Innovations in Hyaluronic Acid

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Hyaluronan (HA), also known as hyaluronic acid, is a high molecular weight carbohydrate polymer that is found in all tissues. However, more than 50% of all the HA in the body occurs in skin, where it is found in both the epidermis and the dermis. Special fixation techniques are necessary to measure epidermal HA, as it tends to leach out during ordinary histological procedures.[1]

HA levels are constant in skin throughout life, and while the HA in newborn skin is mostly soluble, this solubility decreases with age as the HA becomes more tissue/protein bound and less accessible for extraction.[2] Free HA at the body's pH is the most highly charged molecule in biology. Such HA surrounds itself with a large volume of water in an attempt to neutralize that charge. This space-filling property is the basis of the youthful appearance of skin provided by HA. The HA of infant skin is mostly "free HA," while that in an elderly person is tissue- and protein-bound in a state that does not accrue solvent water. HA must be free to attract the voluminous solvent water, therefore increasing the volume of skin and decreasing the appearance of age. It appears unable to do so when it is tissue- and protein-associated.

HA is critically important to dermatologists and to cosmetic scientists, as it maintains the moisture in skin, and loss of accessible HA results in wrinkling and changes associated with aging. Replacement of such HA is the goal of many cosmetic and cosmeceutical techniques and reagents. A number of concepts have emerged since the last update of HA appeared in *Cosmetics & Toiletries*.[3] New insights are appearing rapidly, and this column will summarize recent HA concepts.

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Hyaluronan Degradation

A 70-kg (approx. 154 lb) individual has approximately 15 g of HA, a third of which has a daily turnover. In the bloodstream, HA has an even more remarkable half-life of 2–5 min. The reason why the body exerts so much energy to produce HA only to degrade it with such speed remains a mystery; however, HA can be degraded enzymatically or oxidatively.

Enzymatic HA degradation: The mechanism for enzymatic HA degradation is a group of enzymes called hyaluronidases. Of the genes that code for hyaluronidases in the human genome, i.e., HYAL1–4, PH-20 and HYALP1,[4] only two, HYAL1 and HYAL2, appear to be active in HA turnover in somatic tissues. PH-20 is sperm-specific and involved in fertilization.

Three of these sequences are tightly clustered on chromosome 3, with a similar cluster on chromosome 7. This suggests that following the original sequence, two duplication events occurred resulting in three genes, with a subsequent en masse duplication, from three to six. An analysis of these events is summarized in an article in press[5], and will also be presented in the second portion of this series.

The hyaluronidase-like family of proteins has many unusual properties. For example, no hyaluronidase enzymatic activity has ever been detected for HYAL3 using a variety of sensitive assays. It may not be an enzyme in the usual sense. It may have adhesive or anti-adhesive effects. Other members of the family have activities far different from their enzyme properties. HYAL2 in some species are membrane receptors for cancer-causing retroviruses.[6] Two additional novel functions of HYAL2 are formation of the glycocalyx, the intimate pericellular matrix around cells, and control of the interaction between the HA receptor CD44 and the cytoskeleton.[7] Though enzymes often have functions beyond their enzymatic activities, some of which may be even more important than their enzymatic activities, it is difficult to assay for such additional activities, which are discovered more by accident than by design.

Oxidative HA degradation: Another mechanism for breaking HA chains is through free radicals such as reactive oxygen species.[8] These play an important role in HA turnover but are more difficult to study, and it is uncertain what the proportions are between enzymatic and oxidative degradation of HA.

During inflammation, an enzyme in white cells termed *myeloperoxidase*

provides an oxidative burst, generating free radicals capable of degrading HA chains. Oxidative destruction of HA is an unexplored area in skin physiology. Further, it is likely that UV light can generate free radicals that then break HA chains.[9, 10] This is another area that requires further investigation.

Upregulation of HA deposition can be documented following exposure of hairless nude mice to UV light. The resulting HA must be of the small inflammatory molecular size, and may account for the edema connected with sunburn, as edema fluid contains predominantly HA. This effect occurs within five minutes of exposure (unpublished experiments). Intriguingly, such changes can be detected for as long as six months following exposure, as documented by immunohistochemical examination of skin biopsies.

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Such enhanced HA deposition is presumably a stress response, and perhaps protection against further injuries, comparable to the heat shock proteins that protect against subsequent heat exposure. All such hypotheses are just that and require testing.

Multifunctional Hyaluronan

Hyaluronan is a monotonous unadorned polymer consisting of the alternating sugars glucuronic acid and N-acetyl glucosamine, connected exclusively by beta-linkages. There is no sulfation or other substitutions, unlike all other glycosaminoglycans (GAGs). Additionally, HA is not bound to a core protein. It is synthesized on the cytoplasmic surface of the plasma membrane, while all other GAGs are synthesized by way of the endoplasmic reticulum and the golgi apparatus.

HA can possess a number of functions based on size.[11] High molecular mass HA is found in resting normal tissue. It is anti-angiogenic, anti-inflammatory and immunosuppressive. Fragmented HA has a variety of size-specific functions, most of them reflecting tissues under stress. Short HA chains are highly inflammatory, angiogenic and immunostimulatory.

Alpha Hydroxy Acids

Since ancient times, fruit extracts have been applied to the face for beauty reasons. Alpha hydroxy acids (AHAs) can be found in many foods, including: citrus fruits (citric acid), apples (malic acid), grapes (tartaric acid), almonds (mandelic acid), milk (lactic acid) and sugar cane (glycolic acid). These AHAs are well-known in the cosmetic industry to reduce wrinkles and other signs of aging. The claim that these AHAs enhance the overall look and feel of skin has some legitimacy, as these materials stimulate HA deposition in the dermis.

Among these, this author has observed mandelic acid to be the most potent by measuring HA production in cell cultures of skin-derived fibroblasts. In that unpublished experiment, the aforementioned AHAs were compared. The HA deposited following AHA stimulation must be of high molecular size, as no inflammation is observed clinically, consistent with the absence of any low molecular sized inflammatory HA fragments.

It is often overlooked that vitamin C (ascorbic acid) is also an AHA. A number of cosmetics and cosmeceuticals in the past have contained high levels of vitamin C with well-documented effects. One of the functions of vitamin C and its derivatives is inhibiting hyaluronidase activity.[12, 13] This may be the mechanism by which vitamin C stimulates HA deposition. It has not been examined if other AHAs are also hyaluronidase inhibitors. Attaching fatty acid chains to these AHAs, as has been done with vitamin C,[13] may be a new technique for creating cosmetics/cosmeceuticals that can permeate the skin barrier.

The effects of these AHAs on the epidermis are entirely different from their effect on the dermis. They are effective exfoliants for the epidermis and are used in a variety of peeling procedures. Glycolic acid is the smallest of these molecules, and therefore penetrates the skin most efficiently. These AHAs affect keratinization in the epidermis, and stimulate the production of a new stratum corneum.

Hyaluronan Synthases

Three isozymes of the enzyme that synthesizes HA, i.e, HAS-1, HAS-2 and HAS-3, are present in the human genome.[14] These are membrane proteins embedded on the inner surface of the plasma membrane. Each one is located on a different chromosome and produces different sized HA polymers. Of the three, HAS-2 is critically important as the only one that is lethal to embryos after being deleted. Conversely, deletion of HAS-2 and HAS-3 results in a normal embryo.

Attaching fatty acid chains to the range of alpha hydroxy acids may be a new technique for creating cosmetics/cosmeceuticals that can permeate the skin barrier.

HAS-1 and HAS-2 are regulated by TGF-β in both the dermis and epidermis, with major differences between the two compartments and between the two isoforms, suggesting that the genes are regulated independently.

Recent evidence indicates that HA also occurs intracellularly.[15] It is not known whether such HA chains are taken up from the extracellular matrix

(ECM) or remain intracellular following their synthesis. This intracellular HA participates in the inflammatory response, but probably has additional functions that have not yet been established.

Conclusion

It is apparent that HA, a deceivingly simple unadorned disaccharide polymer, is actually an extraordinarily complex molecule. It does not reveal its secrets easily; however, as reviewed here, much progress is being made in laboratories around the world. Such new information will continue to have major impacts on both the cosmetic and dermatological industries.

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