# BIOSYNTHESIS OF CONNECTIVE TISSUE POLYSACCHARIDES

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## ABSTRACT

The biosynthesis of chondroitin sulphate exemplifies the formation of connective tissue polysaccharides. Six different glycosyltransferases participate: a chain-initiating xylosyltransferase, two galactosyltransferases, two glucuronosyltransferases, and an *N*-acetylgalactosaminyltransferase. Some of their properties are discussed, particularly their substrate specificities determined with small oligosaccharide acceptors of well-defined structure.

Purification of these enzymes is in its early stages and has been hampered by the membrane-bound nature of most of them. Xylosyltransferase, which is the most soluble, has been purified over 1000-fold by chromatography on an affinity matrix of Sepharose-bound Smith-degraded cartilage proteoglycan.

Structural specificity in the synthesis of biological macromolecules is achieved in a variety of ways. Whereas the structure of a simple protein is encoded in the nucleotide sequence of the corresponding messenger RNA, from which the information is translated via the polyribosomes to a sequence of amino acids, the formation of conjugates of a protein and a non-protein prosthetic group is not under direct template control by the genes. The synthesis of the carbohydrate prosthetic groups of glycoproteins and proteoglycans is a typical example of such a non-template control system. The control exerted in the synthesis of these groups resides largely in the specificities of the many glycosyltransferases which catalyze the sequential transfer of the monosaccharide components, one by one, to the growing carbohydrate chain. The genetic control that is nevertheless possible within the framework of such a system is beautifully exemplified by the biosynthesis of blood group substances: as is now well established, the blood group specificities are conveyed by the addition of specific monosaccharide units at the nonreducing ends of the carbohydrate chains, in reactions which are catalyzed by specific glycosyltransferases. Other examples of genetic control over glycosyltransferases are also known, e.g., the genetic polymorphism of $\alpha_1$-acid glycoprotein is believed to result from the existence of three different sialyltransferases which catalyze transfer to positions 3, 4, or 6 of the galactose residues occupying penultimate positions in the finished molecules[1].

A generalization can be made in regard to the specificities of the glycosyltransferases which is most concisely expressed as the 'one enzyme–one linkage' hypothesis[2]. According to this hypothesis, the specificities of the glycosyltransferases are related to three different structural aspects of the transfer reactions: (1) a transferase is distinguished from all other transferases

by its specificity for the sugar transferred; for practical purposes, this also means specificity for a particular nucleotide sugar; (ii) the enzyme is specific for a particular glycose acceptor, and (iii) a transferase synthesizes only linkages of one particular anomeric configuration and linkage position. As the properties of the various glycosyltransferases have become known in more detail, it is obvious that these generalizations are not absolutely true in all cases. Although the specificity of a particular glycosyltransferase for the sugar portion of a nucleotide sugar appears to be absolute, the nucleotide portion may sometimes be varied without complete loss of donor activity. The acceptor specificity is usually determined by the sugar at the nonreducing terminus of the growing carbohydrate chain; in some instances, the structure of the penultimate sugar and its linkage to the terminal sugar are also important. Some transferases show a preference for large acceptors, whereas small acceptors with the same terminus may be much less active. The linkage synthesized is characteristic of the enzyme, and there is as yet no example of a single glycosyltransferase which can synthesize more than one type of linkage.

A special group of glycosyltransferases are those which catalyze transfer to polypeptide acceptors rather than to sugars. Only a few of these carbohydrate chain-initiating glycosyltransferases have been studied in any detail, but it appears that large molecular weight acceptors are, as a rule, better acceptors than smaller peptides, suggesting that not only the primary sequence around the point of attachment of the carbohydrate group but also the conformation of the protein acceptor is of importance in the transfer mechanism. This and other aspects of the properties of the glycosyltransferases have been discussed in more detail elsewhere[3-5].

From the point of view of biosynthesis, the connective tissue proteoglycans may be regarded only as a special category of glycoproteins, since the pathways by which they are formed are basically the same as those of glycoproteins. Structurally, the connective tissue proteoglycans are also akin to the glycoproteins and are distinguished from these primarily by the presence of relatively large polysaccharide chains with negatively charged repeating disaccharide units. These repeating units are usually composed of a uronic acid and a hexosamine, and *Table 1* gives the composition of the polysaccharides which have been recognized so far. It is seen that two uronic acids, D-glucuronic acid and L-iduronic acid, and two hexosamines, D-glucosamine and D-galactosamine, are the constituent sugars of these polysaccharides. In addition, several other monosaccharides may be present, including sialic acid, mannose, fucose, galactose, and xylose. With the exception of galactose, which is one of the components of the repeating *N*-acetyllactosamine units in keratan sulphate, these latter sugars are not part of the characteristic repeating disaccharide units and occur either as side branches or as constituents of the specific carbohydrate–protein linkage regions.

So far, three types of carbohydrate–protein linkages have been recognized in the connective tissue proteoglycans: (1) an *O*-glycosidic linkage between xylose and serine hydroxyl groups; (2) an *O*-glycosidic linkage between *N*-acetylgalactosamine and the hydroxyl groups of serine or threonine; and (3) an *N*-glycosylamine linkage between *N*-acetylglucosamine and the amide group of asparagine. The first linkage type is found in the chondroitin

*Table 1*. Composition of connective tissue polysaccharides

| Polysaccharide | Amino sugar[1] | Uronic acid | Linkage position | | Sulphate |
|---|---|---|---|---|---|
| | | | Uronidic | Hexosaminidic | |
| Hyaluronic acid | Glucosamine | Glucuronic acid | β-(1 → 3) | β-(1 → 4) | — |
| Chondroitin 4-sulphate | Galactosamine | Glucuronic acid | β-(1 → 3) | β-(1 → 4) | *O*-Sulphate |
| Chondroitin 6-sulphate | Galactosamine | Glucuronic acid | β-(1 → 3) | β-(1 → 4) | *O*-Sulphate |
| Dermatan sulphate | Galactosamine | Iduronic acid | α-(1 → 3) | β-(1 → 4) | *O*-sulphate |
| | | Glucuronic acid | β-(1 → 3) | | |
| Heparin[1,2] | Glucosamine | Iduronic acid | α-(1 → 4) | α-(1 → 4) | *N*-Sulphate |
| | | Glucuronic acid | β-(1 → 4) | | *O*-Sulphate |
| Heparan sulphate | Glucosamine | Iduronic acid | α-(1 → 4) | α-(1 → 4) | *N*-Sulphate |
| | | Glucuronic acid | β-(1 → 4) | | *O*-Sulphate |
| Keratan sulphate | Glucosamine | (Galactose) | (β-(1 → 4)) | β-(1 → 3) | *O*-Sulphate |

[1] The hexosamines are generally *N*-acetylated; however, in heparin most amino groups are *N*-sulphated, with the exception of those in the carbohydrate–peptide linkage region, which are *N*-acetylated; heparan sulphate contains varying proportions of *N*-acetylated and *N*-sulphated amino groups.
[2] Evidence for the presence of some (1 → 6)-uronidic linkages has also been presented.

sulphates, dermatan sulphate, haparin and heparan sulphite; the second is involved in the linkage of skeletal keratan sulphate (keratan sulphate II) to protein, and the third is present in corneal keratan sulphate (keratan sulphate I).

The detailed structures of the carbohydrate–protein linkage regions of the two types of keratan sulphate have not yet been established. However, the xylose-linked polysaccharides all have a specific linkage region which is identical from one polysaccharide to another and has the structure shown in *Figure 1*.

In accordance with the 'one enzyme–one linkage' hypothesis, it may be postulated that the biosynthesis of chondroitin sulphate is catalyzed by six different glycosyltransferases, i.e. (i) a chain-initiating xylosyltransferase which transfers xylose to the protein core of the proteoglycan; (ii) a galactosyltransferase which catalyzes transfer to the xylosyl–protein formed in the chain-initiating reaction; (iii) a second galactosyltransferase which utilizes the first galactose unit as acceptor; (iv) a glucuronosyltransferase which completes the formation of the specific carbohydrate protein linkage region; (v) an *N*-acetylgalactosaminyltransferase and (vi) a second glucuronosyltransferase which catalyze the formation of the bulk of the polysaccharide chain composed of repeating disaccharide units. These enzymes have all been found in cartilage and other mucopolysaccharide-producing tissues[4, 5], and some progress has been made in the separation of the various enzymes, although no single one has yet been purified to homogeneity.

Ideally, the various glycosyltransferases should be studied with the substrates that are present *in vivo*. However, this meets with obvious difficulties, e.g., it is not yet known whether the substrate for xylosyltransferase is the growing peptide chain or the completed core protein. Similarly, the structures of the substrates for the other glycosyl transfer steps are not known, and although it is safe to assume that the endogenous acceptors consist of protein molecules which have been glycosylated to a varying degree, the fractionation and recognition of individual molecular species would be extremely difficult with our current limited methodology. As will be discussed in detail below, exogenous substrates of well known structure have therefore been used to investigate the properties of the various chondroitin sulphate glycosyltransferases. Nevertheless, it is of some interest to dwell for a moment on one of the questions regarding the mechanism of assembly of the macromolecular proteoglycan molecules, i.e. the time sequence of the addition of the many polysaccharide chains. We may formulate the problem as follows: 'Are the polysaccharide chains attached to the core protein in a sequential fashion so that one chain is completed before the growth of the next one starts? Or, are all chains initiated simultaneously and grow in a parallel fashion? Or, does an intermediate situation pertain, so that the growth of one chain begins before the adjacent one has been completed?' As another facet of the same problem we may ask whether chain addition occurs from either the *N*-terminal or *C*-terminal end of the polypeptide acceptor, or whether the process occurs randomly anywhere along the peptide chain. These are questions which are still to be answered, but it may be mentioned that preliminary work in our laboratory favours the

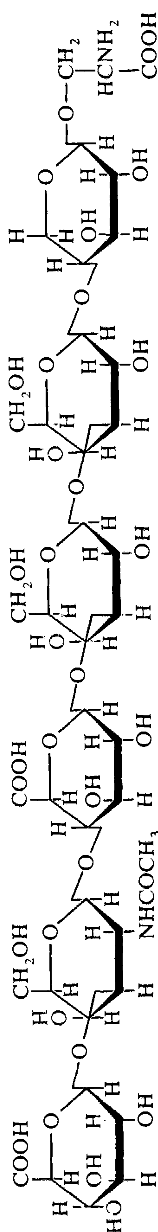

*Figure 1*. Structure of chondroitin sulphate. Only one repeating disaccharide unit is shown. The sulphate group may be located on C-4 or C-6 of the *N*-acetylgalactosamine residue.

third alternative above, suggesting that initiation of a chain occurs before completion of the nearest neighbour (J. R. Baker and L. Rodén, unpublished work).

As a consequence of the difficulties in fractionation and characterization of endogenous acceptors, a variety of simpler exogenous substrates have been used to study chondroitin sulphate and heparin biosynthesis. Mostly, these compounds are oligosaccharides with the appropriate acceptor monosaccharide at the nonreducing terminus, but the simple monosaccharide itself is an adequate substrate for one of the enzymes, i.e. the first galactosyltransferase, which catalyzes transfer to D-xylose. It should be noted, however, that the native, partially glycosylated protein molecules are probably better acceptors than are small oligosaccharides, as is suggested by the relatively high $K_m$ values (about 10 mM) for the artificial exogenous oligosaccharide substrates. The exogenous substrates which have been most commonly used for the various glycosyltransferases participating in chondroitin sulphate synthesis, are listed in *Table 2*. In the following, a brief survey will be given of the substrate specificities of the six chondroitin sulphate glycosyltransferases, as indicated by studies using these and related small oligosaccharide substrates.

*Table 2.* Chondroitin sulphate-synthesizing glycosyltransferases and some of their exogenous substrates

| Enzyme | Exogenous acceptors |
|---|---|
| Xylosyltransferase | Smith-degraded cartilage proteoglycan, L-serylglycylglycine |
| Galactosyltransferase I | Xylose<br>O-β-D-Xylosyl-L-serine |
| Galactosyltransferase II | 4-O-β-D-Galactosyl-D-xylose<br>4-O-β-D-Galactosyl-O-β-D-xylosyl-L-serine |
| Glucuronosyltransferase I | 3-O-β-D-Galactosyl-D-galactose<br>O-β-D-Galactosyl-(1 → 3)-O-β-D-galactosyl-(1 → 4)-D-xylose |
| N-Acetylgalactosaminyltransferase | GlcUA-GalNAc-GlcUA-GalNAc-GlcUA-GalNAc (chondroitin hexasaccharide)<br>GlcUA-(GalNAc-4S)-GlcUA-(GalNAc-4S)-GlcUA-(GalNAc-4S) (chondroitin 4-sulphate hexasaccharide) |
| Glucuronosyltransferase II | GalNAc-GlcUA-GalNAc-GlcUA-GalNAc (chondroitin pentasaccharide)<br>(GalNAc-6S)-GlcUA-(GalNAc-6S)-GlcUA-(GalNAc-6S) (chondroitin 6-sulphate pentasaccharide) |

## 1. Xylosyltransferase

Xylosyl transfer was first demonstrated in cell-free preparations from hen's oviduct[6], mouse mastocytoma[7], and embryonic chick cartilage[8], which catalyzed transfer of xylose from UDP-xylose to endogenous acceptors present in the crude enzyme preparations. In order to gain more information concerning the substrate specificity of the xylosyltransferase reaction, Baker *et al.*[9] tested a number of potential xylose acceptors ranging from serine and simple serine derivatives to the entire protein core of the chondroitin sulphate proteoglycan. Although one small acceptor was found, i.e. the tripeptide, serylglycylglycine, the core protein proved to be a far better

acceptor, with a $K_m$ value of 0.064 mM, expressed in terms of the concentration of serine residues. It may be noted that the native cartilage proteoglycan contains keratan sulphate as well as chondroitin sulphate and that the Smith degradation used for the removal of the chondroitin sulphate chains leaves the keratan sulphate component essentially intact; the artificial acceptor should therefore not be considered identical with the endogenous acceptor which is in all likelihood exclusively of polypeptide nature.

The high acceptor activity of the macromolecular substrate is consistent with observations on other reactions involving glycosyl transfer to polypeptide acceptors. In the formation of salivary gland mucin, the protein core obtained after removal of the sialic acid–*N*-acetylgalactosamine disaccharides was an excellent acceptor for *N*-acetylgalactosaminyl transfer, whereas small peptides from a Pronase digest were completely inactive[10]. Similarly, galactosyl transfer to hydroxylysine residues of collagen proceeds readily with a macromolecular protein substrate, whereas tryptic collagen peptides are only about 25 per cent as active[11].

## 2. Galactosyltransferase I

The substrate specificity of the galactosyltransferases participating in chondroitin sulphate synthesis is illustrated in *Table 3*. It will be noted that the free monosaccharide, D-xylose, is itself a reasonably good substrate, although the enzyme *in vivo* presumably utilizes a xylosyl–protein as acceptor.

*Table 3*. Substrate specificity of galactosyltransferases[4]

| Acceptor | Activity (relative to D-xylose) (%) |
|---|---|
| D-Xylose | 100 |
| L-Xylose | 4 |
| Methyl β-D-xylopyranoside | 99 |
| *O*-β-D-Xylosyl-L-serine | 206 |
| 4-*O*-β-D-Galactosyl-D-xylose | 50 |
| 3-*O*-β-D-Galactosyl-D-xylose | 70* |
| 4-*O*-β-D-Galactosyl-*O*-β-D-xylosyl-L-serine | 40 |
| Lactose | 9 |
| Raffinose | 5 |
| D-Arabinose | 15 |
| D-Glucose | 9 |

Inactive substrates: galactose, 3-*O*-β-D-galactosyl-D-galactose, 2-*O*-β-D-galactosyl-D-lyxose, methyl β-D-xylofuranoside, D-lyxose, D-ribose, D-mannose, maltose, sucrose, L-fucose.
* Characterization of the reaction product with this substrate showed that galactosyl transfer had occurred to C-4 of the xylose residue and not to the nonreducing terminal galactose.

The ability of galactosyltransferase I to recognize and utilize the monosaccharide acceptor itself obviously represents an absolutely minimal requirement with regard to the size of the acceptor structure. If xylose were a common constituent of glycoproteins and could be bound to other sugars than galactose, such a situation could conceivably result in frequent mistakes during the assembly of carbohydrate prosthetic groups. However, this is not

the case, and any xylosyl residue that the galactosyltransferase encounters *in vivo* is almost certainly part of a growing proteoglycan molecule.

### 3. Galactosyltransferase II

The substrate of the second galactosyltransferase carries a galactosyl residue at the nonreducing terminus, and in view of the multitude of glycoprotein structures containing galactose, it is not surprising that this enzyme has a higher degree of specificity with regard to its acceptor structure (see *Table 3*). Transfer does not occur to free D-galactose nor to most oligosaccharides with nonreducing terminal galactose residues. Apparently, the enzyme needs to recognize both the terminal monosaccharide acceptor and also the penultimate sugar, i.e. D-xylose. Slight acceptor activity is observed with lactose as substrate, but it should be remembered that this disaccharide differs from the more natural substrate, 4-*O*-β-D-galactosyl-D-xylose, only by the presence of a primary alcohol group instead of a hydrogen on carbon 5 of the reducing terminal monosaccharide.

### 4. Glucuronosyltransferase I

The glucuronosyltransferase which completes the formation of the specific carbohydrate–protein linkage region likewise requires at least a disaccharide as acceptor, although minimal transfer to free D-galactose also occurs. In addition to the 'natural' disaccharide, 3-*O*-β-D-galactosyl-D-galactose, several other galactose-containing disaccharides may serve as substrates for this reaction, including 4- and 6-*O*-β-D-galactosyl-D-galactose and 4-*O*-β-D-galactosyl-D-xylose (*Table 4*.). Although the activity of the latter is only 9 per cent of that of 3-*O*-β-D-galactosyl-D-galactose, mistakes could conceivably occur in the formation of the linkage region, but molecules with only one galactose residue have never been observed. It is therefore likely that other mechanisms exist which contribute to the orderly addition of the various monosaccharide units to the growing chain. Indeed, it has been suggested by Horwitz and Dorfman[12] that the glycosyltransferases are arranged on the membranes of the endoplasmic reticulum in a sequential fashion so that, e.g., the growing chain will not encounter the first glucuronosyltransferase

*Table 4*. Substrate specificity of glucuronosyltransferase[4]

| Acceptor | Activity (relative to 3-*O*-β-D-galactosyl-D-galactose) (%) |
|---|---|
| 3-*O*-β-D-Galactosyl-D-galactose | 100 |
| 4-*O*-β-D-Galactosyl-D-galactose | 126 |
| 6-*O*-β-D-Galactosyl-D-galactose | 27 |
| 3-*O*-β-D-Galactosyl-4-*O*-β-D-galactosyl-*O*-β-D-xylosyl-L-serine | 167 |
| Lactose | 27 |
| 4-*O*-β-D-Galactosyl-D-xylose | 9 |
| *N*-Acetyl-D-galactosamine | 7 |
| D-Galactose | 2 |

Inactive substrates: raffinose, D-glucose, *N*-acetyl-D-glucosamine, D-xylose, L-fucose.

*Table 5a.* Acceptor and donor specificities of chondroitin sulphate 'Polymerase'[4]

| Donor UPD-nucleotide sugar | Oligosaccharide acceptors with nonreducing terminal glucuronic acid* | | | | | | |
|---|---|---|---|---|---|---|---|
| | Chondroitin | | Chondroitin 4-sulphate | | Chondroitin 6-sulphate | Hyaluronic acid | Desulphated dermatan sulphate |
| | Hexasaccharide | Tetrasaccharide | Hexasaccharide | Tetrasaccharide | Hexasaccharide | Hexasaccharide | |
| UPD-*N*-Ac-galactos-amine | + | + | + | + | + | + | − |
| UDP-Glucuronic acid | − | − | − | − | − | − | |
| UDP-*N*-Ac-glucosamine | | | − | | − | − | |

* Even-numbered oligosaccharides from chondroitin, the chondroitin sulphates, and hyaluronic acid were prepared by digestion with testicular hyaluronidase, which yields homologous oligosaccharides with glucuronic acid at the nonreducing end. Odd-numbered oligosaccharides with *N*-acetylhexosamine at the nonreducing end were obtained from the even-numbered compounds by digestion with β-glucuronidase The desulphated dermatan sulphate tetrasaccharide was isolated from an acid hydrolyzate of the polysaccharide.

*Table 5b.* Acceptor and donor specificities of chondroitin sulphate 'Polymerase'[4]

| Donor UDP-nucleotide sugar | Oligosaccharide acceptors with nonreducing terminal *N*-acetylhexosamine* | | | |
|---|---|---|---|---|
| | Chondroitin Pentasaccharide | Chondroitin 4-sulphate Pentasaccharide | Chondroitin 6-sulphate Pentasaccharide | Hyaluronic acid Pentasaccharide |
| UDP-*N*-Acetyl-galactosamine | − | − | − | − |
| UDP-Glucuronic acid | + | − | + | − |

* See footnote to *Table 5a.*

until both galactose residues have already been added. Such an arrangement would add another dimension of specificity to the assembly of the polysaccharide chains and would prevent any irregularity in structure that could arise from a certain lack of specificity on the part of the individual glycosyltransferases.

### 5. *N*-Acetylgalactosaminyltransferase

The substrate required by this enzyme is an oligosaccharide with a nonreducing terminal glucuronic acid unit (*Table 5*). The identity of the penultimate group is not absolutely essential, since the reaction proceeds equally well with oligosaccharides from chondroitin sulphate and hyaluronic acid which have galactosamine and glucosamine in the penultimate positions, respectively. The size of the acceptor is of importance, however, and although a comprehensive survey of different acceptors has not yet been carried out, it is clear that the smallest members of the homologous oligosaccharide series are much poorer acceptors than, e.g., the hexa- and octa-saccharides.

### 6. Glucuronosyltransferase II

The second glucuronosyltransferase requires a nonsulphated or 6-sulphated *N*-acetylgalactosamine unit as acceptor (*Table 5*). No transfer occurs to a 4-sulphated residue, indicating that the addition of glucuronic acid to the growing chain of chondroitin 4-sulphate has to precede the sulphation of the acceptor *N*-acetylgalactosamine unit. Little or no activity is observed with free *N*-acetylgalactosamine and, as is the case with the *N*-acetylgalactosaminyltransferase, larger oligosaccharides containing several repeating disaccharide units are better acceptors than the smaller members of the homologous series. It is not yet known whether the nature of the penultimate group is of importance for the reaction, since appropriate substrates have not been available for testing; the substrate specificities established so far are summarized in *Table 5*.

## PURIFICATION OF GLYCOSYLTRANSFERASES

None of the glycosyltransferases involved in chondroitin sulphate biosynthesis has as yet been purified to homogeneity. However, some progress has been made recently in the fractionation of xylosyltransferase, which is the most soluble of the glycosyltransferases discussed here. The enzyme has been purified about 50-fold from a high speed supernatant of a homogenate of embryonic chick cartilage by ammonium sulphate fractionation and gel chromatography on Sephadex G-200[13]. Further purification has been achieved by affinity chromatography on Sepharose 4B to which Smith-degraded proteoglycan from bovine nasal cartilage has been coupled (N.B. Schwartz and L. Rodén, unpublished work). The enzyme is quantitatively adsorbed to this affinity matrix from a solution of low ionic strength and may be eluted either by a solution of the Smith-degraded proteoglycan or by merely increasing the ionic strength of the eluant. This procedure has resulted in over 1000-fold purification of the enzyme, but the purified material is not yet homogeneous, as indicated by analytical gel electrophoresis, and further purification is currently in progress.

The purification of other glycosyltransferases which are more firmly bound to membranes has not yet progressed beyond preliminary stages. The major difficulty encountered in the work on these enzymes—their particulate nature—has now been overcome in large part by the development of several procedures of solubilization which hold promise for further rapid developments in this area. Helting[14] showed that treatment of the galactosyltransferases involved in heparin synthesis in mouse mastocytoma by a combination of alkali and detergent (Tween 20) solubilized a considerable proportion of these enzymes. This method has been modified by substitution of Nonidet P-40 for Tween 20 and by an increase of the ionic strength of the solubilizing medium rather than the use of alkaline conditions (N.B. Schwartz and L. Rodén, unpublished work). These methods result in the solubilization of up to 90 per cent of the activities of some of the glycosyltransferases involved in chondroitin sulphate synthesis, and the solubilized enzymes are no longer sedimentable at 100000 × *g*. They appear to have been released in monomolecular form, since they emerge largely in retarded positions when subjected to gel chromatography on Sephadex G-200.

## BIOSYNTHESIS OF OTHER CONNECTIVE TISSUE POLYSACCHARIDES

In the preceding, the six separate glycosyltransferases catalyzing the synthesis of chondroitin sulphate have been described. Four of these enzymes are involved in the formation of the specific carbohydrate–protein linkage region which is also present in several polysaccharides other than chondroitin sulphate, i.e. dermatan sulphate, heparin, and heparan sulphate. It is not yet known whether the linkage regions of these polysaccharides are synthesized by the same enzymes that participate in chondroitin sulphate biosynthesis, and the answer to this question will obviously have to await the purification and comparison of the linkage region glycosyltransferases from several tissue sources.

If the general validity of the 'one enzyme–one linkage' hypothesis is accepted, the existence of certain additional glycosyltransferases may be postulated which are responsible for the formation of the repeating disaccharide units of the remainder of the polysaccharides listed in *Table 1*. It could be predicted from an inspection of this *Table* that the following glycosyltransferases ought to exist:

*hyaluronic acid transferases:* one *N*-acetylglucosaminyltransferase and one glucuronosyltransferase;

*dermatan sulphate transferases*: two *N*-acetylgalactosaminyltransferases catalyzing transfer to glucuronic acid and iduronic acid residues, respectively (the enzyme utilizing glucuronic acid as acceptor might be identical with the corresponding chondroitin sulphate enzyme); one glucuronosyltransferase and one iduronosyltransferase catalyzing transfer to *N*-acetylgalactosamine units (the former enzyme might be identical to glucuronosyltransferase II involved in chondroitin sulphate synthesis);

*heparin and heparan sulphate transferases*: two *N*-acetylglucosaminyltransferases catalyzing transfer to glucuronic acid and iduronic acid units,

respectively; one glucuronosyltransferase and one iduronosyltransferase catalyzing transfer to *N*-acetylglucosamine residues;

*keratan sulphate transferases:* one galactosyltransferase and one *N*-acetylglucosaminyltransferase (these may be identical to glycosyltransferases previously found in studies on glycoproteins of similar structure).

In addition to these glycosyltransferases, several others are presumably involved in keratan sulphate synthesis, since this polysaccharide contains small amounts of sugars which are not part of the characteristic repeating disaccharide structure, i.e. sialic acid, fucose, and mannose, as well as some galactose residues which are located in side chains.

The existence of some of the enzymes listed above has already been substantiated: Malmström and Fransson[15] have demonstrated that *N*-acetylgalactosaminyl transfer may occur to a decasaccharide from dermatan sulphate which contains iduronic acid at the nonreducing terminus. Helting and Lindahl[16] showed that an enzyme preparation from mouse mastocytoma catalyzes transfer of glucuronic acid to a heparin fragment of the following general structure: *N*-acetylglucosamine–uronic acid–*N*-acetylglucosamine–glucuronic acid–galactose–galactose–xylose–serine. The acceptor specificity of this reaction was indicated by the finding that transfer did not occur to a hyaluronic acid pentasaccharide containing a β-linked *N*-acetylglucosamine residue at the nonreducing terminus rather than the α-linked unit which was present in the heparin fragment above.

The existence of some other glycosyltransferases has been suggested by more indirect evidence: hyaluronic acid chains are synthesized by several tissue systems in the presence of both nucleotide sugar precursors, UDP–*N*-acetylglucosamine and UDP–glucuronic acid, but the transfer of a single monosaccharide unit to a well-defined acceptor oligosaccharide has not yet been shown (for a more detailed review, see Reference 4). In a hyaluronic acid-producing system from streptococci, however, glucuronic acid may be transferred to the nonreducing terminal end of an endogenous acceptor, although the same system is not capable of transfer to exogenous oligosaccharide acceptors[17]. It is somewhat disconcerting that the hyaluronic acid-synthesizing systems appear to behave in a manner different from those which catalyze chondroitin sulphate formation, and it must be emphasized that the two polysaccharides could conceivably be synthesized by fundamentally different mechanisms. It is still an open question whether hyaluronic acid might be formed via lipid intermediates of the type involved in the synthesis of certain other polysaccharides with a repeating unit structure, but present evidence speaks against this possibility (see Reference 4).

Until recently, the information available concerning the biosynthesis of connective tissue polysaccharides was entirely consistent with the postulate that each monosaccharide component is formed by transfer from the corresponding nucleotide sugar. However, a different type of pathway has now been indicated for the iduronic acid residues of heparin and dermatan sulphate. In a study of heparin biosynthesis in mouse mastocytoma, Lindahl *et al.*[18] found that the iduronic acid residues are formed by epimerization of glucuronic acid units after the latter have already been incorporated into the polymer. Epimerization occurs only when the polymer is simultaneously being sulphated, but the exact relationship between the two processes is not

yet clear. As an immediate consequence of the discovery of this new pathway, it is no longer necessary to postulate the existence of iduronosyltransferases, and the list of glycosyltransferases given above should be modified accordingly. In an attempt to devise a simple and uniform hypothesis for the synthesis of hybrid polysaccharides which contain both glucuronic acid and iduronic acid, it could also be suggested that epimerization occurs only in internal positions in the molecules, so that the terminal *N*-acetylhexosamine acceptor would always be a glucuronic acid residue; a single *N*-acetylhexosaminyltransferase would then be sufficient for the formation of the hexosamine components of the polysaccharide chain. However, this possibility appears to be ruled out by the finding of Malmström and Fransson[15] of an *N*-acetylgalactosaminyltransferase which catalyzes transfer to terminal iduronic acid residues.

In this brief review, the biosynthesis of chondroitin sulphate has been described in some detail as an example of the mechanisms by which the connective tissue polysaccharides may be formed. However, all polysaccharides of this group are not synthesized by strictly analogous routes, as shown by the recent work on iduronic acid formation. The biosynthesis of hyaluronic acid remains a major problem in this area, and there is a strong possibility that this polysaccharide is synthesized by a pathway different from that established for chondroitin sulphate.

Following the elucidation of a number of reactions involved in polysaccharide synthesis, work in this field is now being directed towards the purification of the individual enzymes and studies of their properties. There are indications that the mechanism of action of the glycosyltransferases may be rather complex, and it has been shown that phospholipids may play an important role in their activity[19]. A complete understanding of the physiology of the glycosyltransferases will require an assessment of this observation in relation to the situation *in vivo* where these enzymes are located in a lipid-rich membrane environment.

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