Peptides, Amino Acids and Proteins in Skin Care?

Editor's Note: Our regular columnist Mindy Goldstein, PhD, welcomes the following "Tech Edge" contribution from colleague Karl Lintner, PhD, of Sederma/Croda.

From the column editor: *The use of peptides with specific functions has increased significantly in personal care products. One is hard-pressed to find a formula on the shelf that does not list a polypeptide in the ingredient list. How are these peptides made? Where do the sequences originate? This month, I welcome Karl Lintner, PhD, from Sederma/Croda to present basic information on the peptides used in skin care products.*

Lintner

Karl Lintner, PhD, is a technical advisor to Sederma and Croda Enterprise Technology. He received a degree in chemical engineering and his doctorate in biochemistry from Vienna University. From 1973 to 1983, he conducted research in biological peptides at the Nuclear Research Centre in Saclay, France, resulting in the publication of more than 30 papers in biochemistry and biophysics—essentially on peptide activity.

From 1983 to 1989, Lintner was laboratory manager, then marketing manager for product development and worldwide technical support with the Henkel Company, Düsseldorf, Germany. In 1990, he joined Sederma as technical director and introduced peptides, synthetic ceramides and the first biotech products as cosmetic ingredients and received numerous patents. From 1997 to the present, Lintner has been managing director of Sederma.

—Mindy Goldstein, PhD

Mindy Goldstein, PhD, received her doctorate in the area of UV and gamma radiation damage to DNA and DNA repair from New York University. She has been awarded patents in encapsulation and controlled release and has been involved in the cosmetic industry since 1987. She currently is the executive director of Research & Development, Patent Formula Review, for The Estée Lauder Companies Inc. in Melville, NY.

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It is difficult today to find a cosmetic or personal care brand without a claim that at least some of its SKUs contain one or more peptides in their formulas. The concept of using active peptides in skin and hair care was slow to catch on but has gained considerable momentum in the industry during the past five years. It would thus be useful to clarify some of the concepts, ingredients and claims in connection with peptides in cosmetics. The relationship of peptides to amino acids and proteins, as indicated by the curious and specifically chosen title of this column, will become evident throughout this explanation.

> The number of possible amino acids is theoretically infinite and practically quite large.

Definitions

Confusion reigns in regard to the use of some technical terms in this field. Because any discussion of cosmetic activities, benefits and claims must be based on precise language, this article begins with some definitions that readers with chemical and biochemical backgrounds may already know.

Amino acids: Amino acids are the building blocks of peptides and proteins. Their molecules have one thing in common: a carbon backbone with at least one amino (-NH_2) and at least one carboxyl (-COOH) group attached. This confers specific electrochemical or charge-related behavior to them, as well as the useful functionality of being able to link into chains; furthermore, the various amino acids are distinguished by the nature of their carbon backbone and the side chains that, in turn, confer additional functions, solubility and linking capabilities to these entities.

Peptides: Peptides are chains of at least two amino acids linked by the eponymous "peptide bond" between the carboxyl group of one and the amide group of the following amino acid. Oligopeptides are chains generally understood to be composed of less than approximately 20 amino acids. Polypeptides have longer chains; for example, insulin has approximately 50 amino acids.

Although no precise cut-off point has ever been agreed upon in scientific nomenclature, most scientists would agree that beyond 100 amino acids and a molecular weight of approximately 10,000 Daltons, the term *protein* is preferred over *peptide*.

Proteins: Thus, proteins are long, usually linear chains of amino acids that can reach 1,000 or more links; collagen for example is approximately that size; fibronectin has a MW of approximately 400 kDaltons (approximately 4,000 amino acids). Unfortunately, the term protein is often used for raw materials that are totally or partially hydrolyzed proteins, or to blends of peptides and/or amino acids**.** To prevent confusion, this imprecise use of the term clearly should be avoided.

Origin and Manufacturing

A few words should be stated about origin and manufacturing.

Amino acids: Amino acids can be synthesized according to the Strecker method[1] and subsequent improvements, which yields amino acids in a racemic mixture of L- and D-stereoisomers; the separation into the biologically predominant and active L-form is possible but costly. Industrial production of amino acids uses either total protein hydrolysis followed by chromatographic separation, or biotechnology processes whereby specific microorganisms are solicited to manufacture a chosen amino acid in high yield.

Peptides: Peptides come in two forms: either they are synthesized as undefined mixtures of protein fragments obtained by partial hydrolysis of collagen, elastin, keratin, wheat or other plant proteins; or they are synthesized one peptide link at a time until a well-defined and well-chosen amino acid sequence is achieved. This latter method, albeit tedious and expensive, leads to reproducible, analytically defined and well-characterized entities with chosen biological activity and usually extremely high potency.

Proteins: Proteins are practically impossible to synthesize by these methods in the laboratory and even less so in industrial scale. They are either carefully extracted from natural sources such as cowhide, bird feathers, human or animal hair, fish muscle or various plants, or produced by specifically chosen, sometimes engineered, microorganisms.

Amino Acids and Cosmetics

Amino acids have the general chemical formula: H-NH-CHR-CO-OH; R being a side chain of variable nature (see **Figure 1**).

Yet too often in the literature, certain amino acid terms are used without being clearly defined. Examples include: the 20 amino acids, "essential" amino acids, and natural and non-natural amino acids.

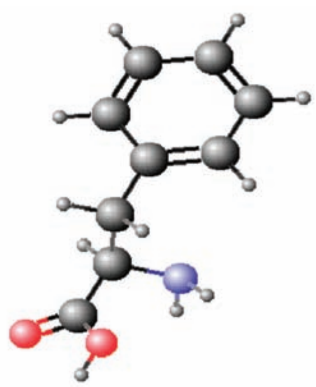

Figure 1. The amino acid phenylalanine

In fact, the number of possible amino acids is much higher than 20. It is theoretically infinite and practically quite large because one can always invent or imagine a new side chain, R, obtained by chemical means.

The so-called "natural" amino acids—those that have been identified in living organisms—number less than 35, of which 20 are particularly important to know. Only those 20 amino acids are coded for in human DNA and used by human cells to build proteins such as enzymes, collagen, elastin, keratin, muscle myosin and actin (see **Proteins and Cosmetics**).

Finally, within those 20 coded amino acids, six are called essential amino acids because humans, contrary to other species, are not able to synthesize these six molecules from simpler building blocks. Therefore, humans must ingest these molecules in one way or another in order to survive.

From a cosmetic science point of view, peptides are far more interesting.

Amino Acids in Cosmetics

Amino acids have been used in cosmetics for many decades, either as rather undefined mixtures of fully hydrolyzed proteins obtained from collagen, elastin, keratin, wheat gluten or milk casein, or as specifically chosen individual amino acids in pure, isolated form.

In skin care, specifically chosen, purified, amino acids such as serine, threonine, alanine and pyroglutamic acid are popular ingredients because they are key components of the natural moisturizing factor (NMF); although, given their zwitterionic charged nature and low lipophilicity, they tend to remain at the surface of the stratum corneum and act only as water-binding molecules. Tyrosine and its derivatives such as acetyl-tyrosine or oleoyl-tyrosine[a] are used as melanin precursors in tanning accelerators.

[a] *Tyr-ol (INCI: Oleoyl tyrosine (and) butylene glycol (and) oleic acid) is a product of Sederma, France. Ty-rol is a trademark of Sederma.*

Carnitine, creatine and taurine—all naturally occurring but not part of the coded 20 amino acids—are promoted as energy stimulating factors, co-factors or precursors, often in analogy to nutritional supplement claims.

Peptides and Cosmetics

Far more interesting, from a cosmetic science point of view, are the peptides, especially those with defined sequence and structure possessing specific biological activity. It is not excluded that partial protein hydrolysates that are "rich in peptides" show some cosmetic activity at sufficiently high concentration; however, their chain length, molecular weight, chemical composition, charge distribution and mechanism of action, if any, are unknown factors.

In contrast to these hydrolysates, synthetic peptides of defined chemical structure—those presently in vogue—have quite a different history and use. Glutathione, a tripeptide with important biological function in humans as an antioxidant and in the transport of amino acids, was discovered in 1921. Since then, hundreds of oligopeptides have been isolated from biological material, identified, synthesized and studied for biological activity. Among the better-known examples are oxytocin, vasopressin, angiotensin and bradykinin that release hormones such as TRH, ACTH, MSH, LHRH, substance P, rigin, enkephalin and endorphins.

The one important concept to understand is the strong correlation that exists between the specific amino acid sequence in the peptide chain and the resulting bio-activity. Most of these peptides act at precise cell receptors and trigger physiological responses in various cells and organs. Changes in the amino acids composing the peptide almost always lead to changes in the potency, type or duration of the activity. An example of this high selectivity is the tripeptides composed of the amino acids glycine, histidine and lysine (Gly-His-Lys) (see **Figure 2**). In the Gly-His-Lys sequence, wound-healing properties are observed through the stimulation of collagen synthesis in fibroblasts.[2] In contrast, the Gly-Lys-His structure has lipolytic activity on adipocytes.[3]

The fabulous thing about peptides of this nature is the fact that they act at nano- to micromolar concentration, so long as they reach their target cells.

How peptides work: Before looking more closely at the cosmetic aspects of peptide use, consider how these peptides work. The general scheme evokes a "key and lock" model whereby a peptide, arriving from blood, lymph, tissue diffusion or some other external source is recognized by a specific receptor molecule or protein at the cell membrane surface. The recognition is due to complementary three-dimensional structures, such as amino acid side chain entities, that fit into pockets of the receptor molecule like a key into a lock. The binding of the peptide to the receptor then triggers conformational changes in this transmembrane structure, which leads to further cascade events in the cell's interior biochemistry. The receptor thus acts like a transistor in electronics: a tiny amount of signal, i.e., the peptide, can lead to macroscopic events including collagen synthesis, lipid metabolism and nerve signal transmission within the cells.

Fundamental peptide biology research and pharmacological investigations into these mechanisms have in many cases confirmed the basic tenets of this model. A variant of the mode of peptide activity is found when the peptide seems to enter the target cell and then interacts with the cell nucleus directly, triggering genetic reactions (DNA transcription) and the like. Because of the signaling nature of the peptides, they are often called *messengers* of biological information.

The origin of peptides: With nature being economical, most of the peptides described in the scientific literature are actually breakdown fragments of larger molecules. For instance, keratinocytes and certain other cells produce a protein molecule called pro-opiomelanocortin (POMC) that consists of 241 amino acids. Depending on the organism's needs, POMC is cleaved by enzymes into one of three peptides: ACTH, MSH and ß-endorphin. ACTH (adrenocorticotropine), consisting of 34 amino acids, acts at surrenal glands in case of stress. MSH (melanocortin), a fragment of 13 amino acids found within the ACTH sequence, leads to increased tanning in melanocytes. ß-endorphin, containing 31 amino acids, controls breathing, digestion and other activities; it in turn can be cleaved to release the pentapeptide enkephalin or natural pain killer.

Peptides act at a nano- to micromolar concentration.

The natural origin of peptides and their high potency at low concentration, coupled to the rapid clearing of peptides in the bloodstream, are of great importance for their use in human applications: low dosage, little remanence and identity to endogenous molecules make peptides a very safe and effective family of active ingredients, especially for cosmetics. Of course, any change in amino acid sequence from its original one, designed perhaps to enhance or modify activity, might require more detailed investigation on potential side effects. In the field of skin care, this aspect is particularly crucial.

Cosmetic aspects of peptide usage: The challenges to using peptides in cosmetic formulas are not negligible, however. The foremost is targeting and bioavailability (i.e., skin penetration): oligopeptides of the nature and structure described do not easily reach their targets. Although low use concentrations of the usually expensive synthetic peptides allow the economic equation to work out for most skin care uses, detection of ppm levels of peptides in complex cream matrices is a challenge. Nevertheless, as stated in the introduction, peptides have become a main staple in skin care formulations because it has been made possible to overcome these hurdles.

Lintner and Peschard[2] have shown that palmitoylation of short peptides improves the skin penetration behavior by a factor 100 to 1000, thus making it possible to achieve wrinkle reduction and other cosmetic effects even at 10 µM concentration.[4–5] Fluorescent tagging coupled to high performance liquid chromatography allows the detection of these milligram (ppm) quantities even in rich cosmetic textures.

What cosmetic activities, properties and claims could then be based on peptides? Foremost, the peptides

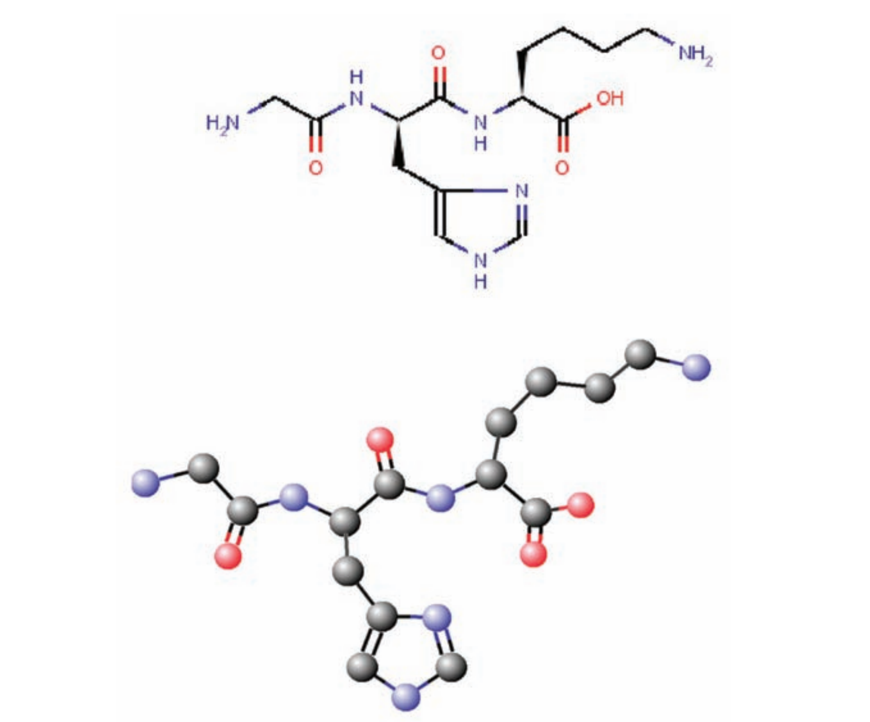

Figure 2. Th tripeptide Gly-His-Lys

derived from wound-healing research are targeted for matrix regeneration because they stimulate collagen, fibronectin, GAG synthesis and allow for wrinkle repair, skin thickening and skin firming. A well-known example is the family of matrikines, peptides that are released by the breakdown of structural proteins such as collagen, elastin and fibronectin, of which the pentapeptide Pal-KTTKS[b],[4–5] the tripeptide Pal-GHK, and the hexapeptide Pal-Val-Gly-Val-Ala-Pro-Gly are prominent representatives.

Other interesting cosmetic activities include the modulation of melanin synthesis in melanocytes, either by stimulating or inhibiting the melanin production for accelerated tanning or so-called skin lightening/toning; the stimulation of lipolysis for slimming and anti-cellulite claims;[3] and anti-inflammatory properties produced by reducing interleukin secretion and even neuronal activity that lends soothing, calming properties to the finished cosmetic formula.[2] Hair growth stimulation and/or hair loss prevention can also be affected with peptides such as Biotinoyl-Gly-His-Lys, which has been demonstrated to stimulate the synthesis of collagen IV and laminin 5.[6]

Small peptides have gained prominence in skin care because they constitute a novel and highly effective approach to today's sophisticated skin care market that is at the same time generally risk-free. Peptides are highly versatile molecules and even by staying within the restriction of approximately dekapeptides (chains containing 10 amino acids), the number of possible peptide sequences based on only the 20 coded amino acids is 20^{10} or 200 billion. Although most of these sequences will likely have no activity, there is still room for research and innovation.

How does one choose the right peptide? It will be a peptide known to the fundamental peptide research community, or it will be a peptide selected from a library of peptides created by efficient, rapid, parallel synthesis coupled to more or less rapid screening methods. Peptide design from molecular modeling is an attractive alternative but would require more knowledge about the structural features of the cellular surface, the receptors or other interactions governing peptide activity. In this author's opinion, there is as of yet little to go on to achieve this.

Limitations of peptide use in skin care may arise from solubility, bio-availability and penetration characteristics, potential toxicological problems (although they should be rare), stability parameters and formulation compatibilities, economic considerations and, last but not least, intellectual property issues because the number of nonpatented oligopeptide sequences is shrinking fast.

Proteins and Cosmetics

Categories of proteins: The intention of this article was to focus on peptides; proteins and their use in cosmetic skin

[b] *Matrixyl (INCI: Palmitoyl-pentapeptide 4) is a product and trademark of Sederma.*

care applications would need at least as much space to be discussed in detail. Briefly, proteins can be placed into four main categories: structural, functional, signal or defense molecules:

- ***Structural:*** Larger structural entities such as collagen, elastin, fibronectin, laminin, myosin, actin and many others participate in the building and maintenance of connective tissue of the three-dimensional arrangement of the organs that constitute the human body, including muscles and skin layers.
- ***Functional:*** Functional proteins, such as enzymes, of which thousands are present in low concentration within cells and the blood stream, can act as chemical catalysts that initiate, regulate and modulate all biochemical processes. Other functional proteins include transport molecules such as transferrine, hemoglobin, myoglobin (**Figure 3**) and lipoproteins.
- ***Signaling:*** Cytokines, chemokines, interferon and interleukins are smaller proteins that possess, like the oligopeptides, a signaling function, triggering a downstream cascade of events.
- ***Defense:*** Finally, antibodies are large protein structures with the specific function of defending the organism against invasion by foreign matter such as bacteria or viruses, allergens and the like.

Cosmetic applications of proteins: Are any of these protein categories useful for skin care applications? Collagen can be extracted in its native, non-hydrolyzed form from cow hide or fish skin and employed as a natural film former and water-binding molecule. Fibronectin, isolated from horse serum, was used for some time for similar purposes. Proteins of high molecular weight also can be obtained from wheat, rice, potatoes and other plants; they are used, for instance, as skin-tightening actives.

Certain enzymes have found some use in cosmetic formulas. The lactoperoxidase/glucose oxidase system, with its antimicrobial activity, was used as an aid in fighting acne-type symptoms on the skin but could also, at higher concentrations, be used in helping to preserve finished products against spoilage. Because of stability concerns, the technology did not find widespread application.

Proteolytic enzymes[c], lipolytic enzymes[d] and more recently, heat- and UV-stable protective antioxidant enzymes[e, 7–8] have found acceptance in skin care.

The other two categories, cytokines and antibodies, present too many challenges in relation to synthesis or extraction from animal tissue, toxicological and pharmacological concerns, to have made an impact in the field of skin care. Cytokines are often mentioned as being the mediators of certain cosmetic activities; however, they are not used as ingredients.

[c] *Keratoline (INCI: Bacillus subtilis ferment) is a product and trademark of Sederma, France.*
[d] *Cyclolipase (INCI: Lipase) is a product and trademark of Sederma.*
[e] *Venuceane (INCI: Thermus thermophilus ferment) is a product and trademark of Sederma.*

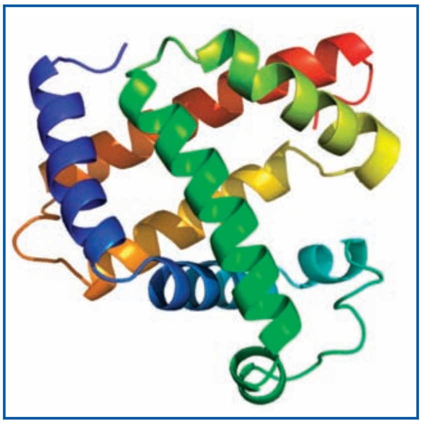

Figure 3. Representation of myoglobin, a functional protein with a chain of 153 amino acids, presented to show the difference in size and structure of a protein as compared to an amino acid or a peptide.

Conclusion

In numerous articles, advertising copy and general media jargon, amino acids, peptides and proteins are too often bunched together as if they were a single entity of cosmetic ingredients with similar activity and usefulness. The present introduction aims to clarify the clear differences between these categories of chemicals, whatever their origin. During the past few years, it has become evident that peptides, if designed well, correctly chosen and intelligently employed, possess by far the greatest potential for beneficial cosmetic use because they have unambiguous, clearly defined specific activity. In addition they are analytically pure, biomimetic, biodegradable, nontoxic and nevertheless highly innovative. Most individual amino acids are too small and simple to show specific biological activity, and most proteins are too big, unwieldy and difficult to obtain to be considered as major players in the field of skin care ingredients.

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