lentigines, which can negatively impact an individual's psychological well-being and overall quality of life.[23,26] The melanocortin-1 receptor (MC1R)/pro-opiomelanocortin (POMC)/cyclic adenosine monophosphate (cAMP)/cAMP-response element-binding protein (CREB) pathway is widely recognized as the primary regulator of melanogenesis as shown in Figure 1.[27]

Acting as a pivotal regulator, microphthalmia-associated transcription factor (MITF) governs the expression of key melanogenic enzymes such as tyrosinase (TYR), tyrosinase-related protein-1 (TRP-1), and TRP-2.[28] Studies have shown that MITF is pivotal in α-MSH-induced melanogenesis, where the cyclic adenosine monophosphate (cAMP) pathway enhances the expression of MITF and subsequent melanogenic enzymes.[27,29] Additionally, epigenetic factors such as microRNAs are also involved in the regulation of melanogenesis.[30] MicroRNAs, including miR-145, have been identified as key regulators in the pigmentation process, influencing the expression of major pigmentation-related genes.[31] Furthermore, autophagy contributes to the regulation of melanogenesis, with evidence showing that autophagy inducers can affect melanin synthesis.[26] Human skin pigmentation is determined by the synchronized actions of two cell types: melanocytes, which responsible for producing melanin, and keratinocytes, which facilitate the even distribution of melanin pigment received from melanocytes.[32] The production of melanin in melanocytes is a negative feedback response to sunlight exposure, which damages DNA and activates the p53 protein as a transcription factor for the large PMOC protein in keratinocytes.[33] PMOC serves as a precursor for alpha-MSH, recognized by the MC1R receptor, which then signals cAMP to MITF as a transcription factor for the TYR enzyme within melanocytes.[34] This bio-signaling

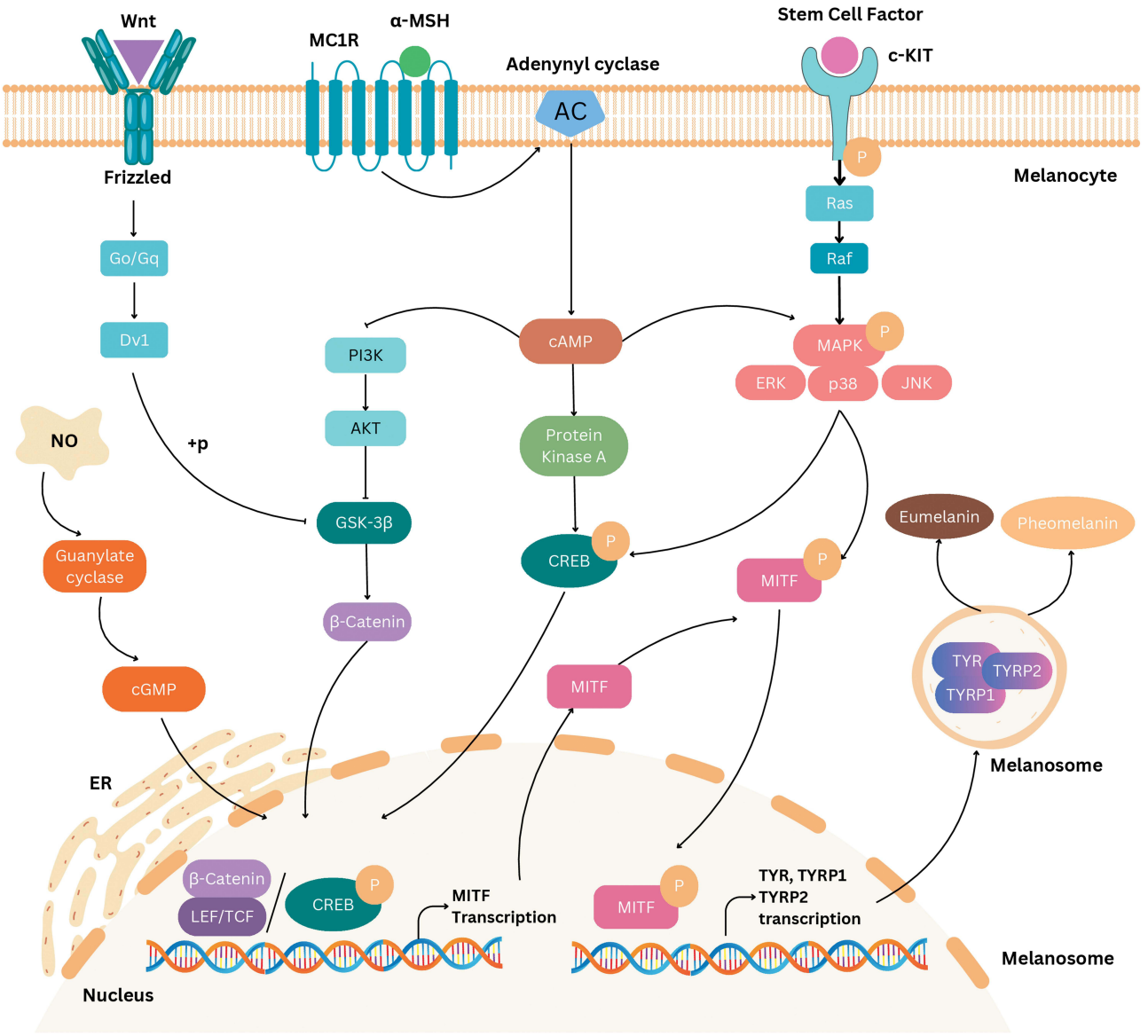


Figure 1 Main signaling pathways involved in melanogenesis regulation. The α-melanocyte-stimulating hormone (α-MSH) binds to melanocortin 1 receptor (MC1R), increasing cyclic adenosine monophosphate (cAMP) levels, which activate protein kinase A (PKA). PKA phosphorylates the cAMP-responsive element-binding protein (CREB), promoting MITF transcription. Additionally, the PI3K/AKT pathway, activated by α-MSH, interplays with the Wnt/β-catenin pathway through phosphorylation of GSK-3β, releasing β-catenin to further enhance MITF transcription. Stem cell factor (SCF) activates the c-KIT receptor, triggering the MAPK pathway, which also phosphorylates CREB and boosts MITF transcription. Other pathways, such as the nitric oxide (NO)/cGMP/protein kinase G (PKG) cascade, are also involved in MITF activation. Together, these pathways ensure MITF activation at both transcriptional and post-transcriptional levels, enabling a coordinated response to external stimuli and the expression of melanogenic enzymes.[27–29]

process results in the formation of melanosomes, which release melanin back into keratinocytes to protect the DNA from damage in the nucleus. As melanin is released from melanosomes into keratinocytes, it results in dark spots on the skin.[35]

The process of melanin biosynthesis in melanocytes begins with L-tyrosine, which is catalyzed by the TYR enzyme into L-3,4-dihydroxyphenylalanine (L-DOPA) and dopaquinone (DQ), both of which act as precursors for melanin synthesis within the melanosome.[36] Melanosomes are classified into two types based on the pigment they produce. Eumelanin, which results in darker skin pigments like brown and black, is produced in eumelanosomes, while pheomelanin, that is responsible for lighter pigments such as red and yellow, is synthesized in pheomelanosomes.[37] This reaction shown in Figure 2.

Eumelanin and pheomelanin differ in their precursors and the enzymes involved in their synthesis. In eumelanin formation, TYR converts L-DOPA to dopaquinone (DQ), which can then lead to either 5,6-indolequinone-2-carboxylic

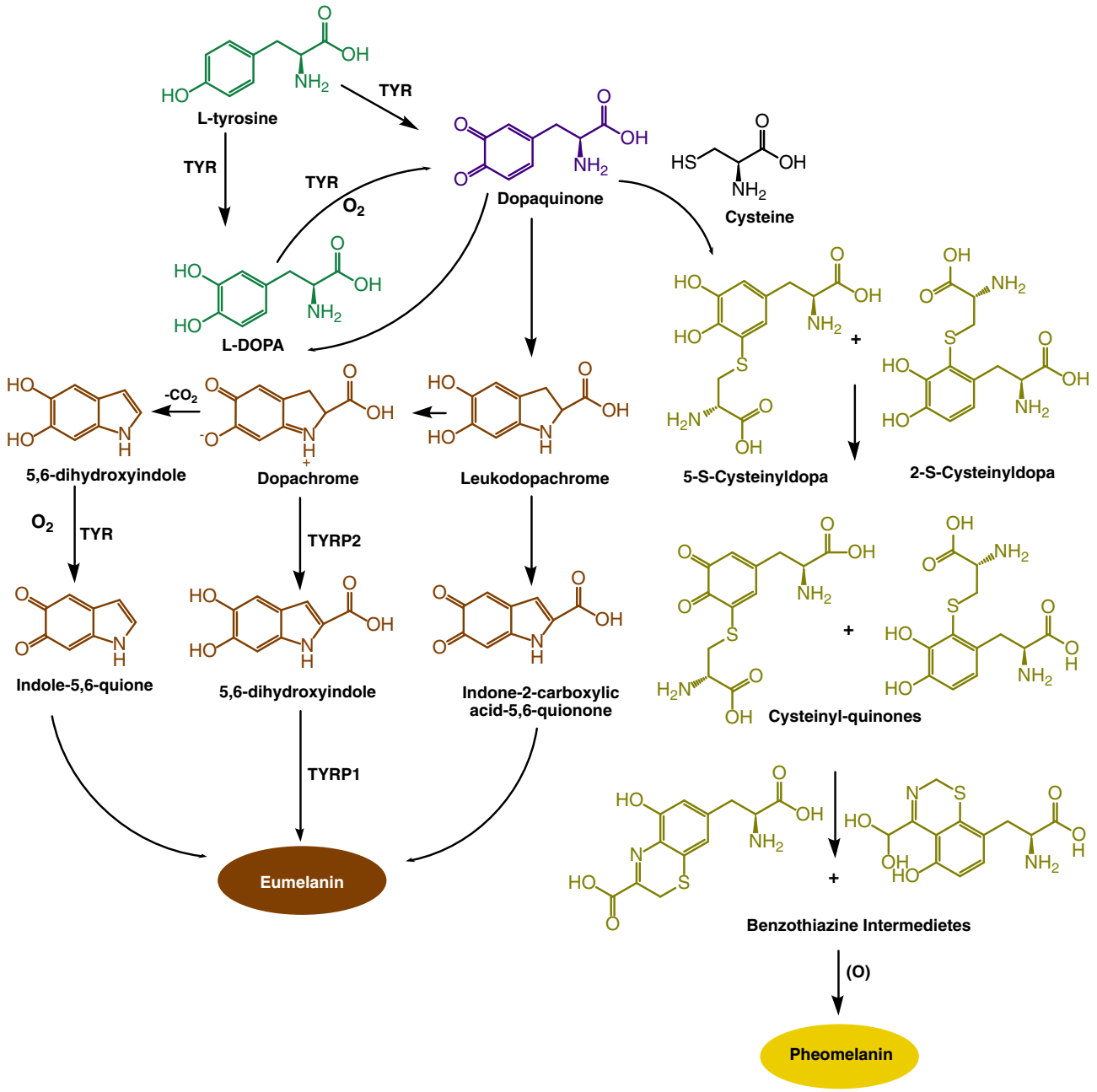


Figure 2 The Raper-Mason pathway for eumelanin and pheomelanin formation, where melanogenesis, or the synthesis of melanin, occurs through a complex series of enzymatic and biochemical reactions.[37,38]

acid (ICAQ) or 5,6-dihydroxyindole-2-carboxylic acid (DHICA) via different pathways. DQ can also form dopachrome, which decarboxylates to produce 5,6-dihydroxyindole (DHI), later converted to indole-5,6-quinone (IQ). Finally, ICAQ, DHICA, and IQ are polymerized by TRP-1 to produce eumelanin, the pigment responsible for dark skin color.[38] In pheomelanin production, dopaquinone reacts with the amino acid cysteine, resulting in substitutions at the 5 and 4 positions to form 5-*S*-cysteinyldopa and 2-*S*-cysteinyldopa, respectively. Dopaquinone then undergoes oxidation, losing a hydroxyl group and converting into a ketone-containing compound known as CD-quinones. These CD-quinones are further transformed into two benzothiazine intermediates, which are subsequently polymerized and oxidized to form pheomelanin, the pigment responsible for lighter skin tones such as yellow and red.[38]

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The key enzymes involved in melanin biosynthesis, as described above, are TYR, dopachrome tautomerase (DCT), TRP-1 with MITF as transcription factor (42). TYR, TYRP1, and DCT are part of the TYR family of proteins. These mature enzymes are transported within the cell to the melanosomes, where melanin is produced. While TYR, TYRP1, and DCT all contribute to melanogenesis, TYR is the only enzyme essential for the process. It catalyzes the rate-limiting step: the hydroxylation of L-tyrosine to L-dopa. MITF, a key regulator in the microphthalmia family, governs gene expression related to melanogenesis. Enhancing melanin production typically involves upregulating TYR expression or activating TYR activity through MITF.[32] TYR enzyme, a metalloenzyme with two copper ions at its active site, is essential for melanin biosynthesis, the pigment responsible for skin color. It has a heterotetrameric structure composed of two light and two heavy chains, underscoring its complex role in melanogenesis, 3D structure of tyrosinase shown in Figure 3.[39]

The light chain of TYR has a lectin-like structure with six antiparallel β-strands forming three trefoil hairpins that cover a six-stranded barrel. The heavy chain consists of thirteen α-helices, eight short β-strands, and connecting loops, with four helices forming the core domain's backbone. The enzyme's tunnel-shaped active site is divided into three regions: a solvent-exposed entrance, a hydrophobic passage, and an inner substrate-binding cavity.[40]

Additionally, two copper atoms in its binuclear cluster, each ligated to three histidines. It exists in three forms: oxytyrosinase (E_{oxy}), mettyrosinase (E_{met}), and deoxytyrosinase (Ede_{oxy}), each with distinct copper arrangements. Oxytyrosinase has two copper (II) ions coordinated by histidines and oxygen, with peroxide bridging the copper centers. Mettyrosinase also contains copper (II) ions but binds hydroxide ligands instead of peroxide. Deoxytyrosinase contains two copper (I) ions with a similar coordination to the met form but lacks the hydroxide bridge. The resting enzyme is mostly mettyrosinase (85%) with some oxytyrosinase (15%). In the monophenolase cycle, oxytyrosinase reacts with monophenol, oxidizing it to o-quinone and transitioning to deoxytyrosinase, which binds oxygen to regenerate

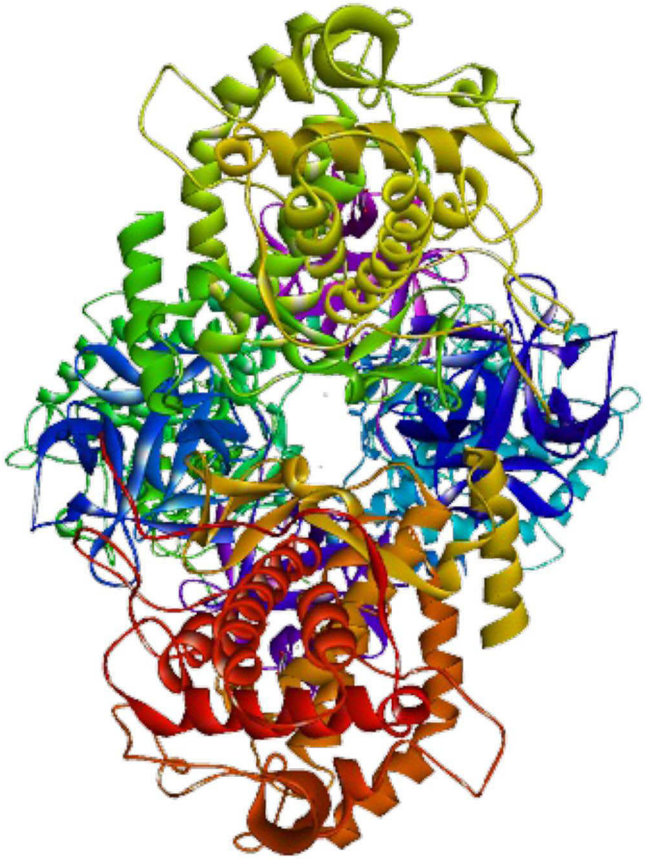

Figure 3 Schematic diagram of the 3D structure of tyrosinase from *Agaricus bisporus*. The structure was retrieved from the Protein Data Bank, with PDB ID: 2Y9X. The visualization was generated using Discovery Studio software.

oxytyrosinase. In the diphenolase cycle, both oxy and met forms react with o-diphenol to produce o-quinone, with the met form reducing to deoxytyrosinase. The monophenolase activity exhibits a lag time due to the need for sufficient catechol to reduce mettyrosinase to the deoxy form. The lag time is influenced by enzyme source, monophenol concentration, enzyme concentration, and the presence of diphenol or metal ions.[41–43] This mechanism is shown in Figure 4.

Excessive TYR production causes skin hyperpigmentation, leading to dark brown spots and irregular grey patches. TYR activity can be inhibited by compounds that block its active site or chelate copper ions. High serine levels can bind copper and affect the C-terminal tyrosine residue and hydroxyl-containing amino acids.[15]

Depigmenting agents, or TYR inhibitors, like ascorbic acid, sulphates, flavonoids, kojic acid, arbutin, hydroquinone, licorice extracts, and synthetic inhibitors, effectively reduce TYR activity. They work through various mechanisms, including acting as alternative substrates, competitive or non-competitive inhibitors, or chelators.[44] Depigmenting agents like hydroquinone are standard benchmarks in skin-lightening treatments and for managing pigmentation disorders such as melasma, age spots, and post-inflammatory hyperpigmentation. Hydroquinone acts as a poor substrate and natural competitor for TYR, inhibiting melanogenesis by producing 1,2,4-trihydroxybenzene.[45] However, due to side effects like

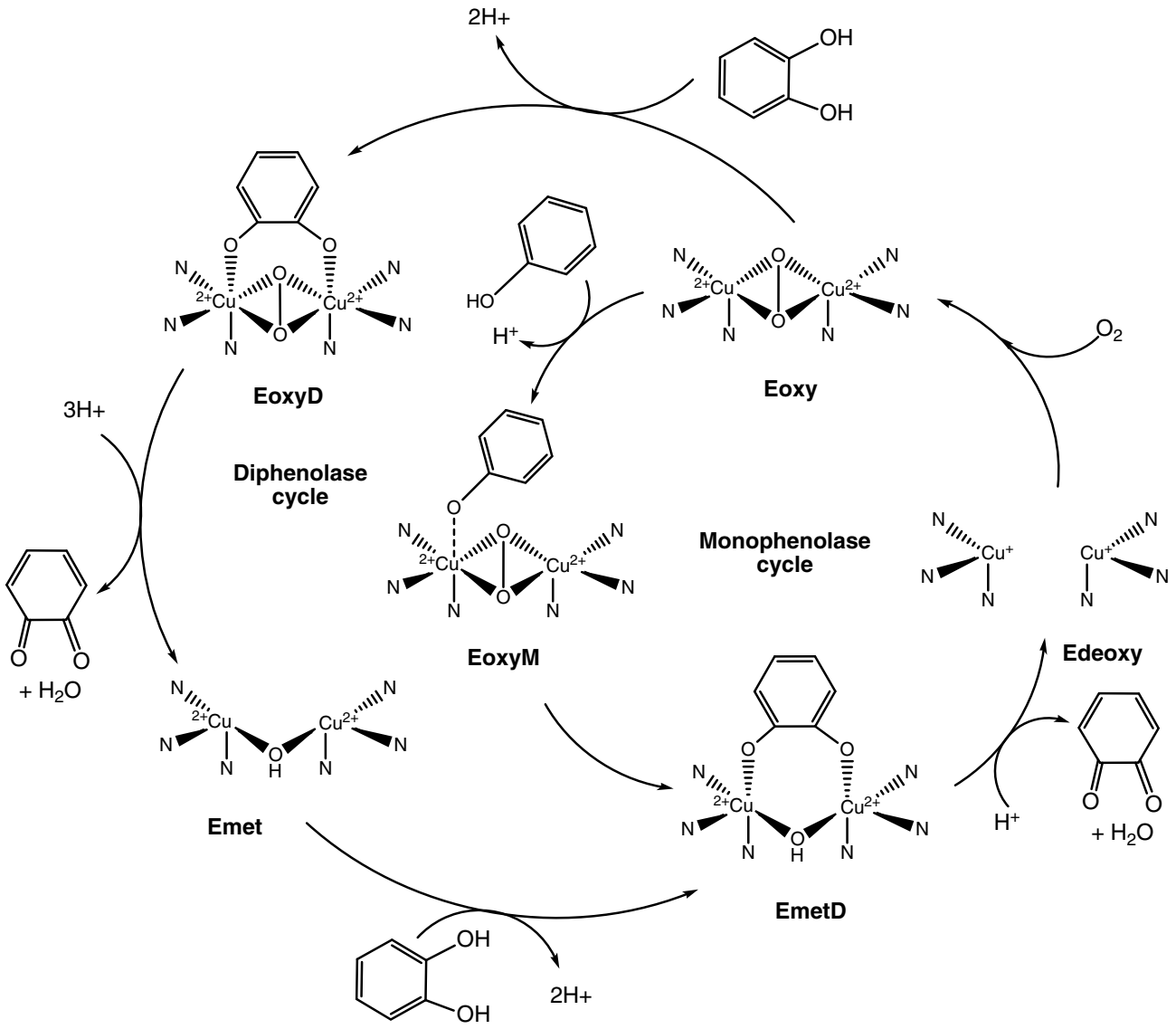

Figure 4 Catalytic cycles of tyrosinase in the hydroxylation of monophenol and oxidation of o-diphenol to o-quinone.[41,42]

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exogenous ochronosis and contact dermatitis, the use of hydroquinone has been discouraged and banned in cosmetic and pharmaceutical products.[46] Besides hydroquinone, kojic acid is also widely regarded as an effective skin-brightening agent. It works by chelating copper (Cu) ions at the active site of the TYR enzyme, thereby inhibiting its catalytic function. However, kojic acid tends to have reduced stability in cosmetic formulations, particularly when exposed to UV radiation.[47] Due to the significant side effects associated with depigmenting agents like hydroquinone and kojic acid, there has been interest in exploring natural alternatives, such as peptides derived from phycocyanin found in the microalgae *Spirulina platensis*.[48] This suggests that further research into novel peptides could provide powerful alternatives to chemical skin-lightening agents by inhibiting TYR enzyme.

Peptide Role in Antimelanogenesis

Several literature studies reveal that peptides can inhibit melanin production in the skin through two main mechanisms. First, peptides suppress the transcription of genes encoding key enzymes in melanin biosynthesis, thereby reducing the amount of enzymes produced. Second, peptides reduce the activity of these enzymes, lowering their capacity to catalyze the transformation of L-tyrosine substrate into melanin. In efforts to reduce melanin production at the gene transcription level, the MITF transcription factor has become the primary target of anti-hyperpigmentation peptides.[49–51] Alcalase®-hydrolyzed sericin peptide suppresses melanogenesis by reducing MITF expression through decreased pCREB/CREB levels without affecting the GSK3β/β-catenin pathway. Although pCREB/CREB signals recover after 24 hours, MITF mRNA and protein levels remain low during this time. Post-translational regulation of MITF by pERK via proteasomal degradation further reduces tyrosinase levels and melanin production in human melanin-producing cells.[52]

The reduction in melanin production can also result from the inhibition of tyrosinase activity, a key enzyme in melanogenesis. This inhibition may occur through various mechanisms, such as blocking the enzyme's active site or chelating Cu^{2+} ions, which are essential cofactors in the oxidation of L-tyrosine to DOPA.[51,53] Haliotis diversicolor tyrosinase inhibitory peptides (hdTIPs) demonstrated anti-tyrosinase activity through in vitro assays (mushroom tyrosinase, cellular tyrosinase, and melanin content tests) and were validated in silico using molecular docking and dynamic simulations. hdTIPs were non-toxic to murine melanoma cells (B16F10) at concentrations up to 200 μM. Among these, the peptide KNN1 (sequence NICEMK) showed the highest inhibition, reducing mushroom tyrosinase activity by 50% at 70.83 μM, which us similar to kojic acid (IC_{50} = 61.65 μM). On the other hand, RF1 (SAPTFFR) showed an ability to inhibit melanin production by 10.70%, which is lower compared to kojic acid at a concentration of 100 μg/mL, achieving 21.08%. Meanwhile, the KNN1 peptide, although known to inhibit tyrosinase in mushrooms, was found to be ineffective in inhibiting cellular tyrosinase or reducing melanin production. In cellular tyrosinase tests, KNN1 only inhibited 18.26%, significantly lower than kojic acid at a concentration of 10 μg/mL, which achieved 42.93%. Molecular docking revealed strong binding affinity (−9.8 kcal/mol) and stability, supported by RMSD (3–5 Å) and RMSF (10–40 Å) analyses over 300 ns, confirming KNN1's potential as a potent and stable tyrosinase inhibitor.[54]

Based on findings related to tyrosinase inhibitory peptides (TIPs), although these peptides demonstrate strong potential as tyrosinase inhibitors with good stability and activity comparable to kojic acid in mushroom tyrosinase assays, their effectiveness decreases significantly in cellular tyrosinase and melanin production tests. This highlights the need for further optimization. Future research should focus on enhancing the biological effectiveness of TIPs through structural modifications, improved cellular uptake, or advanced formulations such as nanoparticles. These steps are crucial to developing TIPs as safe and effective alternatives for tyrosinase inhibition in therapeutic and cosmetic applications.

Other peptides that also inhibit tyrosinase (TYR) activity include the peptide Met-His-Ile-Arg-NH_2, which has been shown to inhibit TYR activity and reduce melanogenesis in melanocytes.[49] Peptide derivatives of Leu-Gln-Pro-Ser-His-Tyr demonstrated strong antimelanogenic effects without causing cytotoxicity, highlighting their potential for treating melanin-related skin disorders.[50] Peptides derived from rice bran protein and abalone peptidome have also been shown to inhibit melanogenesis.[50,54] Coumaric acid- and caffeic acid-conjugated peptides have shown potential in reducing melanin production and TYR activity in human melanoma cells compared to known inhibitors like arbutin.[55]

Additionally, peptides have been found to influence melanosome biogenesis, induce autophagy, and suppress melanin synthesis, resulting in decreased pigmentation.[56] Furthermore, peptides have been explored for their antioxidant

properties and their capability to neutralize free radicals, which can further inhibit melanin biosynthesis.[55] On the other hand, amino acid composition of peptides, particularly arginine, phenylalanine, and hydrophobic or aromatic residues, is essential for tyrosinase inhibition. Sericin demonstrates anti-melanogenic effects by inhibiting mushroom tyrosinase, aided by serine and threonine, which chelate copper at the enzyme's active site. However, structural differences between human and mushroom tyrosinase may cause variability, emphasizing the need for human-specific studies.[52]

The pharmacokinetic potential of peptides can be evaluated by comparing their physicochemical properties—such as molecular weight, hydrophobicity, hydropathy index, hydrogen bond acceptors and donors, and peptide length—to those of established drugs.[57] A detailed examination of the amino acid composition of anti-aging peptides reveals several key characteristics. The first characteristic is "Compact Size", referring to peptides with short amino acid sequences of fewer than 20 residues. These peptides exhibit enhanced absorption efficiency, allowing them to cross the intestinal barrier effectively while maintaining their biological activity in vivo.[58–60] The second characteristic is the "Hydrophobic N-Terminal", which enables peptides to form hydrogen donor interactions with other amino acids, enhancing their anti-aging efficacy. Research indicates that peptides with a hydrophobic N-terminal display increased activity, likely due to their ability to establish strong interactions with surrounding molecules, resulting in better bioavailability and effectiveness in anti-aging applications. Moreover, the hydrophobic N-terminal, particularly those with aliphatic side chains like Glycine, Isoleucine, Leucine, and Valine, has been associated with the inhibition of enzymes involved in aging processes, emphasizing its importance in peptide functionality.[61,62] The third characteristic is the "High Concentration of Polar Amino Acids". The presence of a significant number of polar amino acids in peptides greatly contributes to their capacity to neutralize free radicals. This is achieved through the chelation of polar side chains, enabling peptides to counteract oxidative damage effectively. The abundance of hydrophilic amino acids enhances the antioxidant properties of these peptides, making them potent agents in combating oxidative stress and protecting cells from damage caused by reactive oxygen species.[63] The fourth characteristic is the "Inclusion of Aromatic Amino Acids". These amino acids enhance peptides' anti-aging properties by donating protons to electron-deficient free radicals, resulting in strong antioxidant activity. Previous studies have emphasized the antioxidant potential of amino acids like hydroxyproline (Hyp), leucine (Leu), alanine (Ala), and valine (Val), making them ideal for anti-aging formulations. The potent reactive oxygen species (ROS) and reactive nitrogen species (RNS) scavenging abilities of these peptides, due to the presence of both polar and aromatic amino acids, make them promising candidates for functional dietary supplements or therapeutic agents in combating aging, to synthesize peptides, Solid-Phase Peptide Synthesis is the commonly used method.[14]

Solid-Phase Peptide Synthesis (SPPS) is the most widely used method for synthesizing peptides, especially for short to medium-length peptides consisting of fewer than 50 amino acids at high purity, which are commonly used as tyrosinase enzyme inhibitors.[64] This method involves the attachment of amino acids to a solid resin, with stepwise addition of amino acids and removal of protecting groups to form peptide bonds. The process begins with resin loading, which serves to anchor the growing peptide chain, facilitating selective deprotection and subsequent amino acid coupling. Protecting groups such as Fmoc or Boc are employed to shield the amino or carboxyl groups of amino acids, and are selectively removed using deprotecting agents such as piperidine (for Fmoc) or trifluoroacetic acid (TFA).[65] In a study conducted by Li et al (2024), peptides such as Ala-His-Tyr-Tyr-Asp (AHYYD), Thr-Phe-Ser-Gly-Asn-Tyr-Pro (TFSGNYP), and Lys-Pro-Ile-Trp-Thr (KPIWT) were synthesized using SPPS with a purity exceeding 98% (w/w), as detected by HPLC. After purification, these peptides were identified using ESI-MS (Electrospray Ionization Mass Spectrometry) to confirm the amino acid sequence, ensure purity, and identify peptide fragments following purification. ESI-MS works by ionizing peptides in the gas phase and analyzing the mass of the ions to determine the amino acid sequence.[64] These findings align with the research by Yuxiu et al (2024), which reported that peptides IIAPPERKY, VWDESFKVF, and FAGDDAPRAVFPS, synthesized via SPPS, exhibited purities greater than 95%.[66] Additionally, Lin et al (2024) also reported three peptides—Tyr-Pro-Asn-Pro-Tyr (YPNPY), Gly-Tyr-His-Phe-His-Ser-Tyr-Pro (GY-HFHSYP), and Tyr-Val-Pro-Gly-His-Gly (YVPGHG)—with purities above 98% (w/w), synthesized through solid-phase synthesis.[67] SPPS offers advantages in efficiency, excellent control over peptide sequences, and the ability to produce peptides with non-natural amino acids, enabling the design of peptides with specialized properties, such as enhanced stability or biological activity. However, this method faces challenges when synthesizing longer peptides, where efficiency tends to decrease due to the accumulation of errors during amino acid incorporation or the formation of secondary structures that hinder synthesis.[68] In the case of longer peptides, secondary structures such as

alpha-helices or beta-sheets can impede further amino acid coupling, slowing down the process and reducing yield.[68] Despite these challenges, SPPS remains the predominant method for synthesizing short to medium-length peptides and continues to be widely used in the production of therapeutic peptides, diagnostics, and structural research.

Molecular Weight

Recently, studies have demonstrated that the size, molecular weight, and composition of peptides can significantly impact their ability to inhibit TYR activity.[69] The inhibitory potential of peptides against tyrosinase (TYR) is closely linked to their amino acid composition and structural features, with certain residues playing pivotal roles in enhancing binding affinity and enzyme inhibition. For instance, a C-terminal tyrosine residue is particularly critical, as it significantly contributes to TYR binding and inhibition by altering the enzyme's conformation.[48] Similarly, the inclusion of basic residues like arginine, paired with non-polar amino acids such as proline, alanine, valine, and leucine, has shown strong inhibitory effects on TYR activity.[69,70]

In addition to composition, molecular weight is a crucial determinant of TYR inhibition and skin delivery. Many anti-aging peptides exceed the optimal molecular weight threshold for skin penetration (500 Da) and often have partition coefficients (log P) outside the range necessary for effective absorption.[71] These properties limit their ability to penetrate the skin, significantly reducing transdermal delivery, especially for compounds above 500 Da, regardless of their hydrophilic or hydrophobic nature.[72]

Structure-activity relationship studies further highlight the importance of peptide size in TYR inhibition.[25] Peptides with sequences such as DGGR, DGD, NAGE, LVGE, and GSEG, with ranging from 0.5 to 2.0 kDa in molecular weight, have been identified as effective TYR inhibitors by forming hydrogen bonds with specific amino acid residues on the enzyme.[73] Additionally, the inhibitory mechanism of peptides has been associated with their capacity to chelate copper ions, which are essential for TYR's catalytic activity.[74] Peptides derived from sources such as fish scale gelatin and chicken egg white have demonstrated that smaller peptides (400–600 Da) exhibit higher metal-chelating activities, highlighting the diverse origins and functional potential of bioactive peptides.[75,76] The relationship between the molecular weight of reported anti-melanogenesis peptides and their activity to inhibit the TYR enzyme can be observed in Table 1.

Short-sequence oligopeptides, especially those containing aromatic residues like tyrosine and phenylalanine, demonstrate robust TYR inhibition due to their ability to bind effectively to the enzyme and stabilize the peptide-TYR complex.[78] The study evaluated tyrosinase inhibitory peptides derived from *Spirulina platensis phycocyanin*,

Table 1 Selected Examples of Anti-Melanogenesis Peptides: Summary of the Relationship Between Molecular Weight and Binding Energy and Its Activity in Inhibiting the Tyrosinase (TYR) Enzyme

Sources	Amino Acid Sequence	Molecular Weight (Da)	Binding Energy (kcal/mol)	TYR Inhibition IC50 (mg/mL)	Ref
Spirulina platensis microalgae's phycocyanin	AFGRFR	752.86	−187.1	13.9 ± 0.6	[48]
	MAACLR	663.85	−143.1	84.7 ± 0.4	
	RCLNGRL	831.00	−176.7	84.3 ± 0.5	
	RYVTYAVF	1018.16	−206.8	57.3 ± 4.7	
	SPSWY	638.68	−162.6	83.4 ± 2.6	
	GRF	378.43	−128.2	83.4 ± 2.6	
	AADQRGKDKCARDIGY	1766.95	−151.9	53.5 ± 9.1	
Hydrolyzed proteins from Fengdan peony (*Paeonia ostii*) seed meal	SFAPRFD	838.91	−7.7	110 ±0.05	[72]
	HYGR	531.57	−7.6	96 ± 0.02	
	SPGRLP	625.72	−7.5	158 ± 0.07	

(*Continued*)

Table 1 (Continued).

Sources	Amino Acid Sequence	Molecular Weight (Da)	Binding Energy (kcal/mol)	TYR Inhibition IC50 (mg/mL)	Ref
Enzyme Hydrolyzed Royal Jelly	TIPPPT	735.90	≤−6.80	7.59 ± 0.06	[77]
	IIPFIF	852.97	≤−6.80	6.16 ± 0.12	
	ILFTLL	845.05	≤−6.80	9.25 ± 0.11	
Fish Scale Gelatin	WQLTL	660.3729	−8.3	3.86	[25]
	WSVEF	667.3090	−9.2	5.81	
	FDLGFLAR	469.7591	−6.4	4.00	
Colla corii asini	DGGR	404.26	−126.39	0.92	[73]
	DGD	306.05	−123.35	0.50	
	NAGE	390.11	−123.30	0.77	
	LVGE	417.28	−122.28	0.66	
	GSEG	349.10	−122.23	0.69	

emphasizing the potential of short peptides with specific residue combinations. Using in silico docking through HPEPDOCK, peptides like P5 (SPSWY) demonstrated remarkable inhibitory activity, with a binding energy of −162.6 kcal/mol. The docking simulations targeted tyrosinase structures from mushroom and human sources (PDB codes: 5M8T and 2Y9X) and focused on critical residues such as His263, Val283, and His244 within the enzyme's active site. Ligand preparation involved energy minimization, while docking parameters included a grid size of 60 × 60×60 Å and a center at x = −10.09, y = −28.03, z = −43.14 (chain A of 2Y9X). P5's inhibitory efficacy was further validated in vitro, demonstrating superior activity against mushroom and B16-F10 tyrosinases. It achieved an IC_{50} of 12.1 μM for monophenolase activity, outperforming kojic acid (IC_{50}: 47 μM), and reduced melanin synthesis in B16-F10 melanoma cells by 61.8% at 200 μg/mL without cytotoxic effects. These findings highlight the critical role of hydrophobic, aromatic, and basic residues in enhancing binding stability and TYR inhibition. Notably, tryptophan and a C-terminal tyrosine proved essential for stabilizing the peptide-TYR complex, as their removal significantly diminished activity.[48] On the other hand, hydrophobic amino acids such as tryptophan, with its large aromatic indole ring, interact with the active site of tyrosinase (TYR) through hydrophobic and π-π interactions. This is demonstrated in the SDW peptide, where tryptophan forms interactions with Val283 (5.37 Å) and Val284 (4.84 Å), potentially obstructing the enzyme's hydrophobic pocket and reducing its activity.[76]

Furthermore, Li et al (2024) reported that tyrosine at third position of the AHYYD peptide from *Pinctada martensii* nacre forms a hydrogen bond with Ser282 (2.71 Å) and a π-alkyl interaction with His263 (4.23 Å), while the fourth position forms a π-sigma bond with Val283 (3.64 Å) and a π-π stacked interaction with His263 (4.23 Å). Additionally, histidine at position 2 forms a hydrogen bond with Cys83 (2.10 Å), slightly shorter than tyrosine's bond. The shorter the bond, the stronger the interaction, indicating a more stable ligand-receptor complex. The peptide, containing tyrosine, alanine, histidine and aspartic acid shows mixed-type inhibition of tyrosinase with K_i = 0.601 mmol/L and K_{is} = 38.375 mmol/L as indicated by the Lineweaver–Burk double inverse plot, suggesting a stronger affinity for the free enzyme. Moreover, the addition of AHYYD caused changes in the secondary structure of tyrosinase with the rise in the α-helix content, as shown by Circular Dichroism analysis, compared to tretinoin binding.[64]

Moreover, Terminal histidine and a hydrophobic core in peptide structures contribute to the enhanced stability of TYR–peptide complexes, reflected in more negative docking scores.[77] Hydrophobic amino acids like tryptophan, valine, and leucine play a critical role in stabilizing interactions with TYR's active site and lipid membranes. Molecular

dynamics and docking studies reveal that these residues contribute through van der Waals interactions with hydrophobic surfaces and hydrogen bonding with polar residues like histidine and lysine. In the case of TYR-peptide complexes, the presence of terminal histidine and hydrophobic residues in the peptide structure has been shown to stabilize interactions with the enzyme, as evidenced by more negative docking scores. For example, peptides such as GGWH (−9.36 kcal/mol docking score) demonstrate strong binding to TYR, with acidic amino acids and tryptophan enhancing affinity by interacting with the enzyme's metal center and tightly packed with the aliphatic portion of lipids like ceramides and cholesterol within the membrane, further contributing to the stability of the peptide and its ability to inhibit TYR. These hydrophobic interactions not only stabilize the peptide structure but also facilitate passive permeation into the stratum corneum, crucial for transdermal applications.[57]

Besides, the peptide's affinity for the TYR active site is significantly influenced by residues like tryptophan, histidine, and acidic amino acids, which can substitute a coordination group at the enzyme's metal center as said before.[57] The copper-chelating ability of peptides depends on sequence composition, molecular weight, and specific residues, as shown by Colla corii asini-derived peptides like DGGR and NAGE. These tetrapeptides strongly inhibit tyrosinase (IC_{50}: 0.66–0.92 mg/mL) by forming van der Waals interactions with catalytic residues and copper atoms (Cu401). Negatively charged residues like aspartic acid (D) and glutamic acid (E) enhance copper binding through COO^- groups, effectively blocking the active site, while histidine and aromatic residues optimize copper coordination via π-metal interactions. Their small size minimizes steric hindrance, improving access to the active site. In silico studies using (PDB ID: 2Y9X) revealed high binding affinities for DGGR and NAGE, with docking energies of −126.39 and −122.23 kcal/mol, stabilizing interactions with residues such as His61 and Met280. In vitro, DGD exhibited the highest inhibition (IC_{50} = 0.50 mg/mL), followed by LVGE (IC_{50} = 0.66 mg/mL). These peptides demonstrated strong copper chelation and binding to tyrosinase active sites. Their small molecular weight (<500 Da) and high purity (87–96%) further facilitated effective enzymatic interaction.[73]

These findings underscore the potential of designing peptides with negatively charged, histidine, and aromatic residues to enhance tyrosinase inhibition, which can be leveraged for therapeutic and cosmetic applications targeting hyperpigmentation. Simultaneously, incorporating hydrophobic cores and positively charged residues can further stabilize TYR-peptide complexes and improve skin permeability without diminishing tyrosinase inhibitory activity. The ability of peptides to undergo conformational changes, form stable hydrogen bonds, and interact effectively with lipid bilayers presents a promising strategy for enhancing transdermal delivery while maintaining their biological function.

Polarity

The interaction of peptides with membrane receptors and transcription factors involved in melanin production plays a crucial role in inhibiting excessive melanin synthesis. Peptides can penetrate cell membranes to interact with transcription factors like MITF, which control the expression of tyrosinase (TYR), a key enzyme in melanin synthesis. Several receptors on the melanocyte membrane that regulate the expression or activation of TYR include MC1R, c-Kit receptor, endothelin receptor (ETR), estrogen receptor, and Protease-Activated Receptor-2 (PAR-2).[79]

The effectiveness of peptides in inhibiting TYR is significantly influenced by their polarity, which determines their ability to penetrate the plasma membrane and reach intracellular target.[44,80] Highly polar peptides are effective at interacting with hydrophilic active sites of tyrosinase due to their strong affinity for aqueous environments. However, their high polarity often impairs their ability to cross the hydrophobic lipid bilayer of cell membranes, limiting their intracellular accessibility.[80,81] In contrast, peptides with reduced polarity exhibit better membrane permeability, enabling them to interact directly with intracellular components such as transcription factors and enzymes, to inhibit melanin production.[82]

Achieving effective TYR inhibition requires peptides designed with a strategic balance of hydrophobic and hydrophilic amino acids. This balance is crucial for optimizing solubility, membrane translocation, and interaction with TYR's hydrophobic active site.[83] For instance, the peptide PFRMY, with a hydrophobic-to-hydrophilic ratio of 3:2, exhibits strong TYR binding through hydrophobic interactions, while its hydrophilic tyrosine residue enhances solubility and cellular uptake. It demonstrated superior TYR inhibition (IC_{50}: 0.43 ± 0.08 mg/mL) compared to RGFTGM (IC_{50}: 1.61 ±

0.04 mg/mL; ratio 2:4) and showed higher copper chelation (15.7%). In a zebrafish model, PFRMY at 0.10 mg/mL reduced melanin content by 29.82% and TYR activity by 14.69%, comparable to α-arbutin, with better safety and no morphological abnormalities. Docking simulations using Autodock Vina with grid map size of 60×60 ×60 Å and a grid center with ×=− 10.09, y =−28.03, z =−43.14 form chain A tyrosinase (PDB ID:2Y9X), revealed stronger interaction scores for PFRMY (−7.9 kcal/mol) compared to RGFTGM (−7.2 kcal/mol). Additionally, the RMSD of the PFRMY-TYR complex remained below the standard range of 0.08–0.14 nm over 30 ns, confirming high stability within the active site. These findings highlight PFRMY's superior efficacy and stability in tyrosinase inhibition and melanin reduction.[84]

The enhanced performance of PFRMY underscores the potential of peptides as next-generation anti-melanogenesis agents, offering significant improvements over traditional compounds like arbutin. Arbutin, a glycoside derivative of hydroquinone and a widely used anti-melanogenesis agent, is recognized for its safety but demonstrates limited efficacy, achieving only 1.72% inhibition of monophenolase activity at 0.2 mM.[85] In contrast, peptides such as FLF, SPSSS, and WLL not only exhibit higher tyrosinase inhibition with binding energies of −8.4, −8.4, and −8.2 kcal/mol, respectively, but also show broader mechanisms of action. For example, FLF reduces tyrosinase activity by 75.41% and melanin content by 37.34% in zebrafish models. This superior efficacy is attributed to the structural similarity between phenylalanine, a component of FLF, and tyrosine, the natural substrate of tyrosinase. This similarity allows phenylalanine to act as a pseudosubstrate, enhancing FLF's inhibitory effect. Beyond direct enzymatic inhibition, peptides like FLF, SPSSS, and WLL offer multifaceted mechanisms for suppressing melanin synthesis. They downregulate critical signaling pathways, including MITF, TRP1, TRP2, CREB, WNT4, JNK, ERK, and β-catenin, demonstrating their potential as robust therapeutic agents. Furthermore, peptides exhibit superior safety profiles, as evidenced by their low toxicity in zebrafish models with no significant developmental side effects. Their resistance to enzymatic degradation and oxidative stress enhances their stability, making them well-suited for long-term applications compared to conventional agents like arbutin.[82]

These findings underscore the critical importance of balancing hydrophobicity and hydrophilicity in peptide design, supported by docking scores and experimental data. This balanced approach ensures peptides can permeate cellular membranes, engage with intracellular targets, and effectively inhibit tyrosinase, offering a robust strategy for addressing melanin overproduction and advancing therapeutic and cosmetic applications.

The pharmacokinetic properties of individual amino acids play a pivotal role in the effectiveness of tyrosinase inhibitory peptides (TIPs). These properties influence enzyme activity and melanin production by determining how peptides interact with TYR. Hydrophobic amino acids like leucine and phenylalanine, with their aliphatic side chains and aromatic rings, contribute to strong hydrophobic interactions with TYR, enhancing binding and inhibitory capacity.[86] Aromatic residues like tryptophan further strengthen these effects by interacting with TYR's hydrophobic regions, altering the enzyme's hydrophobicity and reinforcing inhibition.[76] Furthermore, hydrophobic amino acids like leucine and proline, amino acids with benzene rings like tryptophan and phenylalanine, enhances TYR inhibition through hydrophobic interactions with the enzyme's active site that facilitated by van der Waals forces, are critical for stabilizing the binding complex.[36,81] Moreover, as peptide length increases, the hydrophobic characteristics tend to dominate over the polar neutral properties.[87]

In contrast, polar, uncharged amino acids such as serine and cysteine modulate TYR activity, showcasing the functional diversity of amino acids in peptide-enzyme interactions.[88] Charged residues, such as lysine and arginine, add another dimension to TIP effectiveness by leveraging their charge properties for stronger peptide-enzyme interactions. These residues serve a dual role: facilitating membrane penetration by interacting with the negatively charged lipid bilayer and directly inhibiting TYR through interactions at the active site. This dual functionality enhances cellular uptake and may induce conformational changes in TYR that reduce its catalytic activity.[78] Studies by Gong et al (2019) also demonstrated that the charge and hydrophobicity of cell-penetrating peptides significantly affect their capability to penetrate Candida albicans cells, highlighting the importance of these properties in peptide functionality.[89] The relationship between the polarity of reported anti-melanogenesis peptides and their activity to inhibit the TYR enzyme can be observed in Table 2.

Research by Herce et al (2009) highlighted that Arginine residues in peptides facilitate the formation of toroidal pores in cell membranes, enhancing permeability by allowing the flow of molecules or ions across the lipid bilayer. Arg

Table 2 Selective Examples of Anti-Melanogenesis Peptide: Summary of Their Polarity to Binding Energy and TYR Inhibition

Sources	Amino Acid Sequence	Hydrophobic (%)	Hydrophilic (%)	Binding Energy (kcal/mol)	TYR inhibition IC50 (mg/mL)	Ref
Tilapia (*Oreochromis niloticus*) skin	PFRMY	60	40	−7.9	0.43 ± 0.08	[84]
	RGFTGM	33.33	66.7	−7.2	1.61 ± 0.04	
Thiosemicarbazone (TSC)-peptide Conjugate	FWY	66.7	33.33	-	6.66 ± 0.5	[36]
	FYY	33.33	66.7	-	6.57 ± 0.5	
Peptides synthesized from natural product derivatives	ADHPF	60	40	−9.0	35.86 ± 2.26	[74]
	ILELPFASGDLLML	71.43	28,57	−9.5	17.26 ± 2.10	
	FDKLPGFGD	44.44	55.56	−7.0	22.78 ± 1.12	
	GYSLGNWVCAAK	41.67	58.33	−9.4	80.04 ± 2.79	
	HIATNAVLFFGR	58.33	41.67	−8.3	80.04 ± 2.79	
	FMMFESQNKDLLFK	50	50	−9.3	28.95 ± 1.47	

residues interact with phosphate groups on the lipid head via hydrogen bonds, creating structural distortions that form transient pores. While this process benefits therapeutic applications like drug delivery and gene therapy, increased $CaCl_2$ concentrations reduce membrane permeabilization by competing with peptides for phosphate group binding. This strategy minimizes cytotoxicity from pore formation, enabling controlled membrane permeability for safer therapeutic applications.[90]

When combined, hydrophobic, polar, and charged residues create TIPs that effectively bind to TYR and modulate its activity. This integration ensures both robust enzyme inhibition and enhanced pharmacokinetic properties, making TIPs invaluable for skin pigmentation treatments and melanin regulation. By aligning their molecular design with specific functional goals, TIPs hold significant potential in therapeutic and cosmetic applications, offering a versatile and innovative approach to addressing pigmentation-related challenges.

In the other hand, there are significant differences between naturally sourced peptides and those synthesized in the laboratory in terms of tyrosinase (TYR) inhibition and skin absorption, particularly in aspects of efficacy, absorption, and safety and stability. Typically, synthetic peptides are derived from natural peptides that have been adjusted or modified to enhance their purity and functional potential. For example, naturally derived peptides like DIP1 (GYSLGNWVCAAK) have been synthesized in abortorium and demonstrate strong tyrosinase inhibitory activity with IC_{50} = 3.04 ± 0.39 mm. However, natural peptides often face challenges in skin absorption, particularly due to their high molecular weight—such as DIP1, which has a molecular weight of 1268.44 Da—that limits its ability to penetrate the skin.[74] Despite this, natural peptides tend to be more compatible with the human body, offering advantages in terms of safety and biocompatibility, although they are more susceptible to enzymatic degradation, which affects their long-term stability.[25] On the other hand, synthetic peptides derived from these natural sources can be structurally modified to enhance skin absorption, such as through cyclization or methylation, which improve their ability to penetrate the epidermis more effectively and resistance to enzymatic proteolysis.[91] Therefore, both natural and synthetic peptides have their advantages and disadvantages, which should be considered based on the specific goals of therapeutic or cosmetic applications. Synthetic peptides are superior in terms of efficacy and absorption, while natural peptides prioritize safety and biocompatibility.

Cyclization

Cyclization of peptides is a well-established strategy to stabilize bioactive peptides,[92] offering increased structural rigidity, enhanced serum stability,[93] and greater resistance to enzymatic proteolysis compared to linear peptides.[94] This process limits conformational flexibility, improving receptor target affinity[95] and interaction with cell membranes, while maintaining pharmacological activity.[96] In addition to boosting stability, cyclic peptides are also known to enhance skin penetration[93] and cellular uptake,[96] making them valuable in medicinal chemistry and peptide modification.

In the field of anti-melanogenesis, peptide cyclization has been employed to enhance peptide activity in inhibiting melanin synthesis.[97] While research in this area remains limited, comparative studies indicate that cyclic peptides generally exhibit superior stability and binding affinity compared to linear peptides. This advantage stems from their reduced conformational flexibility, which enables them to interact more effectively with receptors and cell membranes. For example, cyclic peptides such as massiliamide (Cyclo[YPVP]) isolated from *Massilia albidiflava* and *pseudostellarin F* (Cyclo[GGYLPPLS]) have demonstrated strong inhibitory activity against tyrosinase. Their IC_{50} values—1.15 μM for massiliamide and 50 μM for pseudostellarin F—surpass that of arbutin, a widely used depigmentation agent with an IC50 of 1.2 mM.[98,99] The increased stability and enhanced receptor interactions make cyclic peptides more effective in inhibiting melanogenesis. Furthermore, cyclic peptides like AG9 (Cyclo[VPPAFFPPGF]) exhibit approximately 38% tyrosinase inhibition by blocking substrate access to the enzyme's catalytic site. AG9 achieves this through strong interactions with critical residues such as Gly46, Lys47, Asp55, Asn57, Met61, and Glu158.[41] The structure of the cyclic peptides can be seen in Figure 5.

However, there are cases where linear peptides may outperform cyclic ones. For example, linear peptides can exhibit higher solubility and flexibility, which may make them more effective under specific conditions where greater mobility or

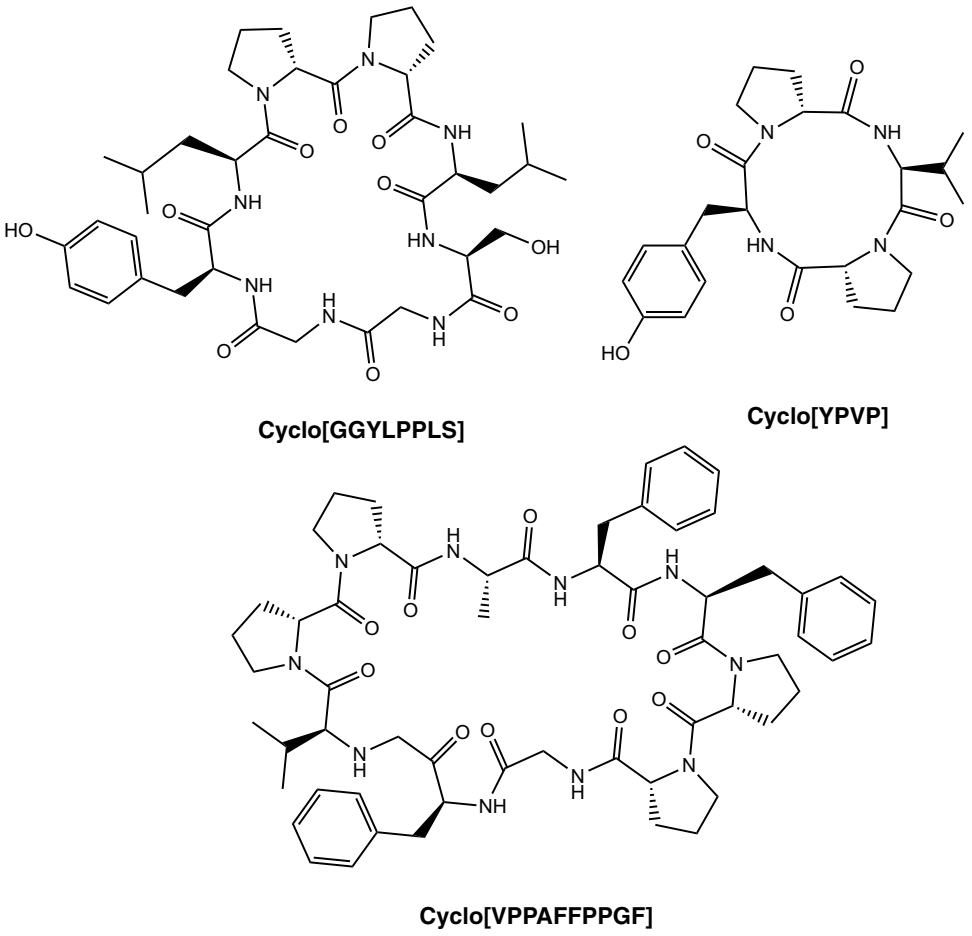

Figure 5 Chemical structure of the investigated cyclic peptides.

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different conformational flexibility is needed for binding or interaction with cellular targets.[100] Additionally, modifications to linear peptides, such as increasing hydrophilicity to improve pharmacokinetics, can enhance their performance in certain biological settings. Nevertheless, cyclic peptides remain more advantageous in terms of stability, pharmacological activity, and targeted inhibition of melanogenesis due to their higher receptor affinity and cellular uptake.[98] Therefore, while linear peptides might outperform cyclic peptides in specific applications, cyclic peptides are generally preferred for long-term stability and stronger melanogenesis inhibition, especially in cosmetic or therapeutic settings. This preference stems from their structural rigidity, which enhances metabolic stability and receptor targeting. The development of conformationally restricted analogues has been instrumental in studying ligand-receptor interactions and structure-activity relationships, further highlighting the advantages of cyclization.

Cyclization also plays a significant role in drug design by improving the stability of peptides during metabolic processes and enhancing receptor specificity.[101] For instance, cyclized peptides, such as cell-penetrating peptides (CCPs), have been shown to effectively traverse the skin barrier and penetrate viable skin layers. These peptides interact efficiently with cell membranes, enter cells through endocytic pathways, and escape from early endosomes to reach the cytosol, thereby enhancing their intracellular delivery.[102] Moreover, cyclization has been demonstrated to improve the penetration of molecules into lipids within the intercellular spaces of the stratum corneum, facilitating deeper skin permeation.[103] When combined with additional modifications, such as fatty acid acylation, the cell-penetrating abilities of cyclic peptides are further enhanced, enabling improved interactions with cell membrane phospholipids and increasing cellular uptake.[104]

Cyclization significantly enhances peptide interactions with cell membranes by reducing hydrogen-bonding potential, increasing lipophilicity, and compacting the peptide structure. Peptide permeability is closely tied to the number of hydrogens bonds the solute forms with water, as these bonds must be broken to cross the membrane—a process that is energetically demanding.[105] Cyclization mitigates this challenge by promoting the formation of intramolecular hydrogen bonds, reducing overall hydrogen-bonding potential and lowering the energy barrier for membrane penetration. This facilitates easier passage through membranes, particularly via the transcellular route.[106] Additionally, cyclization increases the peptide's lipophilicity, enhancing interactions with the hydrophobic core of lipid bilayers. The amphiphilic nature of cyclic peptides, such as those with a Tryptophan side chain near the cyclic ring, further contributes to improved cellular permeation. By balancing hydrophilic and hydrophobic regions, cyclic peptides integrate more effectively with cell membranes, improving their ability to cross these barriers. Furthermore, cyclization reduces the peptide's average radius, resulting in a compact structure that adopts favorable conformations for membrane penetration. Comparative studies have shown that cyclic peptides outperform linear counterparts in crossing cell monolayers through both paracellular and transcellular pathways. This dual-pathway penetration underscores cyclization's role in enhancing lipophilicity, structural compactness, and membrane permeability, making it a critical strategy for optimizing peptide absorption and functionality.[107]

Several approaches to peptide cyclization have been investigated, such as backbone head-to-tail cyclization, on-resin synthesis, chemoselective cyclization, and the use of acylammonium species. These techniques provide efficient methods for constructing cyclic peptides, which are crucial in the development of anti-melanogenic agents.[94,97,108] Moreover, advances in methods for designing conformationally-restricted peptides using computational tools have led to the creation of di-sulfide-crosslinked and backbone-cyclized peptides, pushing the field forward.[109] Overall, peptide cyclization is an effective strategy for improving peptide skin penetration. By increasing stability, conformational rigidity, and cell membrane interaction, cyclized peptides hold significant potential for improving the delivery and effectiveness of therapeutic peptides in skin-related applications.

Additionally, the ability of D-tyrosine-containing peptides to suppress melanin production has been examined. D-tyrosine-containing peptides significantly enhance anti-melanogenic effects by inhibiting melanin synthesis in human melanoma cells (MNT-1). Incorporating D-tyrosine at the N- or C-terminus of pentapeptide-18 derivatives reduced melanin content by 50% at 500 μM and decreased tyrosinase activity by 18% N-terminus (N-D) and 25% C-terminus (C-D). These effects were linked to suppressed tyrosinase and MITF expression and inhibited UV-induced increases in tyrosinase and MITF mRNA levels, without cytotoxicity as confirmed by MTT assays. D-tyrosine improves peptide stability and activity, offering a promising strategy for targeted melanogenesis inhibition in cosmetic and

therapeutic applications.[53] Beyond melanogenesis, cyclization has also been applied to improve peptide targeting of tumor-associated macrophages, demonstrating its potential to expand peptide functionality across diverse therapeutic applications.[110]

Interaction Peptide With Amino Acid in TYR Enzyme

Molecular docking studies have further highlighted the importance of both hydrophilic and hydrophobic interactions between peptides and target enzymes in enzyme inhibition, emphasizing the role of peptide polarity in modulating TYR activity.[111] Using the docking simulation method, we can determine the docking energy, type, and position of the bond between the active compound and the specific target protein. Docking energy serves as a measure to estimate the ligand-receptor interaction. When determining the interaction of inhibitor compounds with target proteins, it is essential to identify specific amino acids and metals (cofactors) as the active sites of each enzyme.[112] Studies on molecular interactions have indicated that H-bonds and van der Waals forces are essential forces in peptide interactions with TYR.[113]

Several studies have commonly utilized the 3D structure of tyrosinase isolated from *Agaricus bisporus* (PDB ID: 2Y9X), as shown in Figure 3. This enzyme is bound by tropolone, a natural compound that acts as a native ligand. Based on the binding visualization between tropolone and tyrosinase, shown in Figure 6, it is evident that tropolone binds to the active site of tyrosinase but does not coordinate the copper ions, suggesting that tropolone forms a pre-Michaelis complex with the enzyme. This indicates that the inhibitor binds to the enzyme prior to the transition to the active reaction state. In Figure 6, the amino acid residues, represented in ball-stick form and colored yellow, are directly involved in the enzyme's catalytic activity, including His296, Val283, Phe264, His263, Asn260, Val248, His244, and His85. The orange compounds represent the copper ions, and the red compound represents tropolone, which acts as the native ligand.

Tropolone interacts with the active site through several key molecular interactions, such as hydrogen bonds with His61 and His85, pi-cation interaction with His263, pi-sigma with Val283, and pi-alkyl with Ala286. Based on these

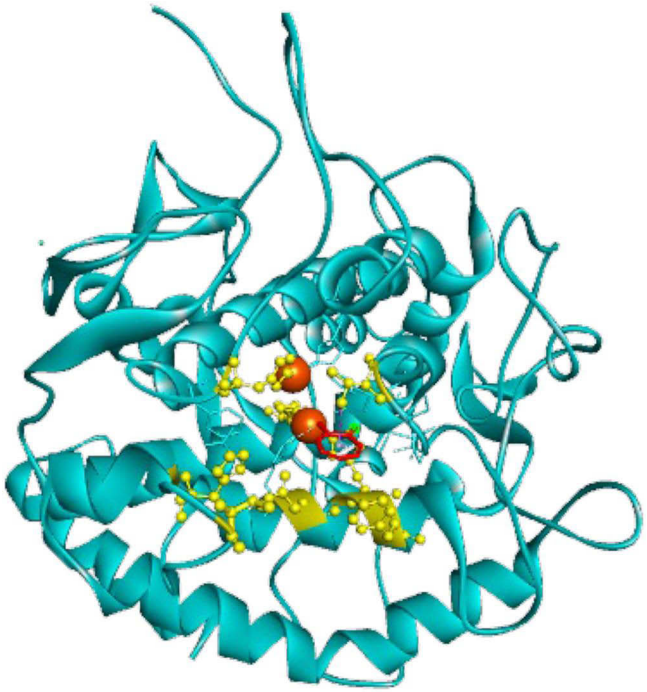

Figure 6 Molecular interaction visualization of tropolone with tyrosinase from *Agaricus bisporus*, derived from PDB data (ID: 2Y9X). The visualization was generated using Discovery Studio software.

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interactions, in silico testing of peptides capable of binding to the catalytic site, such as those involved in tropolone binding, is an important step in identifying potential tyrosinase inhibitors that could reduce melanin production.

For example, the peptide SFAPRFD interacts with tyrosinase mainly through hydrogen bonds, forming 10 hydrogen bonds with residues like His263, Asn260, His259, Ala246, Gly245, His244, Cys83, Gly86, and His85. Other interactions include pi-sigma bonds (involving Val248 and Ala80), a salt bridge (with Glu322), and an unfavorable donor-donor interaction (with His85). Five catalytic residues—His263, Asn260, Val248, His244, and His85—directly interact with SFAPRFD, which ultimately inhibits TYR activity.[72]

In the study by Shen et al (2019), it was found that in the active site of TYR, residues Tyr65, Asn260, His263, and Met280 form hydrogen bonds with the EF-5 peptide (sequence ECGYF). Specifically, the carbonyl group of Met280 forms a hydrogen bond with the phenolic hydroxyl group in tyrosine (2.82 Å). His263 plays a critical role in coordinating copper ions, and the interaction between the oxygen atom of tyrosine in the EF-5 peptide and the hydrogen atom of His263 affects the structural and functional integrity of TYR. Disruption of copper coordination impairs TYR's catalytic function, preventing tyrosine oxidation and melanin production. Furthermore, the aromatic ring of tyrosine in the EF-5 peptide forms hydrophobic bonds with Pro284 and Phe264, potentially obstructing TYR's lipophilic cavity and reducing its hydrophobicity.[114]

Furthermore, short hydrogen bond lengths correlate with higher bond energies, suggesting that the bonds formed between peptides and TYR are highly stable hydrophobic interactions also contribute significantly to the binding strength.[82] For instance, the YYP peptide (binding energy −7.6 kcal/mol) demonstrated a stronger affinity compared to the PYLK peptide (−6.9 kcal/mol). Specific residues like Val283, Phe264, and His85 create a non-polar pocket on the enzyme's A-chain, tightly encapsulating YYP through hydrophobic interactions. Moreover, electrostatic interactions between Glu256 and PPY on the A-chain further stabilize the binding. These observations highlight the pivotal role of hydrogen bonding and hydrophobic interactions in enhancing the strength and stability of the binding complex.[115]

Moreover, peptides may block the hydrophobic pocket in the active site of TYR, inhibiting substrate binding and reducing the enzyme's catalytic function. Notably, the tripeptide DEK demonstrated more potent inhibition of TYR compared to the tetrapeptide GDVA, likely due to its stronger hydrophobic interactions.[100] Peptides containing hydrophobic amino acids, such as tryptophan, can interact with TYR through these hydrophobic interactions, potentially blocking the enzyme's hydrophobic pocket and diminishing its activity.[73]

Based on several previous explanations, critical residues such as His263 and Val283 play distinct yet essential roles in enzymatic function and susceptibility to peptide-mediated tyrosinase inhibition. His263, a histidine residue, is crucial for coordinating copper ions at the enzyme's active site, facilitating the hydroxylation of tyrosine to DOPA. Disruption of this coordination by peptide binding can significantly impair TYR's catalytic activity, underscoring its vital role in the enzyme's mechanism.[116] In contrast, Val283, a hydrophobic residue, likely contributes to the structural stability of the enzyme or aids in substrate binding, though it is not directly involved in catalysis. Peptide inhibitors targeting these residues can hinder TYR activity by destabilizing its conformation or blocking substrate access, even if the residues are not catalytically essential. While residues directly involved in metal coordination or proton transfer are generally more susceptible to inhibition, non-catalytic residues like Val283 can still influence the overall efficiency of inhibition by affecting the enzyme's structural integrity.[115]

Additionally, docking studies have been increasingly employed to predict the potential of peptides as tyrosinase (TYR) inhibitors, with several studies validating computational predictions through in vitro assays. Peptides such as HYGR and SPGRLP, identified through docking simulations, demonstrated strong binding affinities to tyrosinase, with docking scores of −7.6 kcal/mol and −7.5 kcal/mol, respectively. These peptides were further evaluated in in vitro tyrosinase inhibition assays, where HYGR showed the lowest IC_{50} value of 0.96 mmol/L, and SPGRLP exhibited a higher IC_{50} value of 1.58 mmol/L. These findings emphasize the consistency between in silico docking results and actual TYR inhibition activities, supporting the reliability of computational models in predicting peptide efficacy. The alignment of computational predictions with experimental data highlights the utility of in silico screening in the early stages of peptide design, facilitating the identification of promising tyrosinase inhibitors.[72]

Although this molecular interaction analysis provides initial insight into the potential of peptides as tyrosinase inhibitors, further in vitro and in vivo studies are required to confirm their effectiveness. These studies should include

measurements of inhibition percentages, melanin content, and toxicity of the peptides. Moreover, additional research is needed to fully understand the inhibition mechanism of these compounds. Only then can these peptides be considered for applications in the cosmetic field, specifically for reducing melanin production and preventing hyperpigmentation.

Exploring the Mechanisms Behind Peptide-Protein Interaction

The skin is essential to our personal appearance and aesthetics, since it is the first visible signs of aging appear. In an effort to avoid and slow down the inherent aging process, and to maintain a young-looking appearance, consumers are always seeking new and innovative anti-aging skincare products, particularly those with safer and more effective ingredients. Peptides, which are commonly included in anti-aging skincare formulations, are particularly valued for their ability to reduce wrinkles, diminish hyperpigmentation, and boost collagen production. Additionally, peptides are considered less irritating compared to traditional anti-aging cosmetic ingredients. However, despite these benefits, peptides face significant challenges in penetrating the outermost layer of the skin, the stratum corneum, which limits their effectiveness. This difficulty is due to their large molecular size, hydrophilic nature, and high vulnerability to enzymatic breakdown, making it necessary to employ advanced formulation technologies to improve their stability and facilitate their delivery through the skin.[117]

The translocation of peptides across the cell membrane is a complex process that is significantly influenced by the physicochemical characteristics of the peptides and the structure of the lipid bilayer in the membrane. Numerous studies have explored these translocation mechanisms and peptide-cell membrane interactions. For instance, Hertog et al (2004) examined the binding interactions of histatin 5 and histatin 5-derived peptides with liposome membrane, emphasizing the role of peptide-phospholipid interactions in membrane permeabilization and peptide translocation.[118] Henriques et al (2007) examined the CPP translocation, proposing that this process is driven by physicochemical of peptide-lipid bilayer interactions, occurring without the formation of pores.[119] Herce & García (2007) used computational simulations of molecular dynamics to propose a spontaneous pathway for the HIV-1 TAT peptide's translocation through lipid bilayers.[120] Faugeras et al (2022) explored how structural modifications, such as cyclization and methylation, affect peptide penetration through droplet interface bilayers, uncovering a variety of translocation mechanisms, including endocytosis, active transport, and direct translocation.[91] Huang & Garcia (2013) explored the free energy requirements for the translocation of an arginine-rich peptide through a lipid bilayer, hypothesizing that pore formation could facilitate this process.[121] The targeted signaling pathways of reported anti-melanogenesis peptides can be observed in Table 3.

Peptides can influence melanogenesis through several pathways, including PKA/MITF signaling, ERK activation, cAMP-PI3K/Akt, MAPK signaling, and the protein kinase C pathway.[123] These pathways are crucial for regulating melanin production and the activity of key enzymes like TYR. For instance, Silver Carp scale-derived collagen peptides-1 (Hypophthalmichthys molitrix) (SCPs1) have been shown to significantly increase glutathione (GSH) levels while

Table 3 Selective Examples of Anti-Aging Peptide: Summary of Their Targeted Signaling Pathways of Anti-Melanogenesis Peptides

Source of Peptide	Inhibition Pathway					Ref.
	TYR	MC1R	AKT (PI3K)	Apoptosis	CREB	
Quinoa husk peptides	√		√	√		[23]
Silver Carp scale-derived collagen peptides-1 (*Hypophthalmichthys molitrix*)	√	√			√	[122]
α-MSH–Peptide Conjugates	√	√				[123]
Synthetic small peptides		√				[51]
Bee Venom		√				[124]
Tilapia fish scale	√					[126]

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decreasing TYR activity, ROS levels, and cAMP content. It also suppressed the transcription-level expression of MC1R, MITF, TYR, TRP-1, and TRP-2 genes, effectively suppressing the cAMP-CREB signaling pathway to inhibit melanin synthesis.[122] Additionally, Quinoa husk peptides (QHP) not only reduced melanin content through the Akt-signaling pathway in cultured cancer cells and rats but also accelerated apoptosis in A375 cells. Quinoa husk peptides (QHP) reduce melanin content by inducing apoptosis in melanocytes, a mechanism distinct from traditional inhibitors that typically block enzyme activity like tyrosinase or melanocortin receptors. QHP triggers apoptosis in A375 cells through three main pathways: cell membrane damage, DNA damage, and mitochondrial apoptosis. Mitochondrial apoptosis plays a central role by releasing cytochrome c, which activates the caspase cascade, ultimately leading to cell death. While effective at reducing melanin synthesis, this apoptosis-based mechanism raises several concerns. Excessive induction of apoptosis in melanocytes could result in unwanted side effects such as skin depigmentation or tissue damage. Furthermore, since QHP accelerates apoptosis through mitochondrial pathways and causes significant DNA damage, there is potential for harm to surrounding healthy cells if apoptotic signals are not well-regulated. This poses risks to skin tissue integrity and long-term cellular health.[23] Although this apoptosis-based approach offers a novel therapeutic strategy, particularly for hyperpigmentation disorders or melanoma treatments, the safety profile of QHP requires careful evaluation. Further research is needed to ensure that this mechanism does not inadvertently harm normal skin cells or lead to other adverse effects.

A peptide derived from Bee Venom, including melittin, was shown to effectively inhibit melanin production in α-MSH-stimulated melanoma cells, showing that melittin hinders the differentiation of cells involved in melanogenesis. Although melanin primarily functions for shielding from UV-induced harm, it can also diminish radiation and chemotherapy efficacy treatments. Moreover, melanogenesis may accelerate melanoma progression due to its immunosuppressive, genotoxic, and mutagenic effects.[124]

Szabó et al (2024) explored the intricate mechanisms of peptide-protein interactions by focusing on the targeting of the MC1R, which is overexpressed on melanoma cells, using conjugates of α-MSH peptides with the chemotherapeutic agent daunomycin. The study emphasizes the critical role of specific conjugation sites within the α-MSH peptide structure, particularly the attachment of daunomycin to the side chain of lysine residues, in modulating the affinity and efficacy of MC1R binding. These interactions significantly impact the internalization and cytotoxic activity of the conjugates, as evidenced by differential outcomes in cellular uptake, receptor-mediated endocytosis, and subsequent tumor-growth inhibition in both in vitro cell culture systems and in vivo murine melanoma models.[123]

Kim et al (2019) explored the inhibition of melanin synthesis by small peptides identified through a positional scanning synthetic peptide combinatorial library (PS-SCL). Peptides like RFWG-NH_2 and RLWG-NH_2 act as MC1R antagonists, competitively binding to the receptor and blocking α-MSH from initiating the cAMP/CREB/MITF pathway, which regulates melanogenic enzymes (TYR, TYRP1, DCT). This disruption significantly reduces enzyme activity and melanin production. The peptides' efficacy hinges on specific amino acid sequences, with tryptophan contributing hydrophobic interactions and glycine at the C-terminus enhancing flexibility for optimal MC1R binding.[51]

Ju et al (2022) provided an in-depth exploration of the mechanisms underlying peptide-protein interactions by detailing how tilapia fish scale-derived peptides exhibit a strong affinity for copper ions, which are critical cofactors in TYR's active site, an enzyme central to melanin synthesis. The study elucidates that these peptides effectively chelate copper ions through specific interactions involving the amino and carboxyl groups within the peptide structure, thereby inhibiting TYR's enzymatic activity in depending on concentration. In higher doses, the peptides demonstrate more robust copper ion chelation, leading to greater suppression of TYR activity. For instance, at 5 mg/mL concentration, TYR activity is reduced by 59.73%, significantly surpassing the inhibition achieved by the standard control, α-arbutin, at the same concentration. This disruption of TYR function results in a corresponding decrease in melanin synthesis within B16-F10 melanoma cells, with a reduction in intracellular melanin content observed at higher peptide concentrations. The research emphasizes the structural specificity of these peptides, noting that the precise arrangement of functional groups is crucial for their binding efficiency and inhibitory capacity.[126]

Peptide-based cosmeceuticals face significant formulation challenges, requiring precise approaches to ensure stability, bioavailability, and efficacy. Peptides are highly sensitive to environmental factors such as oxygen, light, and moisture, which can lead to oxidative degradation, particularly in residues like methionine or cysteine. Stabilizers such as ascorbic acid and vitamin E are commonly used to mitigate these effects, while maintaining an optimal pH range (typically 4–6) is

crucial, especially for aqueous formulations like serums that are prone to hydrolysis. In creams, peptide stability within biphasic systems requires compatible emulsifiers and stabilizing agents to ensure uniform distribution. Penetration barriers posed by the hydrophobic structure of the skin further complicate formulation, necessitating techniques like lipidation (eg, palmitoyl pentapeptide-3 (Matrixyl), enhance skin penetration and bioavailability, demonstrating efficacy in reducing wrinkles at concentrations as low as 50 ppm)., encapsulation in liposomes or nanoparticles, and the use of microneedles or nanocarriers to enhance penetration into deeper skin layers. Packaging innovations, such as airless pumps and UV-resistant containers, also play a crucial role in protecting peptides from destabilizing environmental exposures.[127]

Experimental evidence supports these considerations. For instance, Peptide-C formulations in amber glass ampoules successfully preserved the stability of biopeptides and vitamin C, resulting in significant improvements in skin cell turnover and wrinkle reduction. Clinical studies demonstrated a 9% reduction in crow's feet wrinkles, an 11% reduction in forehead wrinkles, and a 15.7% reduction in wrinkle surface area after 28 days of application, highlighting the effectiveness of optimized stabilization and delivery techniques.[128] Similarly, Matrixyl® (Palmitoyl-Tripeptide-38) showed substantial wrinkle reduction and improved skin elasticity, demonstrating the potential of lipidation to enhance bioavailability and efficacy. To enhance peptide stability, strategies such as cyclization, incorporation of D-amino acids, and chemical modifications like PEGylation and fatty acid conjugation are employed. For instance, palmitoylation of peptides like Pal-KTTKS improves both skin permeability and metabolic stability by creating a hydrophobic barrier, enabling penetration into deeper layers.[125]

However, several important limitations must be noted. The lack of standardization in evaluating peptide stability and penetration makes it difficult to compare results across studies. Variations in experimental protocols and types of formulations often result in inconsistent data. The reliance on preclinical studies, such as in vitro or animal testing, adds challenges as these results do not always reflect effectiveness in human use. Penetration techniques that appear promising in the laboratory often fail clinically due to the physiological complexity of human skin. Additionally, much of the data comes from industry-sponsored studies, which tend to favor positive outcomes. Dependence on patents and commercial technologies may overlook innovations or alternative approaches from independent research.

The overemphasis on established peptides like Matrixyl® can hinder the exploration of new compounds that might be more effective. The dominance of short-term studies fails to provide insights into the long-term stability and effectiveness of peptides during extended storage or daily use. Environmental factors such as temperature and humidity during storage or use, as well as variations in individual skin pH, are also underexplored despite their significant impact on peptide stability and efficacy. Considering these limitations, more comprehensive research with standardized methodologies, long-term evaluations, and independent approaches is needed to ensure the effectiveness and reliability of peptide-based cosmeceuticals under various usage conditions.

Conclusion

This review emphasizes the critical role of molecular weight, composition, and structure in determining the effectiveness of peptides as tyrosinase (TYR) inhibitors, which are crucial for regulating melanin production and addressing hyperpigmentation. Peptides containing specific amino acid residues, sequences, and chelating properties have demonstrated strong inhibitory effects on TYR. The hydrophobic-hydrophilic balance in peptide sequences is essential for optimal enzyme inhibition, highlighting the importance of a well-balanced composition for effective TYR suppression. Hydrophilic and hydrophobic regions also enable the cyclic peptides to better integrate with the cell membrane, thereby improving their ability to cross the membrane Additionally, peptide cyclization enhances stability, skin penetration, and interaction with cell membranes, making cyclized peptides a promising strategy for improving the efficacy of therapeutic peptides in skin applications.

The review also discusses how short-sequence oligopeptides, particularly those containing aromatic residues like tyrosine and phenylalanine, are highly effective in binding to TYR and stabilizing the peptide-TYR complex. Peptides with key amino acid residues, such as arginine, phenylalanine, and hydrophobic or aromatic residues, significantly contribute to TYR inhibition.

However, structural differences between human and mushroom tyrosinase suggest that human-specific studies are needed to optimize peptide efficacy. The presence of hydrophobic N-terminal residues, such as glycine, valine, leucine, and isoleucine, plays an important role in inhibiting enzymes involved in aging processes, reinforcing the need for further exploration of these residues in peptide functionality. Moreover, peptides with tryptophan, known for its large aromatic indole ring, interact effectively with the hydrophobic pocket of TYR, reducing its activity and further supporting the development of potent TYR inhibitors.

Despite promising results, there are still critical avenues for future research. Further investigations are needed to identify new amino acid residues that can enhance copper chelation and TYR inhibition. Additionally, exploring the clinical potential of peptides, including testing them in human-specific settings and evaluating their long-term safety and efficacy, is crucial. Peptide-based therapies hold great promise for treating hyperpigmentation and other melanin-related conditions, but the transition from in vitro and in silico findings to real-world applications will require rigorous clinical testing.

In conclusion, both synthetic and natural peptides offer distinct advantages—synthetic peptides being superior in terms of efficacy and absorption, and natural peptides excelling in safety and biocompatibility. Tailoring peptide configurations, particularly by incorporating negatively charged, histidine, or aromatic residues, provides a robust framework for developing effective TYR inhibitors. These findings underscore the importance of continued research to optimize peptide therapies for skincare and medical applications, addressing the challenges of melanin overproduction and hyperpigmentation.

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Disclosure

The authors declare that there is no conflict of interest in this work.

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