BIOSYNTHESIS OF HEPARIN AND HEPARAN SULFATE.

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The biosynthesis of heparin has been studied using a microsomal fraction from mouse mastocytoma tissue. During incubation with UDP-[^{14}C]glucuronic acid and UDP-N-acetylglucosamine two distinct polymer species were formed that could be separated by chromatography on DEAE-cellulose. One of these components was identified as a polymer composed of repeating glucuronosyl-N-acetylglucosamine disaccharide units; the other component had a similar structure but was partially N-deacetylated. Addition of 3'-phosphoadenylylsulfate (PAPS) to microsomal fractions containing such preformed, nonsulfated ^{14}C-labeled polysaccharide resulted in the formation of sulfated components that appeared as distinct peaks on ion-exchange chromatography. Structural characterization of the various components showed that they were polymeric intermediates in the biosynthesis of heparin, each representing the product of one or more polymer-modification reactions (see below). Similar components, relating to the biosynthesis of heparan sulfate, could be produced by a microsomal preparation from rat liver tissue. Kinetic studies confirmed that polymer modification occurs in a stepwise fashion, as expected from the observed accumulation of intermediates. A scheme for the overall modification process has been postulated on the basis of the structures of these intermediates, and verified by studies of the individual reactions involved. The following reactions have been established, described in the order of their participation during biosynthesis of heparin.

Deacetylation of N-acetylglucosamine residues - The extent of N-deactylation may be estimated in either of two ways: (a) by the size-distribution of fragments obtained after treatment of partially N-deacetylated polysaccharide with nitrous acid; (b) by the amount of [^{3}H]acetate liberated from a polymer substrate having ^{3}H-labeled N-acetyl groups. An assay method based on (b) has been developed and used to determine the basic kinetic properties of microsomal N-deacetylases from mouse mastocytoma and rat liver. N-Deacetylation of the natural substrate $\left(\left[\xrightarrow{1,4\alpha} \text{GlcUA} \xrightarrow{1,4\beta} \text{GlcNAc} \xrightarrow{1,4\alpha}\right]_n\right)$ occurs in a random fashion and leaves a product retaining about half of the original N-acetyl groups. Further N-deacetylation may occur after sulfation of the exposed free amino groups.

Formation of sulfamino (N-sulfate) groups - The free amino groups formed in the preceding reaction are substituted with sulfate groups. The corresponding N-sulfotransferase may be assayed by a procedure involving transfer of labeled sulfate groups from [^{35}S]PAPS to N-desulfated heparin. Along with N-sulfation additional N-acetylglucosamine units are deacetylated (and N-sulfated). N-Sulfation appears to be completed before any ester sulfate (O-sulfate) groups are introduced into the polysaccharide molecule.

Uronosyl C5-epimerization - D-Glucuronic acid residues are converted to L-iduronic acid units on the polymer level, in a reaction that is strongly promoted by concomitant introduction of O-sulfate groups. An assay method has been devised in which an N-sulfated polymer intermediate, specifically ^{3}H-labeled at C5 of the D-glucuronic acid residues, is used as a substrate; epimerase activity is proportional to the amount of ^{3}H-labeled water formed during incubation. The enzyme has been purified to near homogeneity from high-speed supernatants of mouse mastocytoma and calf liver, respectively. It requires the presence of N-sulfate groups in the polysaccharide substrate but, contrary to the other polymer-modifying enzymes, shows no requirement

for divalent cations. Incubations of doubly labeled substrate having both uniformly ^{14}C-labeled and 5-^{3}H-labeled glucuronic acid residues, or of unlabeled substrate in ^{3}H-labeled water, pointed to a freely reversible epimerization mechanism and also provided some insight into the relationship between O-sulfation and uronosyl C5-epimerization.

Formation of O-sulfate groups - O-Sulfation takes place partly along with uronosyl C5-epimerization and results in the formation of iduronic acid residues sulfated at C2 and glucosamine residues sulfated at C6. Total O-Sulfotransferase activity may be assayed by using a low-sulfated heparan sulfate as acceptor for labeled sulfate groups, with [^{35}S]PAPS as sulfate donor; this procedure does not distinguish between different O-sulfotransferases. The O-sulfation reactions conclude the polymer-modification process, yielding a product with the diverse structural features typical of heparin. However, in addition to this product a partially O-sulfated intermediate was isolated, containing mono-O-sulfated but no significant amounts of di-O-sulfated disaccharide units. Identification of the mono-O-sulfated disaccharide units in this intermediate polysaccharide, as well as in the final product, clearly indicated that sulfation at C2 of the iduronic acid residues precedes sulfation at C6 of glucosamine units.

Some important characteristics of the polymer-modification process should be noted. In each step of this process, the pertinent enzyme is presented with the product from the preceding step; most or all of the modifications introduced are essential for substrate recognition by the enzyme next in turn. Considering the series of modification reactions following the initial polymerization step, it is apparent that the deacetylation reaction represents a key step and is potentially one of the prime targets for regulation; polysaccharide segments escaping N-deacetylation will presumably be withdrawn from the series of reactions which leads to the fully modified, final product. Furthermore, it is apparent that all modification reactions (possibly excepting sulfation of free amino groups) are incomplete, thus adding to the marked microheterogeneity of the products, heparin or heparan sulfate. The regulation of these reactions should be of vital importance in relation to the specific affinity properties of the polysaccharides, expressed *e.g.* in the interaction between heparin and antithrombin III. Some observations of possible relevance to the regulation of the polymer-modification process will be discussed.