THE STRUCTURE OF HEPARIN
PROTON MAGNETIC RESONANCE SPECTRAL OBSERVATIONS[1]

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Heparin is composed of partially sulfated units of α-D-glucuronic acid and 2-amino-2-deoxy-α-D-glucose joined by 1,4-bonds. Extensive studies, which are the subject of a recent review article (1), suggest that formula I represents the basic structure of heparin, although it may contain a low proportion of N-acetyl groups as well as of sugar units and glycosidic linkages other than those indicated. However, samples from different sources vary widely in elementary composition (1, 2) as well as in biological activity (2).

For comparison with the chemical data, we have examined the proton magnetic resonance spectra in deuterium oxide solution of about a dozen heparin preparations. Two typical spectra are shown in Fig. 1. Since protons of a heparin molecule (as in I) occur in a wide variety of magnetically non-equivalent environments, a large number of

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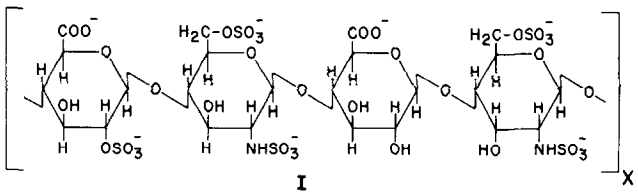
signals (at least eight) are detected, although most of them overlap. The spectra all conform to a common pattern, indicating that the same kinds of protons are present in each heparin, i.e., that each contains the same sugar constituents. However, variations from one spectrum to the next in the relative intensity of a given signal show that the content of any particular proton is not the same in every sample. These variations are independent of such things as ash, moisture and impurities (excepting unknown ones that might produce signals in the same region as heparin protons), and therefore show clearly that there are substantial differences in the ratios of component sugar units. In addition, there appear to be two basic structural types, since about half of the spectra resemble Fig. 1A, and the rest 1B. Noteworthy is the fact that all samples from a given commercial source do not necessarily give the same kind of spectrum. There is no apparent relation between the spectral data and anticoagulant activity.

The sharp signal at 2.02 p.p.m. in spectrum 1B shows widest variations in relative intensity; thus, it is barely detectable in 1A. This signal is attributed to N-acetyl groups since its chemical shift is the same as that of the N-acetyl group of 2-acetamido-D-glucose and of chondroitin sulfate. Certainly, it is not due to ionic acetate which, when present, produces a signal at 1.85 p.p.m.

Anomeric protons undoubtedly account for the three signals (a, b and c) at lowest field in Fig. 1A and 1B, based on their chemical shifts and also their intensities relative to the total integral of the spectrum. Accordingly, the current data support the earlier evidence that heparin contains at least three types of constituent sugars but, in contrast to



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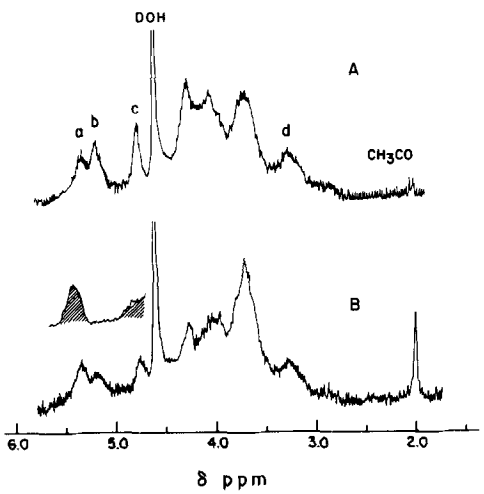

Fig. 1. Proton magnetic resonance spectra at 100 Mcps of heparin samples A and B in deuterium oxide at 35°C. Signal designations apply to both A and B: DOH, deuterium hydroxide; CH_3CO, N-acetyl. Improved resolution was obtained with some samples at 75°C; at this elevated temperature also, upfield displacement of the DOH signal facilitated integral measurements on signals a, b and c. Inset signals represent the lowfield portion of the spectrum for heparitin sulfate.

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formula I, show that the three are present in roughly equimolar proportions. The signal (a) at 5.35 p.p.m. is ascribed to H-1 of the N-sulfated 2-amino-2-deoxy-α-D-glucopyranosyl units,

from comparisons with spectra of model compounds and the fact that it is shifted upfield on N-desulfation (3). For analogous reasons the broad signal (d) centered at 3.25 p.p.m. is ascribed to H-2 of this unit. The α-D-glucopyranosyl units sulfated at position-2 probably account for the signal (b) at 5.20 p.p.m. Comparative data for H-1 of α-D-glucopyranosyl units of dextran sulfate (4) and of some D-glucuronosides, and also its unaltered chemical shift after N-desulfation, support this assignment.

The chemical shift of the third of these signals ((c) at 4.75 p.p.m.) is close to that expected for the unsulfated α-D-glucuronsyl unit depicted in I, but might apply equally well for H-1 of a β-D-uronide sulfated at C-2. However, the signal is strongly influenced by pH, and suffers a downfield displacement during N-desulfation of the heparin. Such behavior suggests that this unit undergoes some facile modification, e.g., one involving migration of an ester group (see ref. 4). The pertinency of this observation is emphasized by the fact that much of the available information on the structure of heparin has been obtained by use of methods involving strongly acidic conditions (1). Since the sugar unit in question accounts for about a third of the polymer units, its characterization is a problem of primary importance.

A sample of heparitin sulfate examined gives a spectrum differing noticeably from those of the heparins. Most striking is the presence of only one broad signal in place of the two lowest field signals for the heparins (inset, Fig. 1). As expected, this sample produces a comparatively strong N-acetyl resonance signal. The spectrum for whale heparin (5) resembles that of the heparitin sulfate more closely than those of other heparins.

The heparin samples strongly adsorb solvents such as ethanol and ether used in their purification. These contaminants are readily detected spectroscopically by their sharp high field triplet signals, and were found to be removed completely by lyophilizing aqueous solutions of the heparins.

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