

Heparan sulfates and heparins: similar compounds performing the same functions in vertebrates and invertebrates?

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Abstract

The distribution and structure of heparan sulfate and heparin are briefly reviewed. Heparan sulfate is a ubiquitous compound of animal cells whose structure has been maintained throughout evolution, showing an enormous variability regarding the relative amounts of its disaccharide units. Heparin, on the other hand, is present only in a few tissues and species of the animal kingdom and in the form of granules inside organelles in the cytoplasm of special cells. Thus, the distribution as well as the main structural features of the molecule, including its main disaccharide unit, have been maintained through evolution. These and other studies led to the proposal that heparan sulfate may be involved in the cell-cell recognition phenomena and control of cell growth, whereas heparin may be involved in defense mechanisms against bacteria and other foreign materials. All indications obtained thus far suggest that these molecules perform the same functions in vertebrates and invertebrates.

Key words

- Heparin, occurrence and function
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- Heparin and heparan sulfate, structure

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Heparan sulfates from mammalian and other vertebrate tissues

Among the sulfated glycosaminoglycans, heparan sulfate, a ubiquitous cell surface component of mammals and other vertebrates, is the one that exhibits the highest structural variability according to the tissue and species of origin (1-12). This class of compounds comprises linear polymers composed of several distinct disaccharide units containing glucuronic or iduronic acid and glucosamine with N- and 6-O-sulfates and

N-acetyl substitutions. The presence of other disaccharide units, which occur in smaller proportions and contain sulfate attached to their uronic acid residues, has also been identified in heparan sulfates (11,12). The order in which these disaccharide units occur in the molecule was first established for the heparan sulfate derived from rabbit endothelial cells in culture (11). Recently the total sequence of the disaccharides from bovine pancreas and the partial sequence of seven other heparan sulfates of mammalian origin have also been established (8,13). It

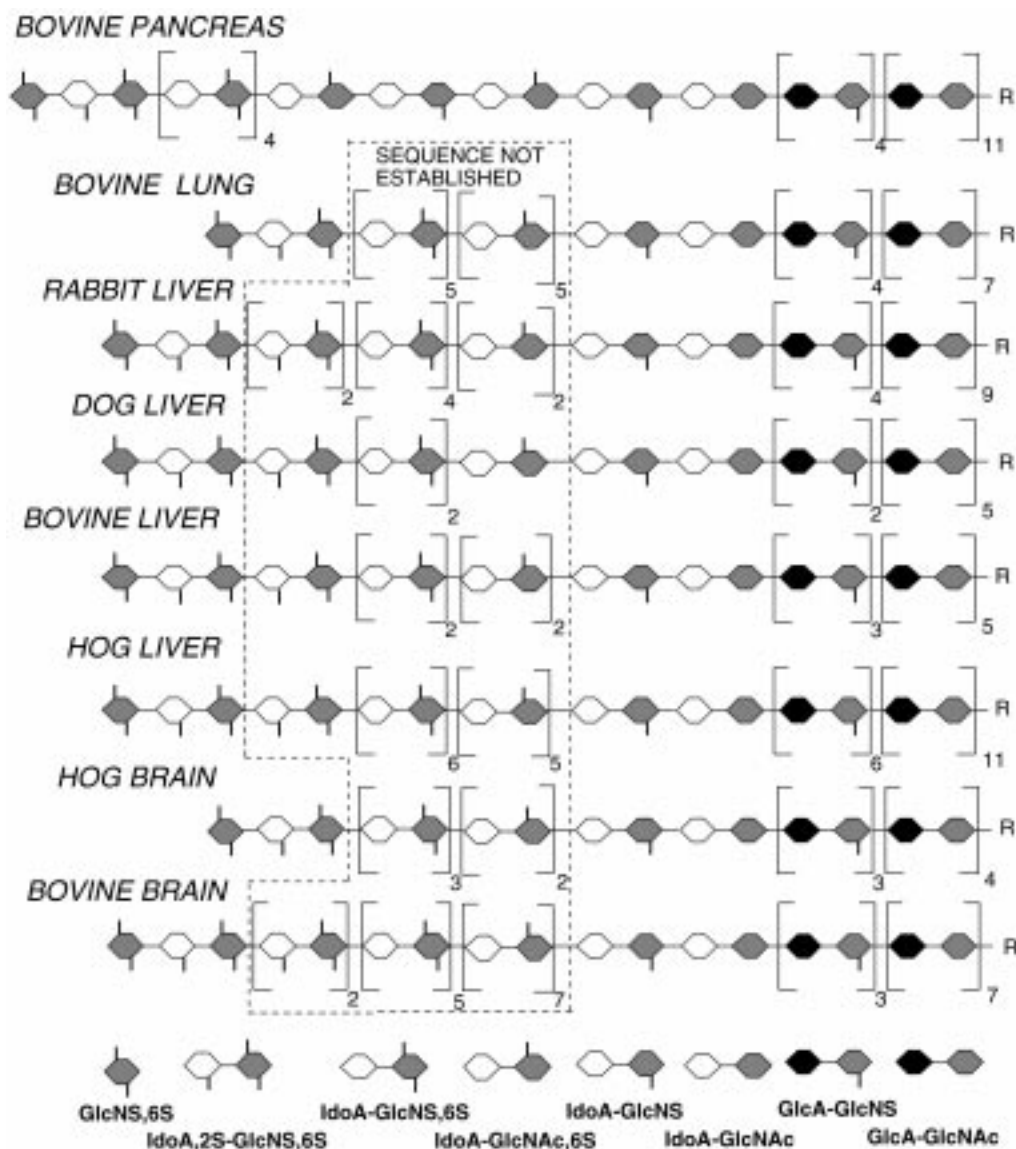
was concluded from these studies that all the mammalian heparan sulfates contain common structural features such as an N-acetylated and an N-sulfated domain consisting of glucuronic acid-containing disaccharides and a more sulfated region consisting of iduronic acid-containing disaccharides. A peculiar tetrasaccharide, namely GlcNAc-(α 1-4)-IdoUA-(α 1-4)-GlcNS-(α 1-4)-IdoUA, positioned between the two regions, was identified in all the heparan sulfates analyzed. It was also shown that the non-reducing ends

of the heparan sulfates contain the monosaccharides glucosamine N-sulfate or glucosamine 2,6 disulfate (13,14). Figure 1 summarizes these findings. Partial sequences of other heparan sulfates of different origins such as liver have also been recently described (12).

Heparan sulfate in invertebrates

By degradation with heparitinases and heparinase from *Flavobacterium heparinum*

Figure 1 - Proposed structures of heparan sulfates from different mammalian tissues. R, Protein linkage region. IdoA, α -L-Iduronic acid; GlcA, β -D-glucuronic acid; GlcN, α -D-glucosamine; GlcNAc, α -D-N-acetylglucosamine; S, sulfate.



as well as electrophoretic migration in different buffer systems of the sulfated polysaccharides extracted from 22 species of the main classes of invertebrates, it was suggested that heparan sulfate-like and/or heparin-like compounds were present in all tissue-organized species analyzed (15). In a more recent survey of more than 50 invertebrates from different classes using the same methodology, it was shown that heparan sulfate was a ubiquitous compound as depicted in Figure 2 (Medeiros GF and Nader HB, unpublished data). Other authors have also reported the presence of sulfated glycosaminoglycan-like compounds in some species of invertebrates (16-24).

These studies were further extended to different tissues of the mollusc *Pomacea* sp (25). Figure 3 shows that all tissues analyzed contain heparan sulfate-like, chondroitin sulfate and other unidentified polymers. A subsequent study using invertebrate species from habitats with different degrees of salinity, including a vicarious one (26), has shown that the concentration of heparan sulfate was directly proportional to the salt concentra-

tion of the habitat (Figure 4).

Conclusive evidence that these heparan sulfates from invertebrates were undistinguishable from the ones of mammalian origin came from the isolation and purification of these compounds from three species of molluscs, namely, *Pomacea* sp, *Tagelus gibbus* and *Anomalocardia brasiliiana* (27). Chemical analyses and enzymatic degradation have shown the presence of the same disaccharide units present in mammalian heparans. This was further confirmed by ¹³C nuclear magnetic resonance spectrometry where, as shown in Figure 5, the heparan sulfate from the mollusc *Anomantidae* sp was undistinguishable from bovine pancreas heparan sulfate (28). As shown in Figure 6, the disaccharide units of this last heparan sulfate were also recently sequenced (29).

A heparan sulfate with some interesting characteristics was also isolated from the brine shrimp *Artemia franciscana*. This heparan sulfate, although containing the same disaccharide units found in the other vertebrate and invertebrate heparans, has a different electrophoretic migration. COSY and

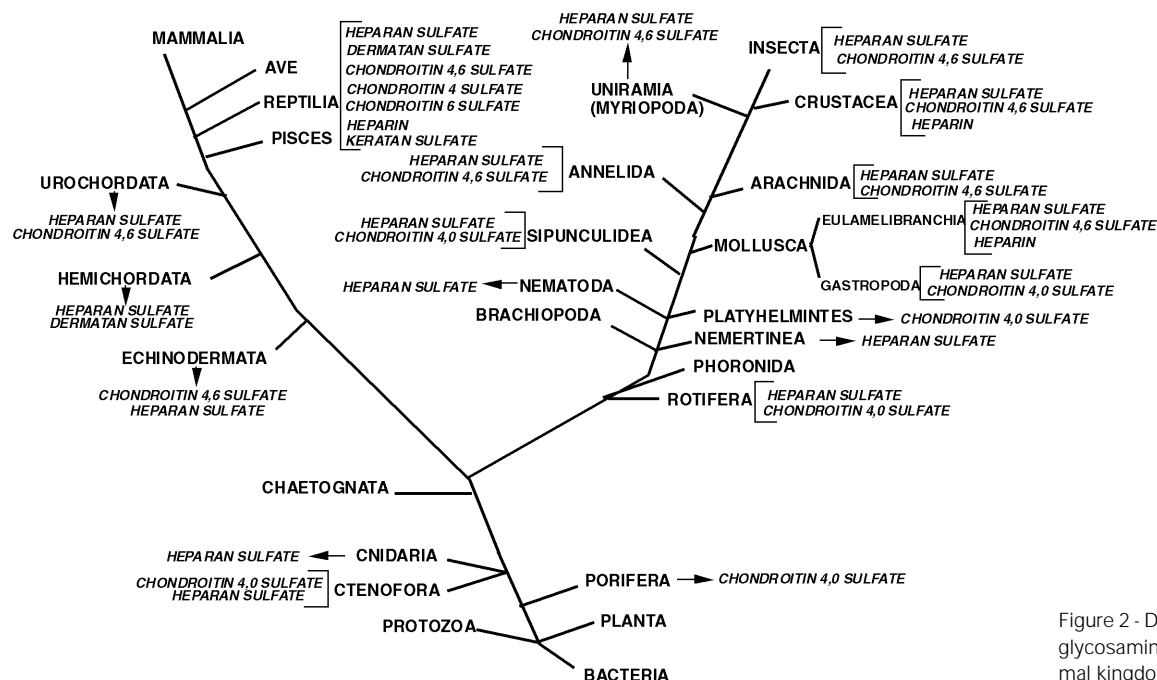


Figure 2 - Distribution of sulfated glycosaminoglycans in the animal kingdom.

Figure 3 - Distribution of sulfated glycosaminoglycans in different tissues of Pomacea sp. CS, Chondroitin sulfate; HS, heparan sulfate; ant. and post. dig. gland, anterior and posterior digestive gland, respectively; other, unknown sulfated polysaccharides.

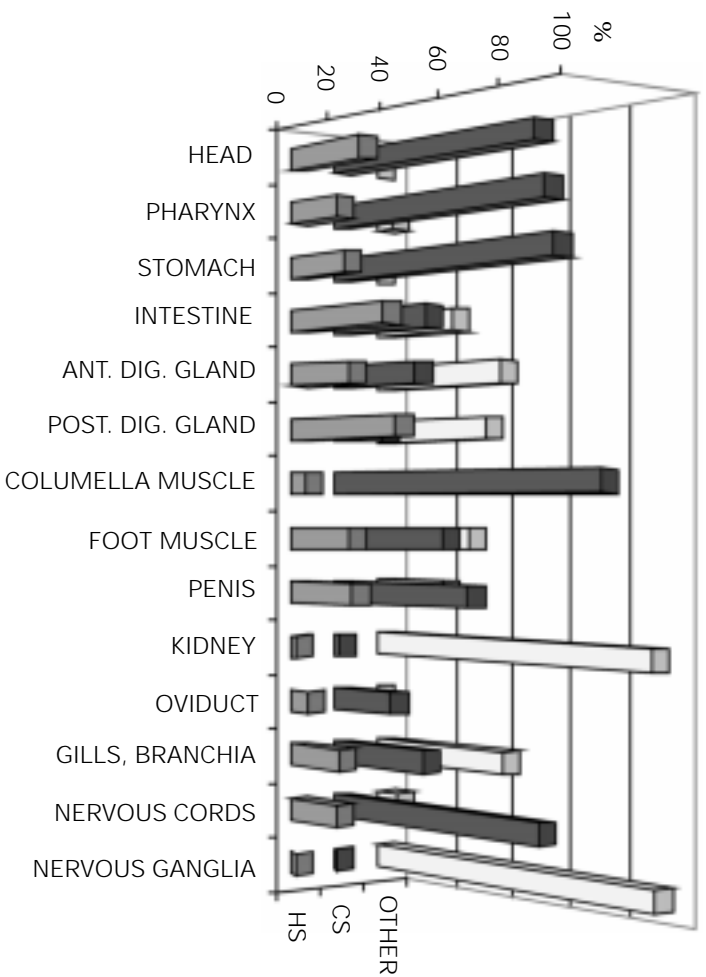
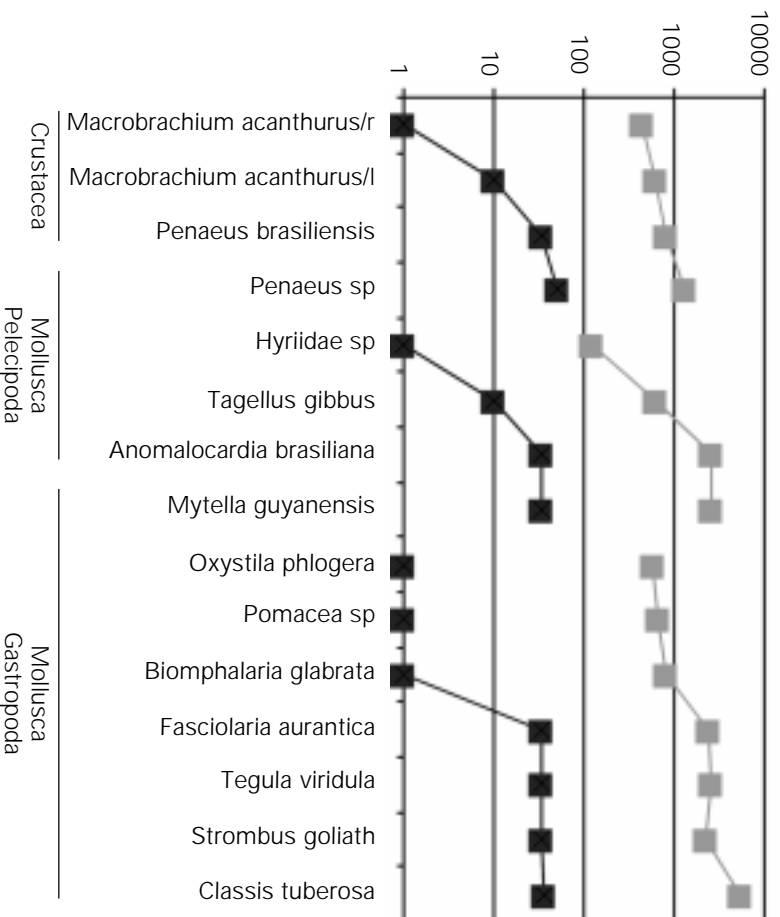


Figure 4 - Sulfated glycosaminoglycans in invertebrates from different habitats. Gags, Sulfated glycosaminoglycans; r, river; l, lagoon.



TOCSY nuclear magnetic resonance (NMR) spectroscopy has shown that this heparan was extremely rich in non-sulfated iduronic acid residues. It was also shown that the content of non-sulfated N-acetylated disaccharide was low and accounted for 3-5% of the total disaccharides of the molecule when compared to those of mammalian origin which accounted for 20-60% of the molecules (30). Another heparan sulfate isolated from the lobster *Homarus americanus* also showed different characteristics from those of heparan sulfates isolated from mammals, such as enrichment in disaccharides containing glucuronic acid residues (24).

Heparin in mammalian and other vertebrate tissues

Unlike heparan sulfate, heparin is present only in some tissues of vertebrates, as shown in Figure 7. For instance, heparin is absent or occurs in small amounts in brain, muscle and kidney of most species (for a review, see Ref. 31). Also, a wide variation in the concentration of heparin was observed when the same tissue of different species was compared. In general, heparin is usually present in tissues that are in direct contact with the environment such as lung, skin and intestine. Of particular significance was the observation that rabbit tissues do not contain heparin. Non-mammalian vertebrate tissues con-

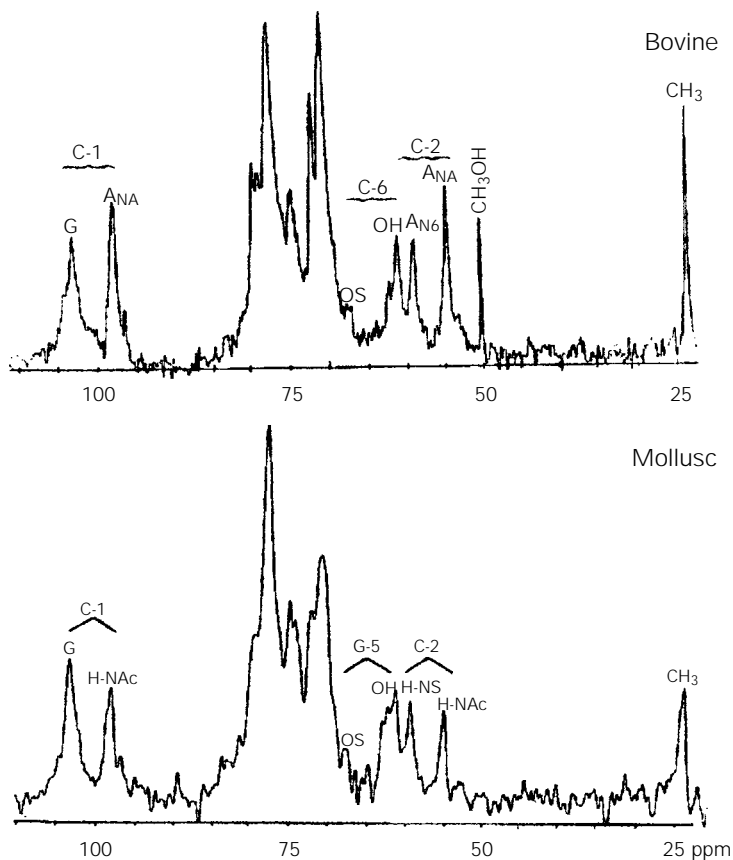


Figure 5 - ^{13}C -NMR of mammalian and mollusc heparan sulfates. G, Glucuronic acid; H-NAC, ANAc, N-acetylated glucosamine.

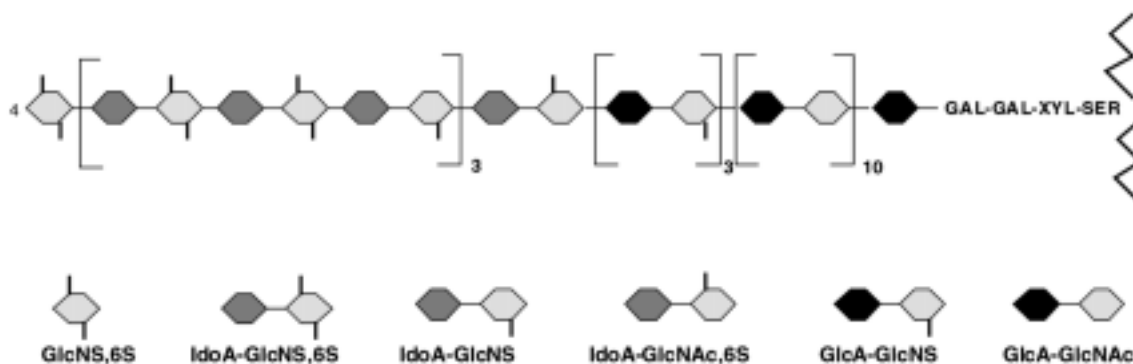
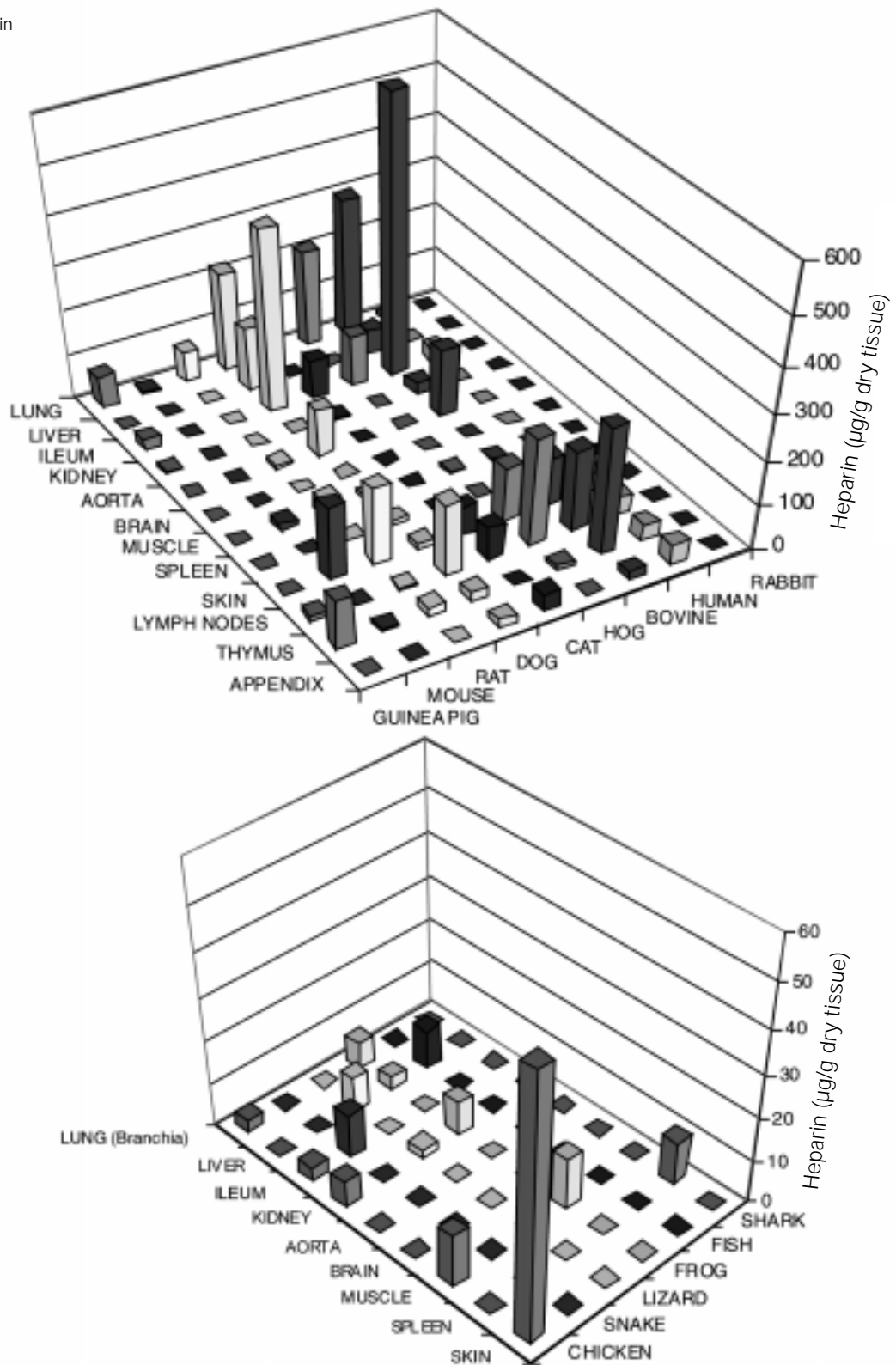


Figure 6 - Proposed structure of heparan sulfate from the mollusc *Anomantidae* sp. IdoA, α -L-Iduronic acid; GlcA, β -D-glucuronic acid; GlcN, α -D-glucosamine; GlcNAc, α -D-N-acetylglucosamine; S, sulfate.

Figure 7 - Distribution of heparin in vertebrate tissues.



tain smaller amounts of heparin when compared to those of mammalian origin. An exception to this rule was the finding that chicken skin contains relatively large amounts of heparin (32).

Heparin in invertebrates

Suggestions for the presence of heparin in invertebrates came from the work of Burson et al. (33). These authors have isolated from the molluscs *Spisula solidissima* and *Cyprinia islandica* a polysaccharide denoted mactin, composed of glucuronic acid, glucosamine and sulfate, which possesses anticoagulant activity. Similar studies have shown that *Anodonta* sp (34), *Anomalocardia brasiliiana* and *Mesodesma donacium* (15) contain similar polysaccharides.

Unlike heparan sulfate, heparin was only found in some species of invertebrates, e.g., molluscs and crustaceans (Figure 2). The distribution of heparin in different tissues of the mollusc *Anomalocardia brasiliiana* (35) has revealed that the highest concentration of heparin was found in tissues that are in direct contact with the environment (Figure 8), similar to the distribution found for heparin in vertebrates. Histological examination of the tissues has shown that heparin is present in special cells forming granules, suggesting that the mollusc also contains mast cells (35).

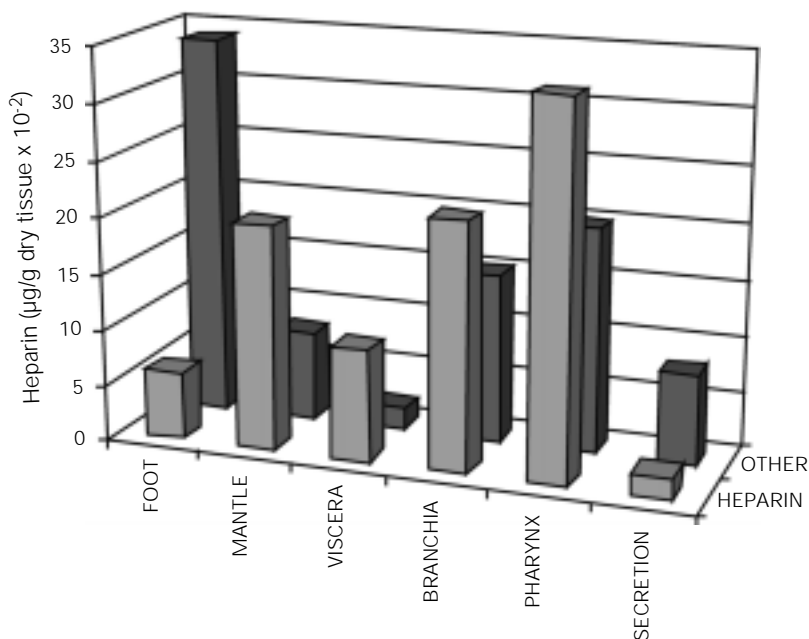


Figure 8 - Distribution of heparin in different tissues of the mollusc *Anomalocardia brasiliiana*. Other, heparan sulfate, chondroitin sulfate and unknown sulfated polysaccharides.

Using heparinase and heparitinase II from *Flavobacterium heparinum*, it was possible to draw a general picture of the structure of heparin, as shown in Figure 9. Heparin seems to be composed of two different regions, one susceptible to heparinase whose action upon the compound produces a trisulfated disaccharide and sulfated tetrasaccharides, and another less sulfated region, which is susceptible to the action of heparitinase II. This last region seems to contain disaccharides with glucuronic acid residues, as judged by

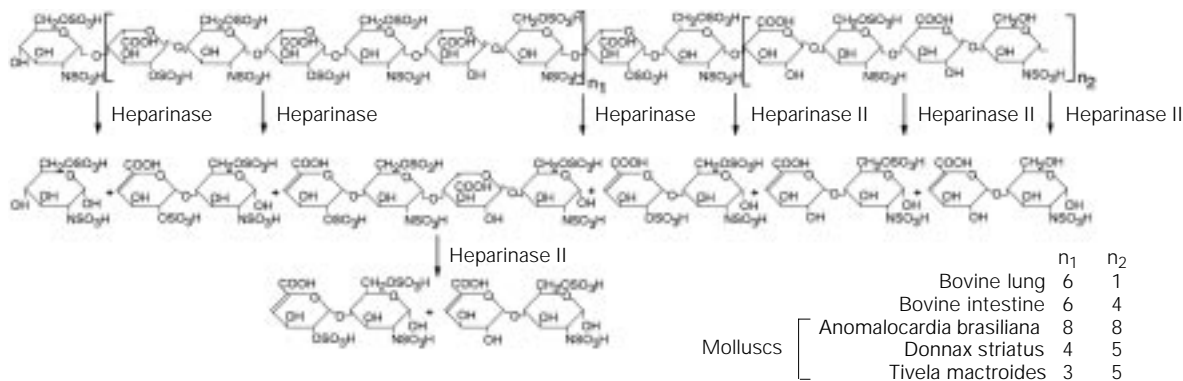
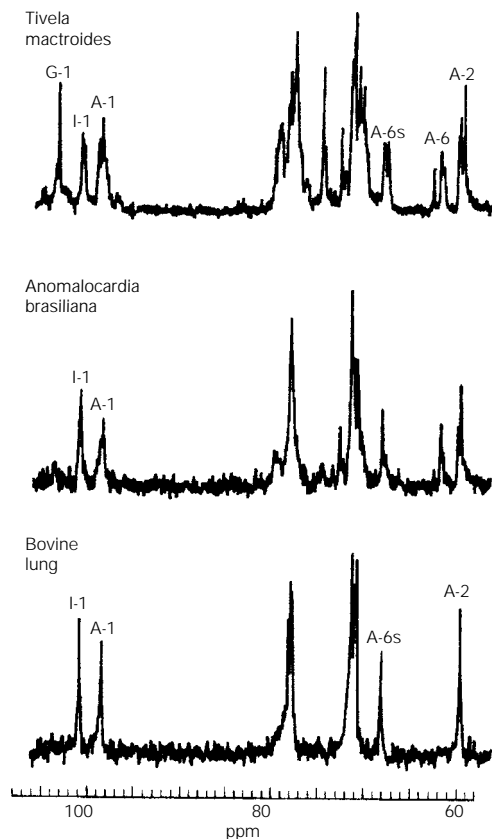


Figure 9 - Proposed structure of heparin in mammals and invertebrates.

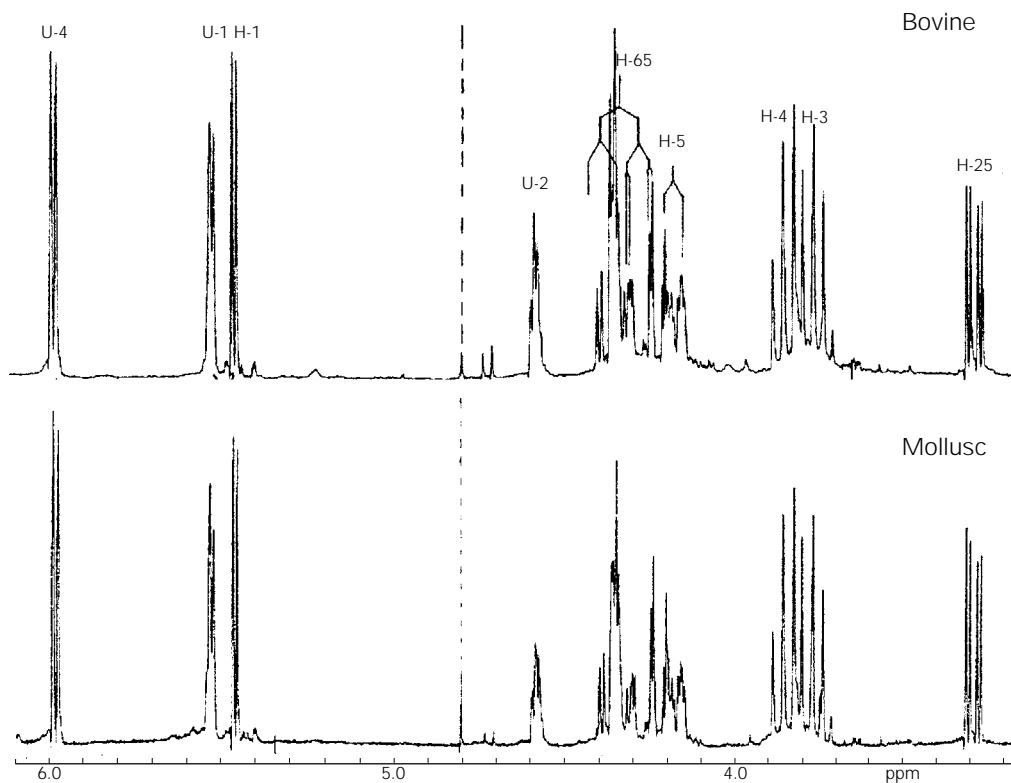
Figure 10 - ^{13}C -NMR of mammalian and mollusc heparins.



^{13}C -NMR spectroscopy (see below). The length and abundance of these two regions vary according to the origin of heparin. Thus, bovine lung heparin is extremely rich in the region susceptible to heparinase (36), whereas bovine intestinal heparin and mollusc heparins contain significant amounts of the region susceptible to heparitinase II (37-41). The estimated abundance of the two regions is shown in Figure 9. Besides the disaccharides depicted in the figure, other disaccharide units which occur in small amounts in the molecule have been identified such as disaccharides containing 3-O sulfated residues in the glucosamine moiety (42) and N-acetylated glucosamine (43).

Besides being susceptible to specific enzymes the heparin from *Anomalocardia brasiliiana* possesses all the other properties characteristic of heparin such as anticoagulant and other pharmacological activities (38,40) and chemical degradation (38). NMR spectroscopy has shown that the mollusc

Figure 11 - ^1H -NMR of IdoA,2S-GlcNS,6S formed from mammalian and mollusc heparin by action of heparinase. Upper panel, Mammalian heparin; lower panel, mollusc heparin.



heparin was undistinguishable from those of mammalian origin (41). Figure 10 shows the [^{13}C]-NMR spectroscopy of heparins obtained from two species of molluscs compared to a mammalian heparin. Note that the main chemical shifts are present in the mammalian and mollusc heparins. The one derived from *Tivela mactroides* also contains signals attributed to the nonsulfated uronic acid residues. The [^1H]-NMR spectroscopy of the main repeating disaccharide unit obtained from mollusc and mammalian heparin by heparinase shown in Figure 11 indicates that they contain the same signals with identical chemical shifts, confirming the identity of these heparins.

Conclusions

These studies indicate that heparan sulfate is a ubiquitous compound of animal

cells, whose structure has been maintained throughout evolution, showing an enormous variability regarding the relative amounts of its disaccharide units. Heparin, on the other hand, is present only in a few tissues and species of the animal kingdom in the form of granules inside organelles in the cytoplasm of special cells. Thus, the distribution as well as the main structural features of the molecule, including its main disaccharide unit, have been maintained throughout evolution.

These and other studies (9,44,45) have led to the proposal that heparan sulfate may be involved in the cell-cell recognition phenomena and control of cell growth, whereas heparin may be involved in defense mechanisms against bacteria and other foreign materials (31). All indications obtained so far suggest that these molecules perform the same functions in vertebrates and invertebrates.

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