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STRUCTURE AND CONFORMATION OF POLYSACCHARIDES BY NMR SPECTROSCOPY

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NMR spectra provide a wealth of information on primary structure and conformation in solution of polysaccharides which is unparalleled by that obtainable by any other single spectroscopic method. Confining this discussion to the nuclei most commonly studied by NMR (^1H and ^{13}C), each non-equivalent hydrogen and carbon of a polysaccharide produces an NMR signal at a characteristic resonance frequency (usually expressed in terms of 'chemical shift'). Also the shape of each signal (from which the parameters 'coupling constants' and 'relaxation times' can be derived) are characteristic of the chemical environment of the corresponding hydrogen and carbon. All the NMR parameters are affected by the stereochemistry of such an environment (Perlin & Casu, 1982).

In favourable cases, all the ^1H and ^{13}C signals of a polysaccharide are completely resolved. More commonly, a number of signals overlap to some extent, limiting the information which can be extracted from the spectra. Signal overlap is made more severe by signal broadening associated with the viscosity of polymer solutions. Working at high magnetic fields (>200 MHz for ^1H) is becoming increasingly common in polysaccharide research.

Although a high field is especially desirable for ^1H spectra, ^{13}C spectra also greatly benefit from increasing the magnetic field, since minor but important details in the structure of heteropolysaccharides can then be shown. Since resolution problems are less severe for ^{13}C than for ^1H spectra, the use of ^{13}C spectroscopy for characterising polysaccharides is now becoming routine. Besides providing a most specific fingerprint of a polysaccharide, a ^{13}C spectrum gives rapid information on purity and structural heterogeneity, as well as on type and relative proportion of positional linkages (Perlin & Casu, 1982; Gorin, 1981).

Advances in NMR instrumentation, especially as regards computer control systems, make it possible to enhance digital resolution, observe 'hidden' resonances in partially resolved spectra and measure relaxation times routinely. All the ^1H resonances were

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recently assigned in disaccharides (Morris & Hall, 1981) and polysaccharides (Gatti, 1982) using 'two-dimensional' (2D) techniques. End-residues in highly branched mannans were characterised taking advantage of differences in relaxation times associated with a much greater segmental mobility with respect to 'internal' residues (Gorin & Mazurek, 1979). Relaxation times are becoming increasingly important in studies of molecular dynamics of polysaccharides (Yokota *et al.*, 1978) (Fig. 1).

A major breakthrough in NMR was the advent of the cross-polarisation/magic angle spinning (CP/MAS) technique, which permits 'high-resolution' spectra of solid samples to be obtained. ^{13}C CP/MAS spectra of celluloses (Maciel *et al.*, 1982; Saito *et al.*, 1981), other polyglucoses (Saito *et al.*, 1981) and chitin (Gagnaire *et al.*, 1982) were recently reported, showing significant differences for different polymorphs. These spectra are of obvious interest for direct comparison with spectra in solution (Fig. 2).

The need for further resolution and increased solubility often requires partial depolymerisation of polysaccharides. Informative spectra were obtained from guaran after controlled hydrolysis with acids (Grasdalen & Painter, 1980) or enzymes (Bociek *et al.*, 1981). Spectra of good quality were also obtained from xanthan after partial cleavage with crude cellulase (Lambert *et al.*, 1982). The ^{13}C spectra of partially depolymerised guaran and related gums (Grasdalen & Painter, 1980; Bociek *et al.*, 1981) and of alginates (Grasdalen *et al.*, 1977) permit the compositional analysis of these polysaccharides. In addition, analysis of complex signals especially sensitive to sequence effects provides information on the statistical distribution of branching points (Grasdalen & Painter, 1980) and of different blocks (Grasdalen *et al.*, 1977). As shown in extensive work on dextrans (Seymour, 1979; Seymour & Knapp, 1980) and antigenic polysaccharides (Jennings & Smith, 1978; Jennings, 1982), ^{13}C-NMR has a great potential for the characterisation of complex branching.

NMR is now currently used also for the characterisation of derivatised polysaccharides such as cellulose ethers (Parfondry & Perlin, 1977). To preserve information on sequence, it is usually preferable to work on products only partially depolymerised. However, advantage can be taken of the much better resolution achievable with monomeric species by hydrolysing all the glycosidic bonds of the polysaccharide derivative. The degree of substitution of carboxymethyl celluloses (CMCs) can be easily determined from the ^{1}H spectra of CMC hydrolysates (Ho & Klosiewicz, 1980). The ^{13}C spectra of similar hydrolysates provide information also on the distribution of carboxymethyl substituents at positions 2-, 3- and 6- of the anhydroglucose residues (Parfondry & Perlin, 1977). Di- and tri-substitution at the same residue are clearly detectable in the ^{13}C spectra of CMC of high degrees of substitution (Casu *et al.*, unpublished) (Fig. 3).

Working out the structure of highly heterogeneous polysaccharides usually requires combined chemical, enzymic and NMR approaches. As an example, the characterisation of the 'active site' of heparin was made by concentrating this site in fragments obtained by affinity chromatography on antithrombin-III after controlled degradation with nitrous acid (Meyer *et al.*, 1981; Casu *et al.*, 1981) or heparinase (Casu *et al.*,

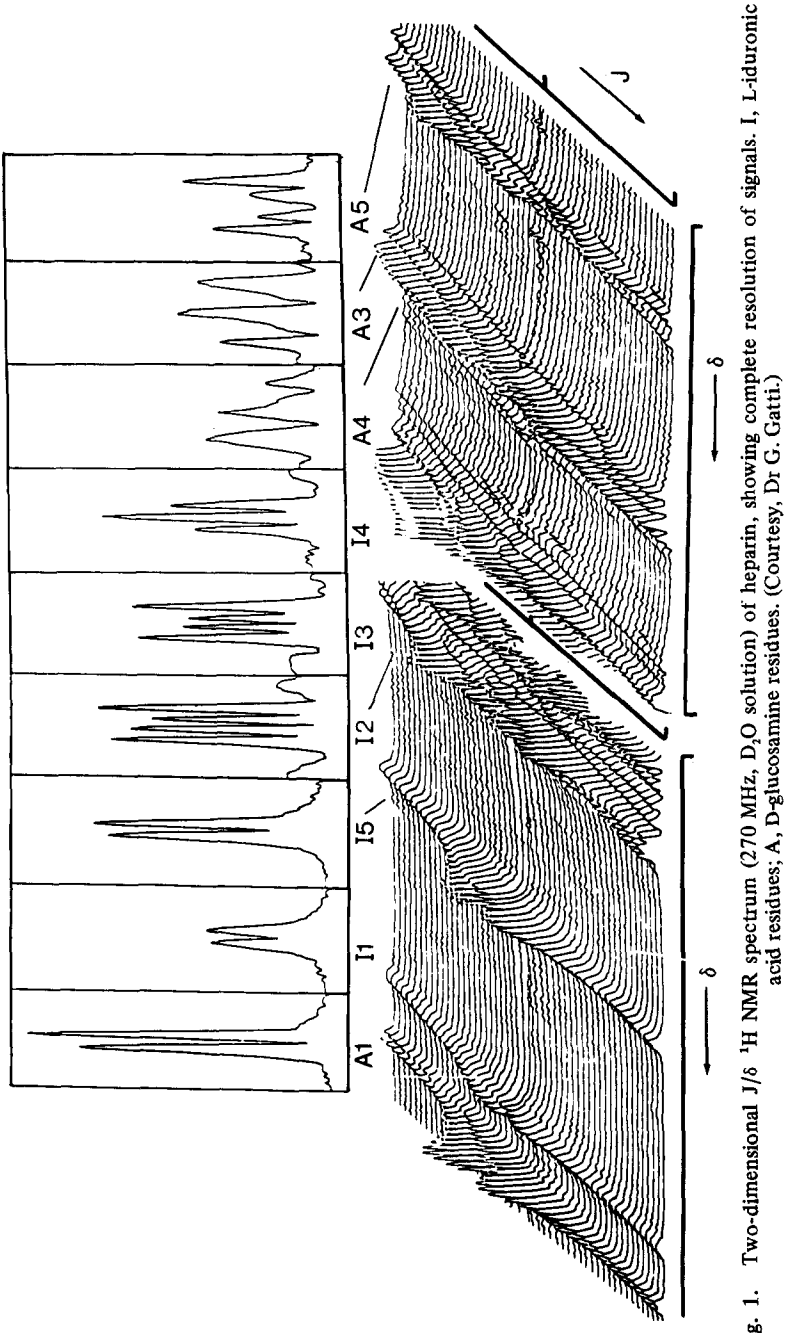

Fig. 1. Two-dimensional J/δ ^{1}H NMR spectrum (270 MHz, D_2O solution) of heparin, showing complete resolution of signals. I, L-iduronic acid residues; A, D-glucosamine residues. (Courtesy, Dr G. Gatti.)

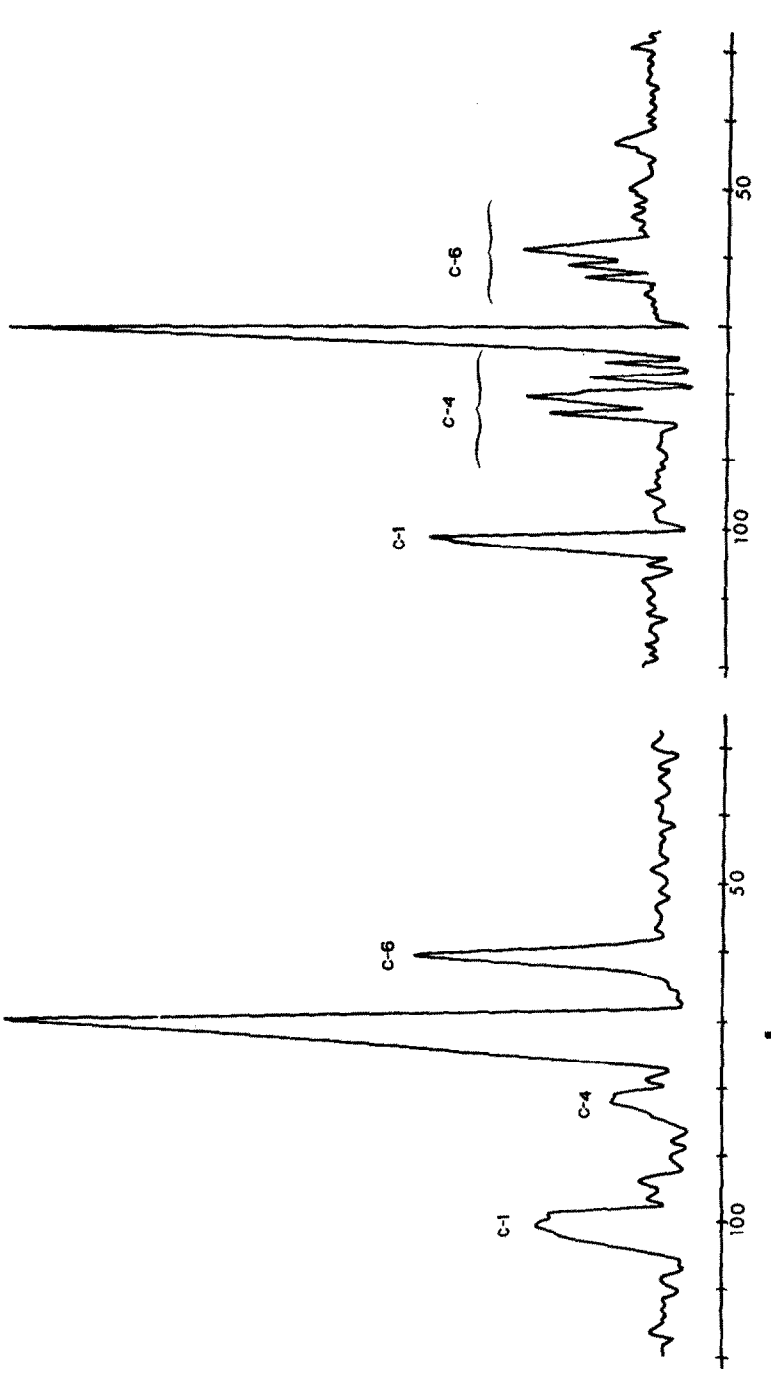

Fig. 2. Solid-state CP/MAS ^{13}C NMR spectra (75 MHz) of amylose (A) and β-cyclodextrin (B). Signal splittings are attributed to the presence of different conformers. (Courtesy, Drs G. Allegra & V. Meille.)

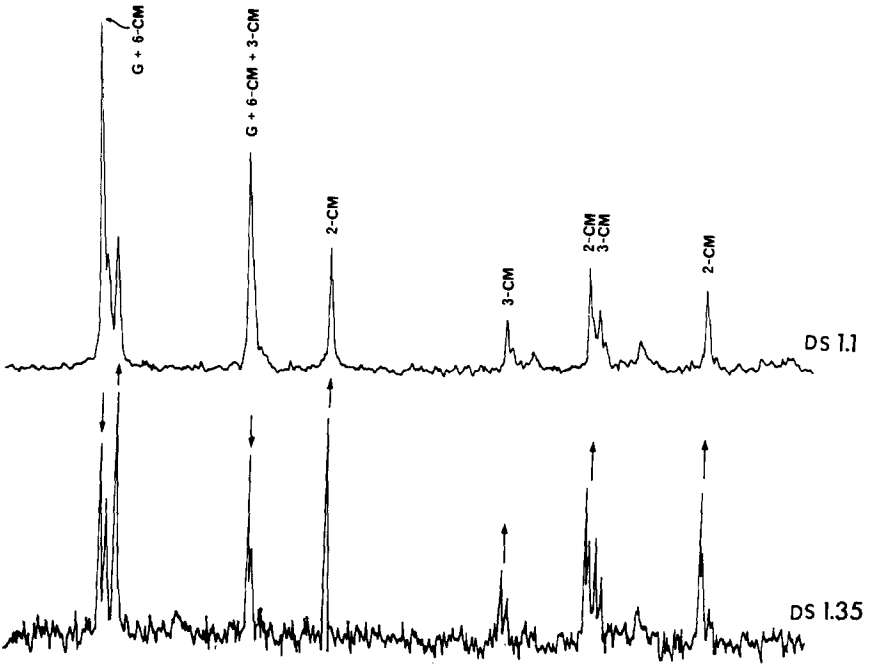

Fig. 3. Partial ^{13}C NMR spectrum (25 MHz, D_2O solution) of acid hydrolysates of carboxymethylcellulose, showing variation of C1, C2 and C3 patterns for increasing degrees of substitution (Casu *et al.*, unpublished).

1981). The C-2 signal of trisulphated D-glucosamine, an essential component of the active site, is being used as a physico-chemical parameter correlated with the antithrombotic activity of heparin (Casu *et al.*, submitted) (Fig. 4).

The NMR studies on conformation in solution of monomeric residues in polysaccharides largely rely on obtaining approximate values of C(H)–C(H) dihedral angles from interproton coupling constants (Perlin & Casu, 1982). Such an analysis usually requires completely resolved ^1H spectra and computer simulation of the coupling pattern. Well resolved ^1H spectra of glycosaminoglycans (whose structure is largely accounted for by disaccharide repeating units, with COO^- and SO_3^- substituents that magnetically de-shield, to different extents, the various hydrogens) were obtained by working at high field and using resolution enhancement techniques (Gatti, 1978). The controversial conformation of L-iduronic acid residues was found to be a slightly distorted form of the 'alternate' $^1C_4$ chair in both heparin (Gatti *et al.*, 1979*a*) and dermatan sulphate (Gatti *et al.*, 1979*b*).

As discussed in a recent critical review on glycosaminoglycans (Casu, 1982), studies on the chain conformation of polysaccharides are mostly based on chemical shift

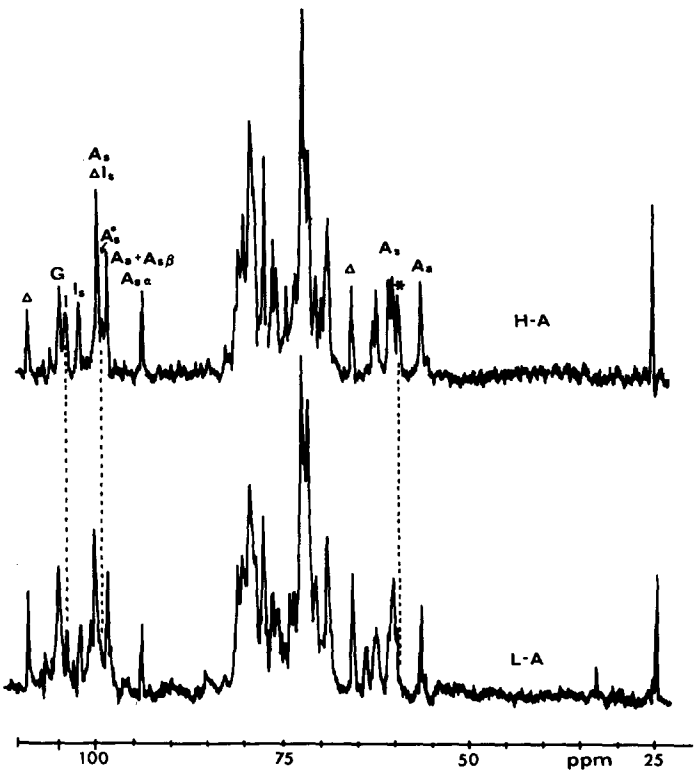

Fig. 4. ^{13}C NMR spectra (20 MHz, D_2O solution) of octasaccharides obtained by partial cleavage of heparin with heparinase, affinity chromatography on antithrombin-III and gel filtration. H-A, high-activity fragment; L-A, low-activity fragment. Signal labelled with an asterisk is from C2 of *N*-3,6-trisulphated D-glucosamine residue (Casu *et al.*, 1981).

arguments which can be weak on account of the many factors affecting this NMR parameter (Perlin & Casu, 1982). Although at present limited to disaccharides and cyclodextrins, the analysis of coupling between ^{13}C and ^{1}H across the glycosidic bridges appears the most promising approach for evaluating inter-residue torsional angles (Perlin & Hamer, 1979). It is predicted that such an approach will become common practice when coupling patterns are simplified by special decoupling or selective deuteration techniques.

Changes in chemical shift can be more safely used to monitor changes in chain conformation whenever these changes can be clearly associated with changes in inter-residue hydrogen bonding. This effect can be felt at the level of carbons to which the OH (or NH) groups involved in hydrogen bonding are attached, or on the hydrogens appended to these carbons, as observed for the alkali-induced transition of sodium hyaluronate (Welti *et al.*, 1979). A more direct approach is to study the OH (or NH) signals. Unfortunately, these signals are not observable in the ^{1}H spectra in D_2O (the

most common NMR solvent for polysaccharides), and studies in H_2O are still difficult to perform. Studies of OH resonances in dimethyl sulphoxide indicated retention, in this solvent, of the inter-residue $O_2H..O_3H$ hydrogen bond in amylose and its oligomers (Casu *et al.*, 1966; St. Jaques *et al.*, 1976). In a recent study of deuteration resistant NH signals of hyaluronic acid oligomers in dimethyl sulphoxide, strong support was given to the 'super-H-bonded' chain model predicted on account of periodate oxidation and other studies (Scott *et al.*, 1981).

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