Chapter 36: Peptides and proteins

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BASIC CONCEPTS

- Amino acids are the building blocks of peptides that link together to from proteins.
- Peptides are biologically active communication tools that direct skin functioning.
- Engineered peptides are a new category of active skin ingredients usually applied in a moisturizing vehicle.
- Gene chip array analysis can be used to evaluate the effect of engineered peptides in fibroblast cultures.

1 Introduction

Peptides, proteins, and amino acids are often mislabeled and the terms applied as if they were interchangeable, yet they are different in their characteristics, uses, biological activities, and cosmetic potential. After defining peptides and proteins, the first part of the chapter discusses the specificities of these molecules and their physiologic, biological function, particularly in the skin; what can they do, what are the obstacles to their use in cosmetic products and how these obstacles can be overcome. In the second part, concrete examples of peptides and proteins in dermocosmetics, in particular for the "antiaging" sector, is discussed before concluding with an outlook for the future of this ingredient category in skincare.

Definitions

It is important to understand the differences between amino acids, peptides, and proteins.

Amino acids

Amino acids are the building blocks of which peptides and proteins are made. They are small molecules, with a molecular weight of 100–200 Da, characterized by the fact that both an amino group (NH_2) and a carboxylic acid group (COOH) are attached to the central carbon atom which also carries further quite variable structures, known as side chains, by which the different amino acids are distinguished (Figure 36.1).

Of the essentially unlimited theoretical number of amino acids that can be imagined on paper, only 20 (e.g. alanine, proline, tyrosine, histidine, phenylalanine, lysine, glutamine) are incorporated into peptides and proteins via the genetic code. Individually, these amino acids in isolation have no specific intrinsic biological activity. Within cells, they exist in a pool from which they can be called upon to make peptides and proteins or, sometimes, biogenic amines, such as serotonin or dopamine. In the upper layers of the skin, they are part of the natural moisturizing factor (NMF) where they participate in the skin water holding capacity contributing to both osmolytic and hygroscopic properties.

The specific interest in amino acids lies in their ability to function as an acid (pH < 7) and amine (pH > 7) simultaneously and the chemical fact that reacting an acid with an amine leads to the formation of an amide, which is a peptide bond, whereas this linkage is achieved in living cells by enzymatic means. The result of linking two or more amino acids in a linear chain is called a peptide, when the length of the chain is less than approximately 100 amino acids, or a protein when the chain is longer.

Peptides

The general terminology uses prefixes to describe the type of a peptide. For example, when the peptide is made of two amino acids, such as tyrosine and arginine written as Tyr-Arg, it is called a dipeptide. Three amino acid combinations yield a tripeptide, four amino acid combinations yield a tetrapeptide, etc. "Oligo" stands for a "few" so that oligopeptides can have 2–20 amino acids linked in a chain. The term polypeptides is used to mean many peptides, although these latter distinctions are not strict and not governed by official rules.

The most important characteristic of a peptide, besides its length determined by the number of amino acids in the chain, is its sequence. The sequence is the precise order in which the various amino acids are linked together. Both

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glycyl-histidyl-lysine and glycyl-lysyl-histidine are tripeptides, composed of the three amino acids glycine, histidine, and lysine. However, the fact that these amino acids are linked in the Gly-His-Lys sequence in the former and in Gly-Lys-His sequence in the latter is crucial. The former peptide, usually abbreviated GHK, stimulates collagen synthesis in fibroblasts [2], the latter GKH stimulates lipolysis in adipocytes [3]. The primary function of most peptides is to bring a biochemical message from place A in the body to place B allowing effective communication.

Proteins

A peptide chain of more than approximately 100 amino acids is termed a protein. However, interleukins, cytokines, and interferon are also sometimes referred to as peptides, even though they possess a much higher molecular weight (Figure 36.2). Sometimes the distinction between the two categories relies more on the function of the molecule rather than the size.

Proteins can be categorized by their function, roughly into the following:

- Structural proteins: building tissue, such as collagen, elastin, fibronectin and many others;
- Enzymes: very specific proteins that catalyze biochemical reactions, such as superoxide dismutase (SOD), chymotrypsin, tyrosinase;
- Transport proteins that bind to a specific substrate and carry it along in the body (e.g. hemoglobin as oxygen carrier, ferritin for iron transport, lipoprotein for lipids, including cholesterol);
- Difficult to categorize proteins with highly specific functions: receptors such as protein G, genetic regulators such as peroxisome proliferator-activated receptor (PPAR), antibodies, coagulants, histones.

Proteins with individual molecular mass of hundreds of thousands of Daltons often autoassemble into large complexes of even larger size with very complex mechanisms of activity.

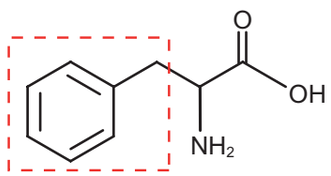


Figure 36.1 Phenylalanine, one of the 20 proteinogenic amino acids. The "side chain" which is characteristic of each amino acid (here a phenyl group) is shown in the box.

Biological functions of peptides and proteins in the skin

Peptides

Single amino acids very seldom have specific biological functions other than being present in the cytoplasmic pool for enzymes to be picked up and processed in one of many ways. Peptides perform many important biologic functions. The word hormone comes from Greek and means messenger. Hormones can be classified as peptide hormones or steroid hormones. Both steroids and peptides act in similar fashion. Some disturbance, either internal or external, leads to the release of a small amount of peptide in a cell, blood, gland, or in some other organ. The peptide then travels in the body until it interacts with a target receptor either on the cellular surface or within the cell nucleus after having penetrated the cell wall. This interaction triggers further

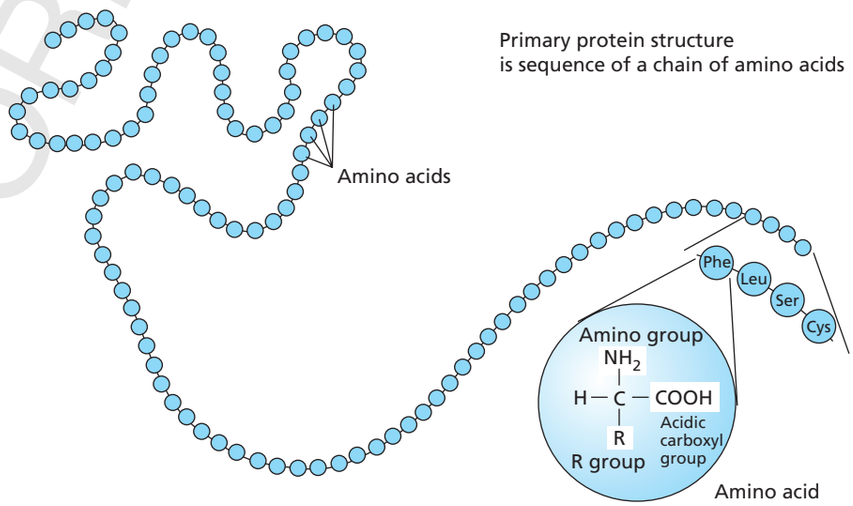


Figure 36.2 A protein chain composed of amino acids.

activity at the site, destined to respond and correct the initial disturbance.

This mechanism of action is usually characterized by three items:

1 Peptides circulate and act at their target sites at extremely low concentration levels, generally in the nanomolar (10^{-9} mol/L) level.

2 Each peptide sequence has, at its rather specific target, a highly specific binding affinity and carries a specific message such that its activity is quite specific. The highly simplified concept of "key" and "lock" (i.e. peptide and receptor) interaction is used to explain this potency and specificity.

3 Peptides have short lifespans in the organism because proteolytic enzymes break them down quickly in order to avoid overload at the target site.

Well-known biological activities of peptides in the human body are, for instance: regulation of blood sugar concentration (insulin); blood pressure regulation (angiotensin, bradykinin, calcitonin gene-related peptide [CGRP]); lactation and birthing (oxytocin); diuresis (vasopressin); pain repression (endorphins, enkephalin); tanning (α-MSH); radical scavenging (glutathione); other peptides include vasointestinal peptide (VIP), substance P, the inflammatory undekapeptide, and hundreds more. The ubiquitous nature of these peptides and their myriad activities clearly indicate their importance.

The relevance of some of the peptides in skin and skincare is of interest. As the term "antiaging" is not defined, it is interpreted here in a rather broad way to represent anything that helps the skin look younger. The rest of this chapter focuses on peptides for antiaging purposes.

Antioxidant peptides

Glutathione (γ-glutamyl-cysteyl-glycine)

This tripeptide GSH is one of the "oldest" members of the peptide family, with respect to its discovery, analysis, and confirmatory synthesis. It contains an –SH bearing, cystein amino acid which confers antioxidant activity to the molecule (Figure 36.3). The level of glutathione concentration in the body decreases notably with age, which may be a cause and a symptom of aging both at the same time [4]. The less GSH that is present, the more damage generated by free radicals; hence less glutathione reductase may be present to regenerate GSH. Besides affording this protective, antioxidant activity, for which there is *in vitro* but very little documented clinical evidence of skin benefits (for a medical study, see [5]), GSH may also have so-called "skin whitening" effects, as described by Villarama and Maibach [6].

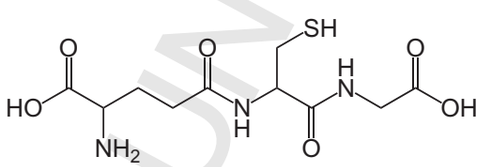


Figure 36.3 Glutathione (γ-Glutamyl-cysteyl-glycine).

Carnosin (β-alanyl-L-histidine)

This dipeptide also contains an unusual structure, in that β-alanine is used instead of the standard α-alanine. The histidine moiety in carnosin is of particular interest. Enzymatic breakdown of this peptide can lead to the production of histamine, a potent inflammatory agent, but also useful and necessary in the preparations for wound healing (see below).

Carnosin has been proven to scavenge reactive oxygen species (ROS) formed from peroxidation of cell membrane fatty acids during oxidative stress. 4-Hydroxy-2-trans-nonenal (4HNE) is one of the toxic end products of lipoperoxidation by free radicals. While the reaction of HNE with glutathione (GSH) is a well-recognized pathway of detoxification in biological systems, the quenching ability of carnosin towards HNE was studied by Aldini *et al.* [7]. Carnosin, although less reactive than GSH, significantly quenched HNE (48.2 ± 0.9% HNE consumption after 1 hour). The results indicate that beside GSH, histidine-containing dipeptides could be involved in the detoxification pathway of reactive species from lipid peroxidation. Carnosin is also shown to be useful to counter the effects of glycation (the non-enzymatic binding of sugars to proteins), leading to cytotoxic advanced glycation end products (AGE).

Nagai *et al.* [8] showed that carnosin may indeed promote wound healing, at least indirectly, as exogenous carnosin is degraded in the body by carnosinase into β-alanine and histidine which is then transformed by histidine decarboxylase to yield histamine. β-Alanine was found to stimulate the biosynthesis of nucleic acids and collagen, whereas histamine is considered to enhance the process of wound healing by stimulating effusion at the initial stage of inflammation.

Neuropeptides

The skin and the brain are derived from the same initial embryonic tissues [9]; thus, it is not surprising that many peptides that are found to exist and possess activities in the brain are also found in the skin.

Calcitonin gene-related peptide

Calcitonin gene-related peptide (CGRP) contains 37 amino acids presently known to be the most potent vasodilatating substance. Additionally, other activities were discovered, such as stimulation of cyclic adenosine monophosphate (cAMP) levels and sweating, as the peptide is clearly involved in inflammatory response, and has recently been found to contribute to migraine headaches. It is released from nerve cells, including those of the epidermis. Curiously, atopic dermatitis is inversely correlated with CGRP plasma levels. Certain fragments of the peptide, however, have been

shown to competitively inhibit these activities and may prove to be of interest in skincare applications, such as in anti-redness and antiperspirant products.

Bombesin

Bombesin is a 14 amino acid neuropeptide which activates three different G-protein-coupled receptors known as BBR1, BBR2, and BBR3. With respect to skin-related activity, Baroni *et al*. [10] studied the effect of this neuropeptide on tissue regeneration and wound healing of the skin, on migration, proliferation, and differentiation of keratinocytes in an *in vitro* experimental model, on a mechanically injured human keratinocyte monolayer. Different mediators involved in wound repair, cell proliferation, and motility, and bombesin's direct effect on wound repair by observing the wound closure after mechanical injury, were studied. The data suggest that bombesin may have an important role in skin repair by regulating the expression of healing markers. Bombesin also increased cell growth and migration.

Other neuropeptides of interest include neuropeptides Y (NPY), PYY, and PP which act on a family of protein G receptors. NPY contains 36 amino acids and is present in the central nervous system. Among other (appetite regulating) activities, NPY acts on adipocytes and favors obesity. NPY inhibitors have been used in cellulite treatment whereas stimulation of NPY in facial skin might be of interest in redensifying the hypodermal layers (lipofilling).

Pro-opiomelanocortin

Pro-opiomelanocortin (POMC) should be considered as a protein, given its size of 241 amino acids. It is coded for by a gene found in the anterior pituitary gland. It is also secreted by cells of the hypothalamus, some neurons, and by keratinocytes and melanocytes of the skin and scalp. However, this protein does not seem to have a function of its own. Depending on the cell in which it is produced, it is broken down by endopeptidase enzymes into smaller fragments, or peptides, which have specific functions in the target cells.

The 241 amino acid chain contains the sequences of the immunomodulatory and inflammation mediating peptide adenocorticotropic hormone (ACTH; corticotropin with 39 amino acids), the melanin synthesis stimulating hormone α-MSH (melanocortin, previously called melanocyte-stimulating hormone with 13 amino acids), and its slight variant called β-MSH as well as the lipolytic peptide β-lipotropine (90 amino acids) which contains within its sequence β-endorphin (31 amino acids), and the pentapeptide enkephalin (Tyr-Gly-Gly-Phe-Met) which constitutes the first five amino acids of endorphin.

Of interest in skincare are the fragments α-MSH and its derivatives or analogs, as this peptide may be used to help even out skin tone by either stimulating melanogenesis (tanning) or by reducing the amount of pigmentation (skin "whitening"). β-Endorphin and particularly its N terminal pentapeptide fragment enkephalin may have skin soothing activities, as these molecules can be detected in the epidermis, localized close to nerve endings. A particularly interesting neuropeptide is kyotorphin, which has not yet been detected in the skin, but seems to act in similar ways on the skin as it does in the brain [11, and see below].

Matrikines

The term matrikines is used to describe fragments of matrix macromolecules endowed with stimulatory, tissue repair activity [12]. Katayama *et al*. [13] describe the minimum size fragment of procollagen I still able to induce collagen neosynthesis in human lung fibroblasts; the very hydrophilic pentapeptide Lys-Thr-Thr-Lys-Ser is such a molecule. The tripeptide Gly-His-Lys, found in different parts of broken down collagen and in some serum proteins, also stimulates collagen synthesis in human skin fibroblasts, as found by Maquart *et al*. [2]. The tetrapeptide Arg-Gly-Asp-Ser, a sequence found within the fibronectin structure responsible for the binding affinity of this protein to collagen and to cell membranes, is able to help cells migrate during the wound healing process. In order for this migration to occur, the cells must detach and then move through tissue to where they are needed [14]. This migration is guided by a concentration gradient of peptides, such as Val-Gly-Val-Ala-Pro-Gly, which are fragments of elastin. This migration phenomenon is better known as chemotaxis [15]. Schematically and very simplified, this event is illustrated in Figure 36.4.

Obstacles to peptide use in dermocosmetics

The incorporation of peptides in dermocosmetics can be challenging. Some of the hurdles confronted with peptide formulation include: skin penetration, stability, toxicity, analysis, and cost.

Skin penetration

The stratum corneum is not the primary target for peptides, as they need viable, living skin to receive their message. It is necessary for a peptide to cross the cutaneous barrier in order to reach the viable epidermis (keratinocytes), the basal layer (melanocytes, nerve cell endings), the dermis (fibroblasts), and even the hypodermis (adipocytes). Even small peptide molecules, such as the dipeptide carnosin, are too hydrophilic and electrically charged to penetrate any further than the first or second layer of the stratum corneum. The larger the peptide (beyond six or seven amino acids), the less likely it is to reach the deeper layers of the skin. Thus, the long peptide sequences mentioned above, such as CGRP, POMC, and similar structures, do not function as active ingredients in cosmetic formulas.

Lintner and Peschard [16] have shown that the attachment of a lipophilic chain (fatty acid of sufficient length) to smaller peptides can increase the penetration rate by a factor of 100 or more. Similar effects were confirmed by Leroux

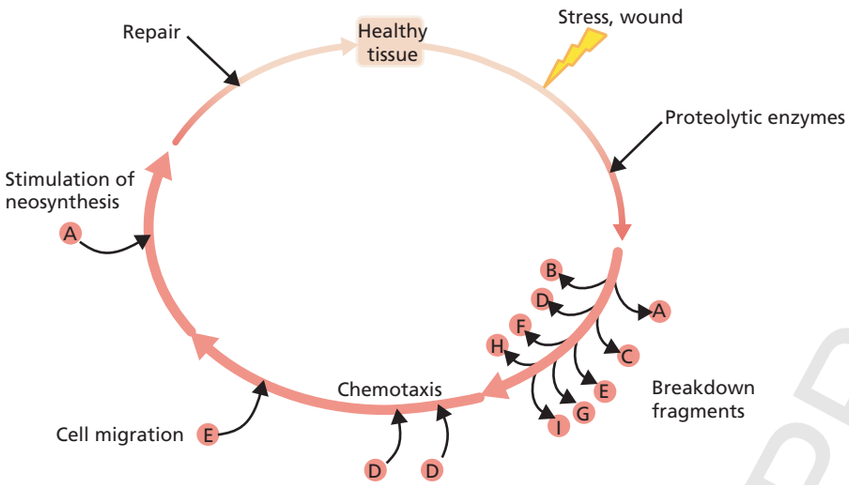


Figure 36.4 A tissue protein (e.g. collagen, elastin, fibronectin) is broken into fragments by enzymatic hydrolysis, either during normal tissue renewal, or as a consequence of induced damages (free radicals, burning, mechanical wound). The breaking up of the protein does not occur randomly, nor sequentially from one or the other end; various pieces of amino acid strings are generated, which when small enough, are readily available to act as "messengers" in the surrounding tissue, and will act as chemoattractants, transport aids, and stimulants to trigger neosynthesis of the necessary tissue molecules to renew/repair the three-dimensional structure.

et al. [3]. This technique of vectorizing the peptides has its limits because the longer the peptide chain, the less penetration the fatty acid will produce. Another limitation is the interference of biologic activity by acylation of the peptide. The N terminal ionic charge may be of importance for triggering effects at the target site or otherwise interfering with the peptide's properties. For example, the antioxidant activity of carnosin turns into pro-oxidant activity when the peptide is modified to become palmitoyl-carnosin (F. Vissac, unpublished results). Alternatively, attaching a poly-arginine chain to the peptide can help molecules penetrate the stratum corneum [17]. Liposome formulations may also help carry the peptide through the barrier, but little if anything has been published in this respect.

Stability

Unfortunately, peptides have limited chemical stability. In aqueous environments, such as those frequently encountered in cosmetic applications, hydrolysis may occur. Experience shows that the longer the peptide, the more fragile it becomes. The choice of excipients and stabilizers can help overcome this obstacle [18]. However, the question of peptide stability, an increasingly important consideration, must be considered.

Analysis

Detecting the presence of a peptide in a formulation 6–12 months after product manufacture can be difficult when the peptide is present in micromolar or less concentrations (p.p.m. level). Special analytical techniques, such as derivatization, mass spectrometry, and fluorescence spectrometry, have to be individually developed for each peptide. This is not always possible and/or very costly and sometimes proves an insurmountable hurdle.

Toxicity

Generally, the smaller the peptide, the less likely it is to show untoward effects. Peptides, in contrast to proteins, are hardly big enough to elicit allergic reactions, but specific undesirable cellular effects may occur with unknown sequences. It is advisable to use peptides with a biomimetic amino acid sequence, as the likelihood of toxicity is close to nil when the peptide is almost identical to human peptides. Nevertheless, proper safety evaluation of newly developed peptides, especially if the peptide is modified by acylation or esterification, is necessary.

Cost

Peptides of defined sequence and high purity (>90%) are expensive to produce; although extraction from some protein hydrolysates is theoretically possible, most peptides used in cosmetic applications are synthetic (i.e. made in a step by step process from the individual amino acid building blocks). It is noteworthy that the amino acids themselves are frequently of natural plant or fermentation origin. However, the very high potency of the peptides compensates for their cost and makes it possible to employ them at efficient level in all types of skincare formulas, because they are used at the p.p.m. level in finished cosmetics. Therefore, in spite of the formulation challenges, peptides have become popular, widely used active ingredients for antiaging skincare products, discussed next.

Examples of concrete applications of peptides in antiaging skincare

Numerous peptides are available for incorporation into cosmetic formulations. This section illustrates the manifold possibilities for using peptides in dermocosmetics.

Matrikines

The best known matrikine peptide used in skin care is the pentapeptide Pal-KTTKS, derived from the Katayama *et al.* [13] discovery discussed above. A DNA array study on this molecule indicated that mostly genes implicated in the wound healing process were upregulated in cells incubated with the peptide. Furthermore, the palmitoylated peptide stimulates not only the synthesis of collagen I, but also of collagen IV, fibronectin, and glycosaminoglycans in monolayer culture of normal and aged human fibroblasts and in full thickness skin (Mondon, unpublished data). This peptide was tested in vehicle controlled clinical trials where it proved to thicken the skin, improve the epidermal–dermal junction, and macroscopically reduce fine lines and wrinkles [19–21].

The peptide Pal-Gly-Gln-Pro-Arg (Pal-GQPR), although technically not a matrikine because it is derived from a matrix macromolecule, is a fragment of the natural circulating protein IgG and stimulates macromolecule synthesis in cell culture demonstrated in Figure 36.5. This peptide also contributes to the reduction of basal and UV-induced IL-6 release in keratinocytes and fibroblasts, as demonstrated in Table 36.1.

The matrikine tripeptide Gly-His-Lys (GHK) has been mentioned as a wound healing and skincare ingredient for dermocosmetic formulas [2], especially when associated with copper ions. In its palmitoylated form (Pal-GHK) it is more active, even in the absence of copper, and can mimic the effects of retinoic acid [25]. The *in vitro* synergy between the tri- and tetrapeptide (Pal-GHK + Pal-GQPR) [22] led to an investigation of the combination in a clinical, vehicle controlled, blind study on 23 panelists (Mondon, unpublished data). Twice daily application of an oil-in-water (O/W) emulsion containing 4 p.p.m. of Pal-GHK and 2 p.p.m. of Pal-GQPR against placebo showed significant wrinkle reduction, an increase in skin firmness, and visible smoothing after 1–2 months (Figure 36.6).

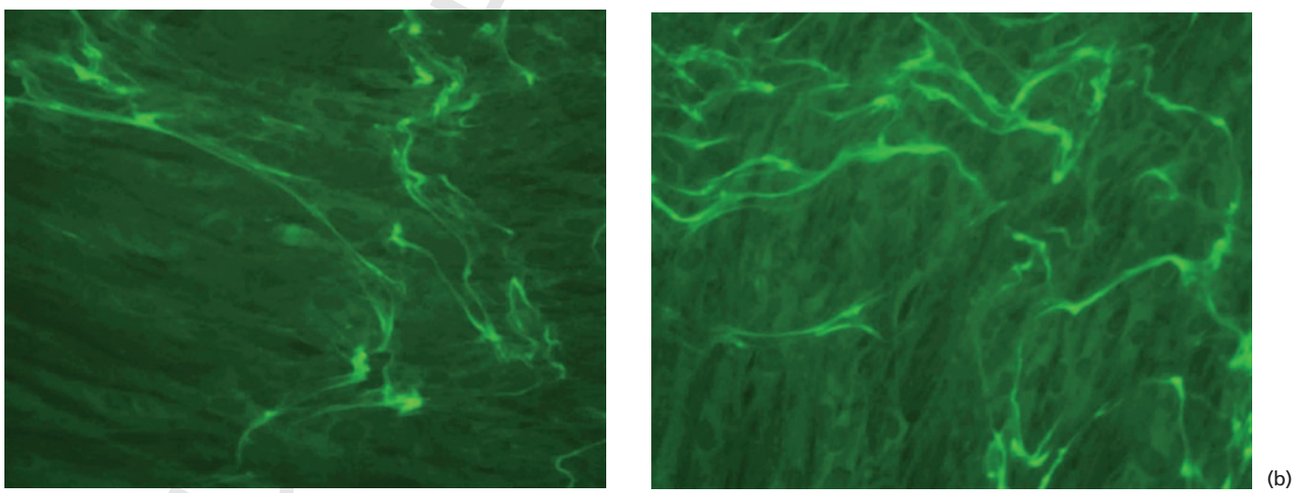
In another formulation, the peptide GHK was coupled to biotin, instead of palmitic acid, in order to strengthen the affinity of the peptide to hair keratin. This biotinyl-GHK peptide was then tested on hair growth *in vitro* (Figure 36.7) where 2 p.p.m. of the peptide increased hair length by 58% (identical to minoxidil), and 5 p.p.m. achieved 120% increase.

The production of the mitotic marker Ki67 and stimulation of collagen IV and laminin 5 syntheses were also investigated. Confirmation of the improved anchoring of the hair to the follicular infundibulum came from a clinical trial to study hair loss in alopecia patients where a significant

Table 36.1 Variation in IL-6 levels in presence of Pal-GQPR.

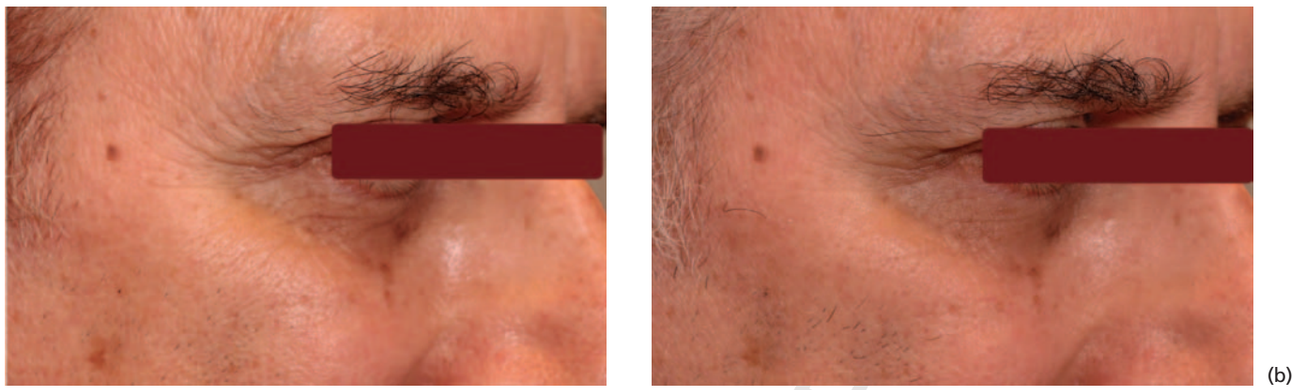
Pal-Gly-Gln-Pro-Arg (ppm)	Decrease of basal IL-6 (% of baseline level)	Decrease of UVB-induced IL-6 (% of baseline)
10	–15.6 ± 8.2	–33.2 ± 12.8
15	–20.0 ± 6.6	–37.3 ± 13.0
30	–24.6 ± 17.6	–60.3 ± 8.5
45	NT	–70.6 ± 9.8
65	NT	–85.5*
85	NT	–86.5*

NT, not tested.
*n = 2.



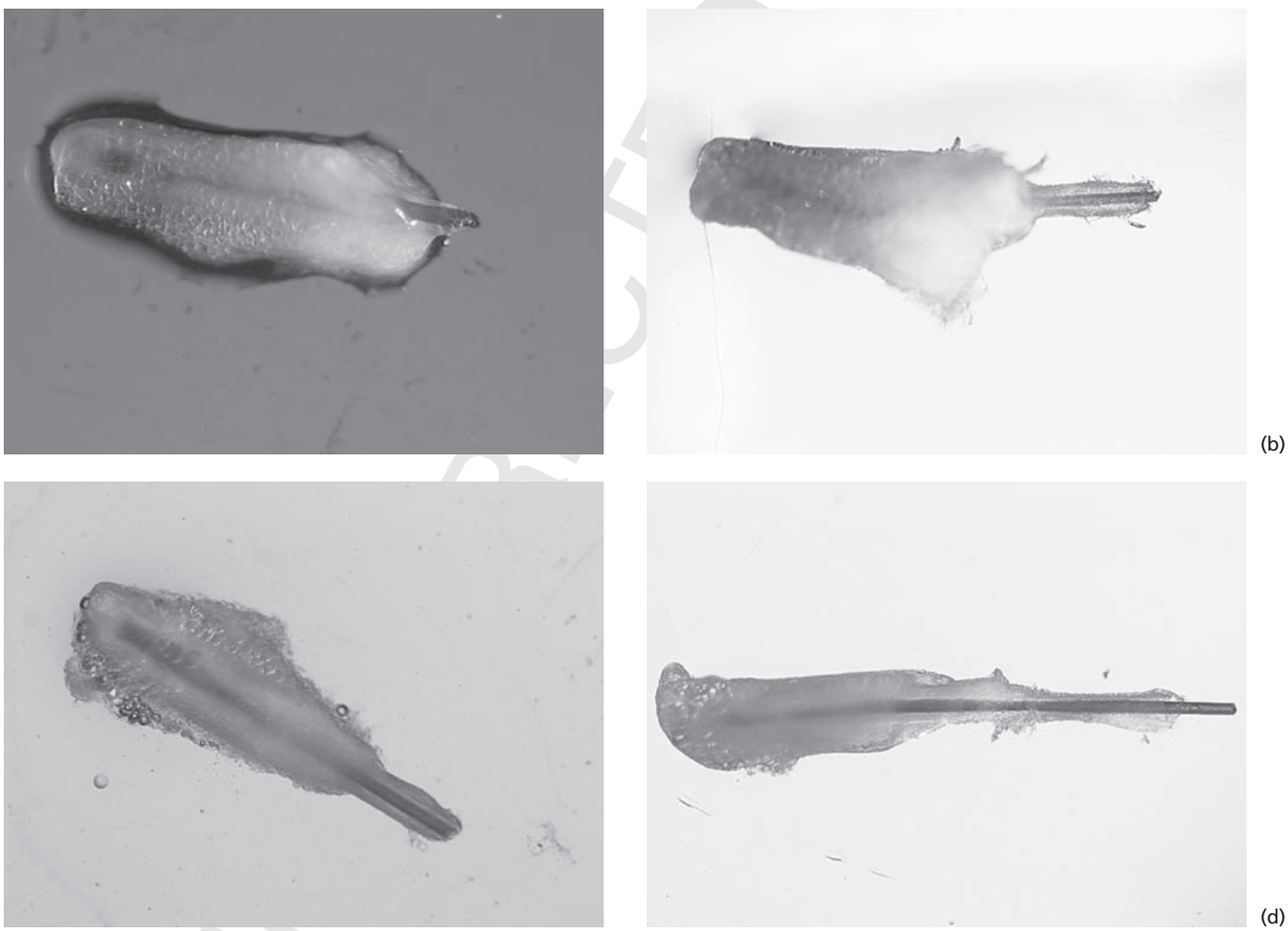
(a) (b)

Figure 36.5 Immunofluorescence staining of collagen I in a normal human skin fibroblast culture after 3-day incubation: (a) control; (b) with 6 p.p.m. of Pal-GQPR.



(a) (b)

Figure 36.6 Wrinkle improvement after 2 months' application (twice daily) of a cream containing 4 p.p.m. Pal-GHK and 2 p.p.m. of Pal-GQPR. (a) Before. (b) After.



(a) (b) (c) (d)

Figure 36.7 Hair follicles in survival medium, incubated 14 days; (a & b) control; (c & d) 5 p.p.m. Biot-GHK.

improvement of the anagen : telogen ratio was observed, in line with histologic observations on plucked hairs from the panelists [23].

Neuropeptides

Neurotensin, VIP, NPY, substance P, and CGRP, although endowed with potent biological activity, are not candidates for cosmetic applications because of their size and irritation potential. This is not the case for the β-endorphin enkephalin and the kyotorphin peptide complex. The dipeptide Tyr-Arg, which is known as kyotorphin, has been shown to be analgesic via enkephalin release in mouse brain [11]. The modified peptide, also known as *N*-acetyl-tyr-arg-hexadecylester, demonstrates improved skin bioavailability and stimulates the release of β-endorphin in keratinocytes. It is also able to reduce skin sensitivity to external thermal, chemical, and mechanical stress. A double-blind, vehicle controlled study using a lie detector established that this peptide, at 300 p.p.m. in an O/W emulsion, was able to diminish skin electrodynamic response to mechanical trauma induced by wiping the skin with sandpaper [24]. Sensitivity of the skin to thermal trauma induced by a heat probe and chemical trauma induced by topical capsaicin is also decreased after application of the peptide [16]. The peptide also inhibits *in vitro* muscle contraction.

Injections of derivatives of the *Botulinium* neurotoxin have been approved in a number of countries to diminish glabellar wrinkles. To develop a peptide with similar effects, Blanes-Mira *et al.* [25] synthesized the hexapeptide N-Ac-Glu-Glu-Met-Gln-Arg-Arg-NH_2 (N-Ac-EEMQRR-NH_2), a fragment of the SNAP-25 molecule [26]. It is reported to inhibit neurotransmitter release, apparently as a result of interference with the formation and/or stability of the protein complex that is required to drive Ca^{2+}-dependent exocytosis, namely the vesicular fusion known as SNARE complex, similar to what happens with *Botulinium* neurotoxin injections. The authors also claim that the peptide, formulated at the concentration of 10% = 100 000 p.p.m. in an O/W emulsion, reduced wrinkle depth up to 30% upon 30 days treatment on a panel of 10 human female volunteers [25].

Proteins

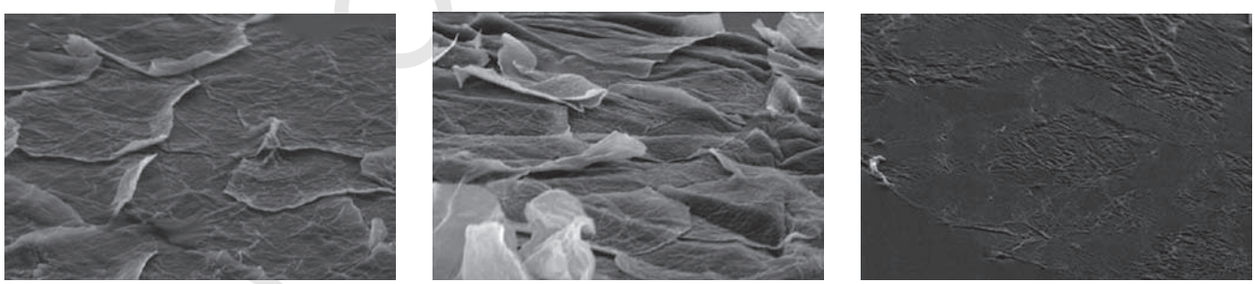
Structural proteins are building blocks for the organs and tissues of the human body. Collagen, one of the most abundant protein families, as well as keratin, elastin, fibronectin, actins, together with glycoproteins and proteoglucans, arrange themselves in finely tuned, three-dimensional structures to form muscles and skin. These proteins undergo a constant renewal process, even in the absence of external disturbances. These structural proteins are in contrast to enzymes, which fulfill an entirely different function. Enzymes speed up biochemical reactions, which would otherwise occur too slowly for the body to function. Enzyme function is highly specific. This specificity results from the precise amino acid sequence, which not only aligns the correct atoms and side chains in the right order, but also directs the precise folding pattern of the enzyme protein, thus guaranteeing its biological function. Enzymes are catalysts acting at low concentrations. The most important families of enzymes are proteolytic, lipolytic, antioxidant, DNA repair, and those involved in protein synthesis and gene regulation. A variety of enzymes have been employed in dermocosmetic products.

Proteolytic enzymes

Proteolytic enzymes are used as an alternative to α-hydroxy acids for superficial peeling of the skin surface, but care must be taken with the dosage. Figure 36.8 illustrates the proteolytic smoothing effect obtained with these enzymes.

T4 endonuclease V

T4 endonuclease V, isolated from *Escherichia coli* infected with T4 bacteriophage, has been shown to repair UV-induced cyclobutane pyrimidine dimers in DNA. Applied topically, liposomes containing T4 endonuclease V reduced the



(a) (b) (c)

6 **Figure 36.8** Scanning electron microscope (SEM) pictures of skin treated with an occlusive patch for 2 hours; (a) control cream pH 7; (b) cream with AHA to pH 3.5; (c) cream with 2% proteolytic enzyme solution (10 proteolytic units/mL).

incidence of basal cell carcinomas by 30% and of actinic keratoses by 68% without adverse effects and no evidence of allergic or irritant contact dermatitis. Although the photoprotective effect of T4N5 has been investigated only in xeroderma pigmentosum patients, it may be also be effective for normal skin [27]. Cosmetic products based on this concept are in the current marketplace.

Superoxide dismutase

Superoxide dismutase, an antioxidant enzyme, is present at the surface of the skin. Adding this enzyme to cosmetic formulations to strengthen the natural defense system is tempting, although the transmutation of the superoxide anion to hydrogen peroxide, without further detoxification of the peroxide, is not necessarily sufficient for protecting the skin. Furthermore, the bovine blood, yeast, or biotechnologically derived SOD does not guarantee sufficient stability to survive manufacturing procedures and shelf life in cosmetic consumer products.

A novel alternative is based on extremozymes (enzymes produced by extremophile bacteria, such as *Thermus thermophilus*). Mas-Chamberlin *et al*. [28] have shown that these enzymes are heat and UV stable, possessing both SOD and catalase mimicking activity protecting the skin against UV-induced free radical damage. A 6-month clinical vehicle controlled blind trial under tropical conditions (Figure 36.9; Tables 36.2 & 36.3) on the island of Mauritius [29] demonstrated the visible and measurable benefits of protecting the skin in preventive manner, as opposed to treating photodamaged skin with peptides.

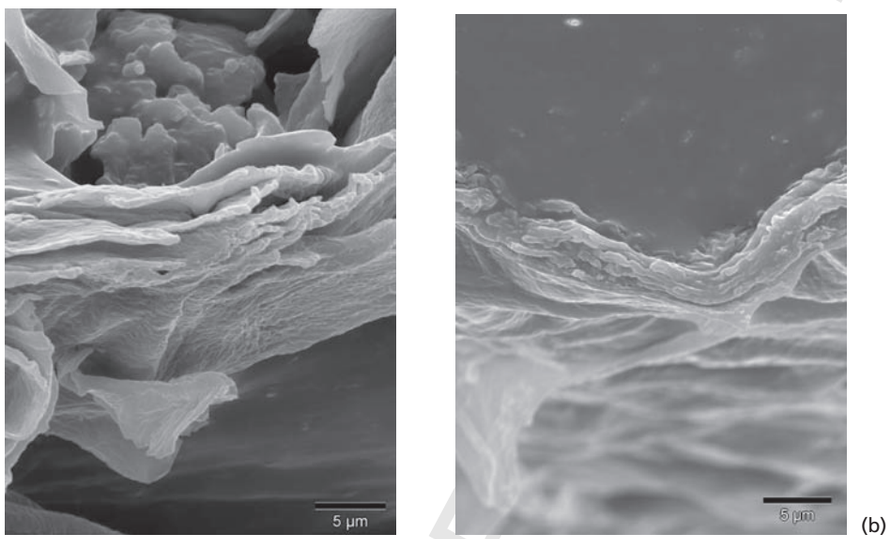


Figure 36.9 Scanning electron microscope (SEM) pictures of stratum corneum strippings: skin exposed to 6 months' tropical climate, treated with moisturizer. (a) Control formula; (b) extremozyme-containing moisturizer. (Reproduced with permission of Soap Perfumery and Cosmetics.)

Table 36.2 (a) Changes in transepidermal water loss (TEWL) after exposure to tropical climate (25 panelists); group treated with extremozyme formula.

Time	Variation ($g/m^{-2}/h^{-1}$) (mean ± SEM)	% Change (mean)	Significance
Week 4 vs. T0	–1.0 ± 0.7	–6%	NS* (p = 0.192)
Week 12 vs. T0	+0.8 ± 0.9	+5%	NS (p = 0.391)
Week 24 vs. T0	+0.2 ± 0.9	+1%	NS (p = 0.855)

*NS, not significant.

Table 36.3 Changes in transepidermal water loss (TEWL) after exposure to tropical climate (25 panelists); group treated with placebo formula; clearly the increase.

Time	Change ($g/m^{-2}/h^{-1}$) (mean ± SEM)	% Change (mean)	Significance
4 weeks vs. T0	+0.9 ± 0.8	+7%	NS (p = 0.258)
12 weeks vs. T0	+1.3 ± 0.7	+10%	NS (p = 0.081)
24 weeks vs. T0	+1.4 ± 0.7	+11%	Borderline (p = 0.057)

Conclusions

Peptides, more than proteins, have become the "buzz" word of dermocosmetics (sometimes called "cosmeceuticals" or "active" cosmetics) during the last 5 years. This chapter presents some justification for this success. Although the list of peptides naturally occurring in the human body is long, allowing for further biomimetic peptide development for skincare, the possibility to create derivatives, analogs, and other variations on a theme is enormous and exciting. For example, the number of possible pentapeptides based on the 20 proteinogenic amino acids is 20^5 or 3 200 000. As understanding of cellular mechanisms, gene regulation, receptor activity, and metabolic interactions increases, many new peptides may appear in dermocosmetics.

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