NUCLEAR MAGNETIC RESONANCE STUDY OF SULFATED MUCOPOLYSACCHARIDES

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The application of high resolution nuclear magnetic resonance (NMR) spectroscopy to the structural investigation of sulfated mucopolysaccharides has shown that this provides a convenient and powerful method for characterization of glycosidic linkages, for determination of the position of the sulfate group, and for obtaining information on the mean repeating unit and on branching. In some cases, it is also possible to deduce completely new information about polysaccharide conformations which may not be available through any of the other methods. The advantage of the method is that all the information is obtainable from a solution of unchanged sample. This is especially so in the case of polymers consisting of uronic acid, hexosamine and sulfate because of the difficulties met in the isolation of partial degradation products.

In the present study, chondroitin sulfate A from whale cartilage, chondroitin sulfate C from shark, heparin from bovine lung and heparin from whale intestine were used as model compounds. Chondroitin sulfates were products of Seikagaku Kogyo Co. supplied through the courtesy of Dr. T. Furuhashi. Fr-I from whale heparin separated

by the chromatography on Dowex 1 was kindly given by Dr. K. Nagasawa. The fraction had an anti-coagulant activity, 192 I. U./mg.; $[\alpha]_D^{23}$, +75.3°; sulfur/nitrogen, 1.53 (Nagasawa, et al., 1964). The sample behaved as a single component in chromatography on many types of anion exchangers. Electrophoresis and ultracentrifugation showed the preparation to be highly homogeneous.

NMR spectra were obtained on a Varian A-60 NMR spectrometer. Sodium 2, 2-dimethyl-2-silapentane-5-sulfonate (DSS) was used as an internal reference. All samples were dissolved in D_2O for observation after a twice repeated process of dissolving in D_2O and freeze-drying. Spectra are reproduced in Fig. 1. The chemical shifts are expressed in terms of $-\delta$ in ppm.

Chondroitin sulfates

Chondroitin sulfate A and C differ in the position of the sulfate group and this is apparent from their spectra. The intense signal at -3.8 ppm (peak 3) appearing in the spectrum of chondroitin sulfate A arises from the protons on C-6 of galactosamine. This signal is subjected to a down-field shift by the sulfate group located at C-6, in the case of chondroitin sulfate C; consequently a new peak appears at -4.1 ~ -4.2 ppm (peak 5) and peak 3 is correspondingly reduced. The signal for protons on the sulfate-bearing C-4 of galactosamine in chondroitin sulfate A is expected to appear around -4.4 ~ -4.5 ppm and peak 6 may be assigned to these protons. In agreement with a β-linkage, no equatorial C-1 proton is observed. Axial C-1 protons, expected to appear around -4.6 ppm, could not be observed separately from the absorption of the proton resonance of HDO. The measured ratio of the sum of the areas of peaks 2—6 to the area of peak 1

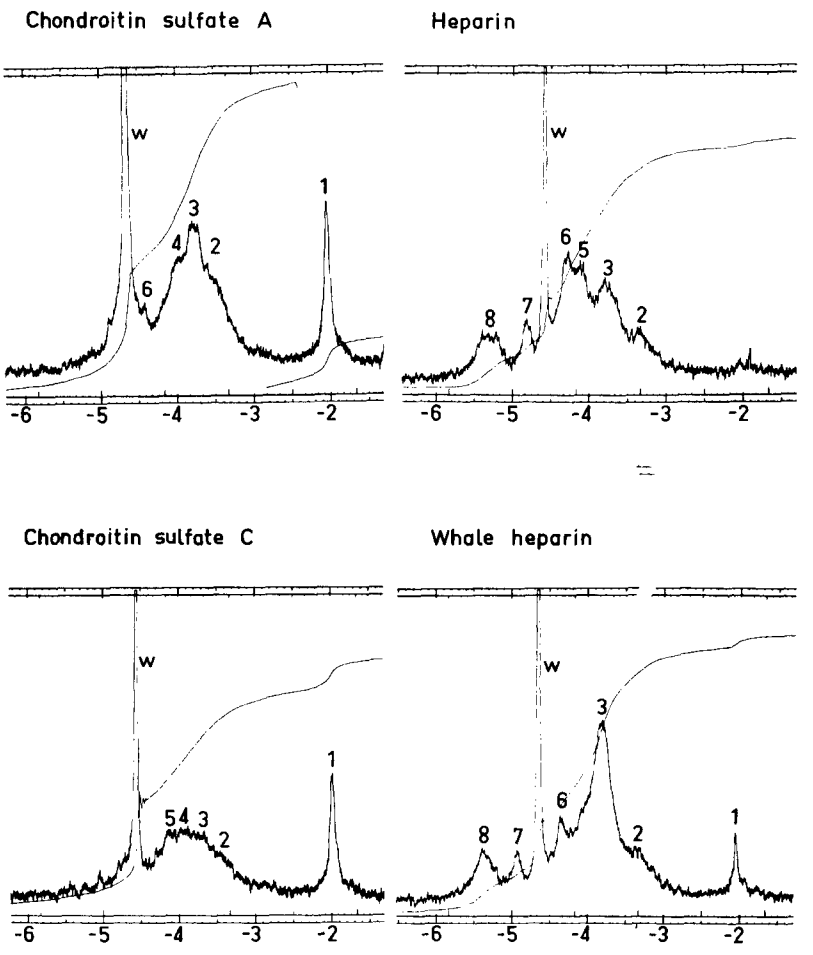

Fig. 1. Proton magnetic resonance spectra of chondroitin sulfate A and C, heparin, and whale heparin in D_2O.

(assigned to acetate methyl protons) is 3.3 in accord with the ratio 10/3 calculated for the disaccharide consisting of a glucuronic acid and an N-acetylgalactosamine.

Heparin

A low-field peak 8 (-5.4 ppm), which is not found in the spectra of chondroitin sulfates, can be assigned to the equatorial C-1 protons in heparin: the linkage has α-configuration. The correctness of the assignment is supported by taking the ratio of the area of peak 8 to the sum of

the areas of peaks 2—7. The measured ratio is 5.2 compared to a ratio of 5.0 calculated from the working structure (Wolfrom, et al., 1963). Peak 7 (-4.9 ppm) is also found in the spectra of heparins and assignable to the C-2 protons of N-sulfated glucosamine. Peak 5 is again assigned to the protons on the sulfate-bearing C-6 and is formed at the expense of peak 3; peak 6 (-4.3 ppm) may arise from the protons on the other sulfate-bearing carbons.

Whale heparin

An acid mucopoly saccharide isolated from whale organs and named "whale heparin" or "ω-heparin" has been characterized by Nagasawa et al. (1964) and Yosizawa (1964). A characteristic of this heparin is its particularly high anticoagulant activity and the low sulfate content. The presence of an acetyl group, revealed by chemical analysis and located on the amino group of glucosamine (Yosizawa, 1964), results in the appearance of peak 1 (CH_3-absorption of acetyl group); on the basis of the homogeneity tests mentioned above, it is concluded that N-acetyl-glucosamine is an integral component of whale heparin. As to the type of the glycosidic linkage and the position of the sulfate group, no conclusion was given by the above authors. It was therefore of interest to find how much information could be obtained from an analysis of the NMR spectrum.

In the spectrum of whale heparin, the greatest difference from that of heparin is in the intensity of peak 3 (-3.8 ppm), indicating that C-6 of glucosamine is not sulfated in this heparin. The presence of peak 8 indicates that the linkage is α. The ratio of the area of peak 8 to the area of peak 1 is 8/3 showing that one acetyl group is present for every eight α-linkages. The ratio of the sum of the areas of peaks 2—7 to the area of

peak 8 is found to be 6.2. This should be 5 if whale heparin has the same structure as heparin. The large value of the ratio of non-anomeric to equatorial anomeric protons could be explained by the presence of β-linkages. A structure, which appears to be in good agreement with the spectral analysis, is one involving one β-linkage for every eight α-linkages and consisting of a unit composed of four uronic acid, four N-sulfated glucosamine and one N-acetylglucosamine residues. The possibility of sample impurities being the source of the β-linkages is ruled out from the homogeneity of the sample used.

If one considers a structure in which a uronic acid is linked to N-acetylglucosamine by a β-(1→3) linkage, a precedent may be found in hyaluronic acid. Considering also the possibility of a common enzyme participating in the formation of this linkage in the process of biosynthesis, whale heparin could be a polymer which mainly consists of α-(1→4)-linked glucuronic acid and N-sulfated glucosamine, and contain, in the proportion given above, N-acetylglucosamine to which glucuronic acid is joined with a β-(1→3) linkage. The β-linkage could be a branch-point and such a branching structure may be responsible for the high anticoagulant activity exhibited by whale heparin.

A more detailed account of this and other related studies on mucopolysaccharides will be reported elsewhere.

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