Hyaluronan: its nature, distribution, functions and turnover

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Hyaluronan is a polysaccharide found in all tissues and body fluids of vertebrates as well as in some bacteria. It is a linear polymer of exceptional molecular weight, especially abundant in loose connective tissue. Hyaluronan is synthesized in the cellular plasma membrane. It exists as a pool associated with the cell surface, another bound to other matrix components, and a largely mobile pool. A number of proteins, the hyaladherins, specifically recognize the hyaluronan structure. Interactions of this kind bind hyaluronan with proteoglycans to stabilize the structure of the matrix, and with cell surfaces to modify cell behaviour. Because of the striking physicochemical properties of hyaluronan solutions, various physiological functions have been assigned to it, including lubrication, water homeostasis, filtering effects and regulation of plasma protein distribution. In animals and man, the half-life of hyaluronan in tissues ranges from less than 1 to several days. It is catabolized by receptor-mediated endocytosis and lysosomal degradation either locally or after transport by lymph to lymph nodes which degrade much of it. The remainder enters the general circulation and is removed from blood, with a half-life of 2–5 min, mainly by the endothelial cells of the liver sinusoids.

Keywords: biosynthesis, concentration, degradation, hyaladherins, physiological functions, structure.

The nature of hyaluronan

Hyaluronan (HYA; syn. hyaluronic acid, hyaluronate [1]) is one of a group of polysaccharides typically found in the connective tissues of vertebrates, which were formerly known as acid mucopolysaccharides and are now designated glycosaminoglycans (Table 1). Glycosaminoglycans are unbranched single-chain polymers of disaccharide units containing N-acetylhexosamine and hexose. The second sugar is a hexuronic acid in all except keratan sulphate, which contains galactose instead.

Glycosaminoglycans other than HYA share several other characteristics. All contain sulphate groups, and their polysaccharide chains are relatively short (<50 kDa, commonly 15–20 kDa). Their synthesis takes place in the endoplasmic reticulum and Golgi bodies, and they are substituted in peptide cores, often with a variety of other saccharides, to form proteoglycans. Different kinds of sulphated glycosaminoglycans can be joined to a common peptide core, which with accompanying variations in the peptides and other saccharides provides for great variety in proteoglycan structure and consequently, in their potential for reactivity with other compo-
components of extracellular matrix and with various cells. Most proteoglycans are notable for one or more strong associations with fixed matrix structures or cells and are relatively immobile.

HYA (for more extended reviews see [2–5]) is quite distinct from other glycosaminoglycans in most of these respects. Its primary structure contains no peptide, which is consistent with its synthesis in the plasma membrane rather than the Golgi. Although it consists of a single polysaccharide chain like other glycosaminoglycans, its molecular weight (relative molecular mass; \( M_r \)) usually reaches the millions; in normal synovial fluid, for example, the weight-average is about \( 7 \times 10^6 \), which if straightened would extend to \( >15 \mu \text{m} \). Its uniformity of structure would seem at first sight to restrict its biological roles, but this limitation is overcome by the numbers of specific HYA binding sites that have evolved in other matrix molecules and on cell surfaces (see following). Moreover, it has other distinctive attributes arising from its extraordinary molecular mass, which underlie its distinctive role in extracellular matrix.

### Distribution of hyaluronan in mammalian organs and tissues

A painstaking analysis of the body of the rat by Reed et al. [6] should be broadly applicable to other mammals. About half of the HYA was recovered from skin, and a quarter from the skeleton and joints taken together. The rest was almost equally divided between the muscles and viscera (Table 2). Comparative data for a variety of species, tissues and organs are shown in Table 3. The highest HYA concentrations are found in typical connective tissues such as umbilical cord, synovial fluid, skin and the vitreous body. Notable amounts are also present in lung, kidney, brain and muscle but very little in liver. The lowest concentration is found in blood serum. The striking differences that can occur within a tissue are illustrated by the kidney (see also article by Gerdin & Hällgren (pp. 000–000) in this Minisymposium). With the development of specific and sensitive histochemical methods, improved fixation and specific enzymatic controls, an extensive body of data is now available on the distribution of HYA in normal tissues and disease (see for example Refs 2–11 and the following articles in this symposium).

### Biosynthesis and cellular origins of hyaluronan

Hyaluronan is synthesized in the plasma membrane by a membrane-bound protein whose genetic code has recently been determined in bacteria, mouse and human [12–16]. This adds sugar units from

<table>
<thead>
<tr>
<th>Name</th>
<th>Constituent sugars</th>
<th>Sulphate</th>
<th>( M_r )</th>
<th>Proteoglycans</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyaluronan</td>
<td>Glucuronic acid Glucosamine</td>
<td>–</td>
<td>( 10^2–10^7 )</td>
<td>–</td>
</tr>
<tr>
<td>Chondroitin 4-(6-) sulphates</td>
<td>Glucuronic acid Glucosamine</td>
<td>+</td>
<td>( 10–50 \times 10^3 )</td>
<td>+</td>
</tr>
<tr>
<td>Dermatan sulphate</td>
<td>Iduronic acid Galactosamine</td>
<td>+</td>
<td>( 10–50 \times 10^3 )</td>
<td>+</td>
</tr>
<tr>
<td>Keratan sulphate</td>
<td>Galactose Glucosamine</td>
<td>+</td>
<td>( 5–15 \times 10^3 )</td>
<td>+</td>
</tr>
<tr>
<td>Heparan sulphate</td>
<td>Glucuronic and iduronic acid Glucosamine</td>
<td>+</td>
<td>( 10–50 \times 10^3 )</td>
<td>+</td>
</tr>
<tr>
<td>Heparin</td>
<td>Glucuronic and iduronic acid Glucosamine</td>
<td>+</td>
<td>( 5–20 \times 10^3 )</td>
<td>+</td>
</tr>
</tbody>
</table>
nucleotide precursors to the chain on the cytoplasmic aspect of the membrane and translocates the growing chain to the pericellular space. Prehm has observed that the growth of the chain occurs at the reducing end, in contrast with the synthesis of other connective tissue polysaccharides.

It is almost certain that most kinds of vertebrate cells synthesize HYA at some point in their natural history. This capability can be repressed or activated in changing circumstances as in the case of the smooth muscle cell. In the mature organism, synthesis of HYA is most strongly expressed in cells of mesodermal lineage, although it can remain active in others such as those of epidermis [17].

### Hyaluronan in the initial development of extracellular matrix

When fibroblasts, mesothelial or certain other kinds of cell are plated out in tissue culture, they surround themselves in a few hours with a transparent pool or coating of gel-like material that can be visualized by its impressive ability to exclude cells and other particles but cannot be seen with regular histological techniques [18]. This structure disappears with hyaluronidase treatment and must depend, therefore, on HYA for its integrity; initially, at least, it must represent the newly synthesized polysaccharide translocated to the pericellular space. In terms of cell biology, there are many interesting aspects of this coating. It supplies the cultured cell with its own microenvironment; it forms a barrier against damage by immune cells, impedes virus infection and may be important in mitosis. The nature of this coating and its relation to the surface of the cell almost certainly vary with the kind of cell and its synthetic products, which extends its usefulness as a model of matrix genesis.

### Functions of hyaluronan

**Functions arising from its intrinsic properties (reviewed in Ref. 19)**

The carboxyl groups of HYA are fully ionized at extracellular pH. Its osmotic activity is non-ideal and disproportionately high in relation to its molecular weight. For this and other reasons it is capable of profound effects on the distribution and movements of water and plays a major part in water homeostasis.

Recent work has shown that secondary hydrogen bonds form along the axis of the polysaccharide. These create a twist in the chain, impart some stiffness, and generate hydrophobic patches that permit association with other HYA chains, despite their negative charge, and extend its capability of nonspecific interaction with cell membranes and other lipid structures [20]. The stiffness of the HYA polymers promotes an extended random-coil configuration and their long chains ensure that they occupy enormous molecular domains. These begin to overlap and

<table>
<thead>
<tr>
<th>Organ or fluid</th>
<th>Man</th>
<th>Sheep</th>
<th>Rabbit</th>
<th>Rat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Umbilical cord</td>
<td>4100</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Synovial fluid</td>
<td>1400–3600</td>
<td>540</td>
<td>3890</td>
<td></td>
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<tr>
<td>Dermis</td>
<td>200</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitreous body</td>
<td>140–338</td>
<td>260</td>
<td>29</td>
<td>34</td>
</tr>
<tr>
<td>Lung</td>
<td>98–243</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kidneys</td>
<td>93–113</td>
<td>30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Renal Papillae</td>
<td>250</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Renal cortex</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brain</td>
<td>35–115</td>
<td></td>
<td>74</td>
<td>44</td>
</tr>
<tr>
<td>Muscle</td>
<td></td>
<td>54–76</td>
<td>44</td>
<td></td>
</tr>
<tr>
<td>Intestine</td>
<td>27</td>
<td></td>
<td>44</td>
<td></td>
</tr>
<tr>
<td>Thoracic lymph</td>
<td>8.5–18</td>
<td>1–34</td>
<td>5.4</td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td></td>
<td>1.5</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Aqueous humour</td>
<td>0.3–2.2</td>
<td>1.6–5.4</td>
<td>0.6–2.5</td>
<td>0.2</td>
</tr>
<tr>
<td>Urine</td>
<td>0.1–0.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lumbar CSF</td>
<td>0.02–0.32</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma (serum)</td>
<td>0.01–0.1</td>
<td>0.12–0.31</td>
<td>0.019–0.086</td>
<td>0.048–0.26</td>
</tr>
</tbody>
</table>

form an entangled network at levels of 0.5–1.0 g L\(^{-1}\) which may be stabilized by chain–chain interactions. Alone or in conjunction with collagen fibres and other macromolecular elements of the extracellular matrix, this reduces the mobility of HY A itself and determines its permeability to other substances, whether transported by diffusion or hydrodynamically driven bulk flow.

The phenomenon of steric exclusion of other macromolecules is another attribute of the molecular meshwork of hyaluronan. At the normal HY A content of synovial fluid about 15% of the total water volume is unavailable to albumin, so that its true concentration in the remainder of the solution is in fact higher. The degree of exclusion increases with molecular size, and explains in part why the largest plasma proteins are reduced in extravascular fluid to an even greater degree than albumin. It also means that virus antibodies, for example, may have a much greater neutralizing activity than anticipated in the presence of the polymers and thus hamper the recovery of viable virus. Various forms of protein precipitation can also be facilitated.

The consequences of changes in HY A concentration or degradation of its polymers were first demonstrated by Duran-Reynals as ‘spreading factors’ which disseminated India ink, dyes and microorganisms in the skin and proved for the most part to be hyaluronidases. As indicated from the content of this section, many other consequences are now self-evident.

None of the foregoing effects is restricted to hyaluronan, although it undoubtedly makes the major contribution to the structural properties and functions of extracellular matrix in many areas such as synovial fluid, the renal medulla, and parts of the gut and other soft tissues. In these tissues HY A is the dominant glycosaminoglycan and largely free from specific structural binding. The most distinctive property of hyaluronan is its visco-elasticity in the hydrated state. Both the viscosity and the elasticity are anomalous; that is, they are not constant but vary with the rate of shear or oscillatory movement. The viscosity of a 1% solution of HY A with an \(M\), of 3–4 \(\times 10^6\) is about 500 000 times that of water at low shear rate, but can drop 1000-fold when forced through a fine needle (a useful property in medical use). In plain words, the kind of anomaly in HY A can be described as follows. Rapid movement reduces the viscosity, which reflects the force required to overcome internal friction, and increases the elasticity, which stores energy and permits recovery from the deformation. This phenomenon is familiar to physicians as ‘pitting’ oedema in normally soft subcutaneous tissues. Fluid is slowly displaced by sustained digital pressure and gradually returns on release. After brief and rapid indentation to the same depth, the tissue immediately recovers its original form. Although the fibrous elements will normally obscure these changes, they become apparent in oedema when matrix is largely liquid. Apart from its plasma protein content, which is not intrinsically very viscous, HY A then dominates its macromolecular content.

The anomalous viscosity of HY A suggests that it should be an ideal biological lubricant, at least by reducing the work load during rapid movements. It is abundant in the fluids of synovial joints, tendon sheaths and bursae. It is found in the small amounts of fluid in the ‘serous’ cavities (pleura, pericardium and peritoneum) and in the less well-defined planes of tissue movement such as those between muscle bodies and skin. Notably, it persists between the individual fibres, spindles and septa in skeletal and cardiac muscle, but disappears with maturation of the slow-moving smooth muscle fibres of gut and vessel walls. (It reappears when they resume proliferation in disease). The lubricant role of HY A in the soft tissue lining of joints is well established but its contribution on the hard weight-bearing cartilaginous surfaces is less clear. A very thin film can maintain separation of surfaces bearing a high static load [21], but reduction of friction on cartilage probably relies on a complex interplay of its surface with HY A, a particular glycoprotein of synovial fluid, and phospholipid micelles [22, 23]. The functional role of anomalous elasticity is not so easily illustrated, but it seems likely to minimize cellular distortion and hasten recovery from distortion by the myriads of brief mechanical stresses to which the softer tissues of the integument are exposed.

Both viscosity and elasticity are positively related, in a complex way, to molecular weight and to concentration, a point that must be considered in the surgical and medical uses of various preparations of HY A for viscoprotection and viscosupplementation [24, 25].

The effects of specific binding

1 In the extracellular matrix. In 1972, Hardingham & Muir [26] discovered that cartilage proteoglycans, now called aggrecans, bind to hyaluronan. It soon
became clear that HYA plays a critical role in stabilizing cartilage matrix, although it constitutes only a small fraction of the matrix material. Large numbers of aggrecans are bound through a specific region of their peptide cores to a relatively short but extended HYA chain, and the binding is reinforced by small link proteins. Some of the aggregates may be built on HYA chains also linked to specific binding sites on the chondrocytes. The completed macro-aggregates achieve an individual mass in the order of several hundred million Daltons, and are deposited between collagen fibres and attract water by osmosis. It was assumed that this mechanism was restricted to cartilage, but several other HYA-binding proteoglycans (versican, neurocan, brevican and others; see article by Delpech et al., this Minisymposium, pp. 41–8) have been discovered in softer tissues, notably in the brain, and may have a more general role, yet to be elaborated. (For other forms of HYA–protein interaction, see Refs 5, 27.)

On cell surfaces. Specific HYA-binding sites were first formally demonstrated on the virus-transformed mouse fibroblast cell line, SV-3T3, and next on the hepatic sinusoidal endothelial cell, which is largely responsible for the uptake and catabolism of plasma HYA. A particular amino acid-binding sequence appears common to both the matrix and cell-associated binding sites, and some but not all in both groups share other genetic similarities. Toole [28] has grouped them all together as hyaladherins, which will allow further subgroups to be defined as their functions are more precisely defined.

At present, some can be clearly labelled as receptors, since they initiate an active cell response; for example, endocytosis and catabolism in the liver endothelial cell, cell motility in the case of the RHAMM receptor, and possible influence on cellular proliferation in these and others. Others serve to anchor HYA at the cell surface as an acquired glycocalyx, to mediate binding with other cells (especially in the case of immunocytes), or to initiate the formation of a specialized matrix as in the case of cartilage mentioned earlier. A particular class of HYA-binding peptide such as CD44 may function in different ways on different cells. Circumstantial evidence already points to the existence of HYA-binding receptors on a large variety of cells, and a clearer definition of their genetic origins and functions can be expected (see also articles by Toole, pp. 35–40; and Delpech et al., pp. 41–8).

Combined effects

Despite the considerable effects that arise from the specific binding of small amounts of HYA to cells and to other glycosaminoglycans in cartilage and other tissues, the great bulk of HYA exists without such binding, either free as in synovial fluid, or interspersed with fibrous matrices of varied density as in skin and vitreous humour. The observation of HYA pools with different half-lives in the same tissue [29] points to different degrees of mobility, but the overall half-life of HYA is still relatively short in most tissues. Despite the high turnover the intrinsic properties and functions of HYA will prevail.

A common theme in this Minisymposium is the expansion of the extracellular matrix and its enrichment with hyaluronan: in embryonic development; following various forms of injury; in immune reactions; in inflammation; and in the development of cancer. These expansions of the matrix can also regress with equal rapidity, and do not develop any strong specific structural bonds. On the other hand, binding sites and receptors are almost certain to be up-regulated in infiltrating cells, stromal cells and possibly those of parenchyma, to produce a coordinated interaction with the HYA-enriched matrix. The growth factors, cytokines and other mediators generated in these various disease processes may be responsible for the changes in the synthesis of HYA and for any up-regulation of its cellular binding sites and receptors that takes part in the reaction.

The turnover of hyaluronan in normal and pathological states

It is clear from the foregoing that nearly all the body’s hyaluronan content lies within the tissues, where its turnover was thought to occur until it was shown that the lymphatic vessels carry considerable amounts of HYA to the blood stream [7]. In densely structured tissues such as bone and cartilage, it is probable that most of the HYA turnover occurs by metabolic degradation in situ concurrently with that of collagen and proteoglycans, since there is no lymphatic drainage. In skin and joints, some 20–30% of HYA turnover occurs by local metabolism, and the rest is removed by the lymphatic pathways. The tissue half-life of HYA ranges from half a day to 2 or 3, regardless of its route of elimination. On reaching the blood stream, about 85–90% is eliminated in the
liver by receptor-facilitated uptake and catabolism in
the hepatic sinusoidal endothelial cells. The kidneys
extract about 10% but excrete only 1–2% in urine. In
some species, the spleen also has a high avidity for
HYA and an equally rapid capacity for its metabolism
but its contribution depends on its relative size and
circulation. The normal fractional turnover of plas-
ma HYA in humans is about 15–35% per minute,
which explains the low plasma levels in the face of
the lymphatic input. (For reviews on turnover see
Refs 30, 31.)

More recently, the lymph nodes have been found to
have a considerable capacity for extraction and
catabolism of HYA [32]. Comparison of lymph before
and after passage through the nodes shows an
extraction of as much as 90%, which occurs in the
lining cells of the lymphatic sinuses, comparable to
those of the blood vessels in liver and spleen.

Studies of peripheral lymph have also revealed:
1 that its content of HYA is still much lower than
that of the tissues from which it is derived;
2 that it contains, nevertheless, very large polymers
similar to those in the tissues, which suggests that
they are displaced from the tissues hydrodynamically
rather than by diffusion and unlike collagen and
larger proteoglycans, do not require any prior degra-
dation; and
3 that these polymers are preferentially eliminated
in the lymph node, consistent with the higher affinity
for larger polymers demonstrated in experiments on
the hepatic HYA receptor.

It should be noted that specificity of the metabolic
receptor for HYA in the liver is shared with chon-
droitin sulphate. Infusion studies in blood stream and
lymph node confirm that both share the same cata-
bolic pathways, but no clinical significance has yet
been attached to this fact.

All the major factors that affect the plasma levels
of HYA can now be anticipated. In the elimination
pathways, they include the functional capacity of the
specialized liver endothelial cells and the fractional
distribution of cardiac output through the hepatic
sinusoids, with possibly some effects from impaired
renal function; significant disturbances in HYA
metabolism by lymph nodes have not yet been identi-
fied. From the input side, plasma HYA can be elevat-
ed by an increased synthesis of HYA in a great
variety of diseases. It can be raised or lowered by
variation in the many circumstances that alter the
flux of fluid between the lymph and blood stream and
the displacement of HYA that accompanies it.
Changes of these various kinds are illustrated in the
contributions by Engström-Laurent (pp. 57–60),
Lindqvist (pp. 67–71) and Berg (pp. 73–7) to this
Minisymposium.

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