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Note

Diagnosis of mucopolysaccharidoses using ^{1}H-n.m.r. spectroscopy of glycosaminoglycans*

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Degradation of glycosaminoglycans is carried out by specific endoglycosidases, exoglycosidases, and sulfatases. Specific enzyme deficiencies, which are inherited defects, lead to the group of diseases known as mucopolysaccharidoses[1,2] (MPS). Diagnosis has heretofore been made by identification of the enzymic defect in fibroblasts, serum, or leucocytes[3], or by chemical characterization of the excreted material[4]. The former approach is not always easy or definitive, and the latter is relatively tedious and may be ambiguous. ^{1}H-N.m.r. spectroscopy has recently been introduced[5] as a rapid and nondestructive method for the characterization of heparan sulfates and dermatan sulfates from MPS types II, III, and VI. We report herein the extension of this approach to distinguish between MPS subtypes IIIA and IIIB, and to characterize MPS type IV where both keratan sulfate and chondroitin sulfate are excreted. In addition, we have obtained an ^{1}H-n.m.r. spectrum of the excretion product of a patient suffering from mannosidosis, a storage disease where oligosaccharides rather than glycosaminoglycans are excreted. Although it is extremely difficult to assign all the signals in the ^{1}H-n.m.r. spectrum of a polysaccharide[6–9], we could make assignments in certain regions of the spectrum and obtain enough information through "fingerprinting" to identify the glycosaminoglycan present.

The main regions of interest are (a) the "anomeric", H-1 region (δ 4.5–5.75); (b) the *N*-acetyl, CH_3 region (δ 1.8–2.2); and (c) part of the ring-proton region (δ 3–3.5). The anomeric region may be divided into the α region (δ 5–5.75) where signals from α-D-linked sugars occur and the β region (δ 4.5–5). Signals from H-2 and -5 also occur in the β region, but specific assignments are not necessary here for our purposes. The exact chemical shift of signals occurring in the *N*-acetyl region will depend on (a) the sugar to which the *N*-acetyl residue is linked, *e.g.*, 2-amino-2-deoxy-D-galactose or -D-glucose; (b) whether this sugar is linked α or β; (c) the linkage position of the sugar, *e.g.*, 3 or 4; (d) substituents on the sugar ring,

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TABLE I

^{1}H-N.M.R. DATA FOR STANDARD GLYCOSAMINOGLYCANS AND FOR URINARY EXCRETION PRODUCTS

Sample	*Chemical shifts*[a] *(δ)*					
	Principal anomeric signals		*N-Acetyl*	*Ring*	*Excretion product*[b]	*Diagnosis*
	α	*β*				
Heparan sulfate	5.65, 5.44	4.96, 4.85	2.085	3.41, 3.30		
Dermatan sulfate		4.92, 4.86	2.125, 1.96	3.59		
Keratan sulfate		4.76, 4.93	2.08	3.64		
Chondroitin 6-sulfate		4.76, 4.93	2.07	3.64, 3.40		
Patient A	5.65, 5.44	4.85, 4.82	2.085	3.41, 3.30	H.S.	San Filippo A
Patient B	5.65, 5.44	4.85, 4.82	2.085	3.41, 3.30	H.S.	San Filippo A
Patient C	5.65, 5.44	4.96, 4.85	2.105, 2.085	3.41, 3.30	H.S.	San Filippo B
Patient D	(5.65)[c], 5.44	4.94, 4.86	2.125, 2.105 2.085, 1.96	3.59, 3.40	H.S. + D.S.	Hurler
Patient E		4.93, 4.76	2.08, 2.07	3.64, 3.40	K.S. + C.S.	Morquio
Patient F		4.93, 4.76	2.08, 2.07	3.64, 3.40	K.S. + C.S.	Morquio
Patient G	5.21, 5.12	4.79	2.04			Mannosidosis

[a]Relative to the signal of sodium 4,4-dimethyl-4-silapentane-1-sulfonate (indirectly to the signal of acetone in D_2O; δ 2.225). [b]Abbreviations: H.S., heparan sulfate; D.S., dermatan sulfate; K.S., keratan sulfate; and C.S., chondroitin sulfate. [c]Low-intensity signal.

e.g., sulfate group and its position, *e.g.*, 2,4, or 6; and (e) the type of neighboring sugar — uronic acid, amino sugar, or neutral sugar.

Since heparan sulfate is the only glycosaminoglycan that contains an α-D-linked sugar, its presence is easily detected by the appearance of a signal in the α anomeric region (see Table I). In addition, when heparan sulfate is present[2] along with dermatan sulfate (MPS I, II, and VII) or with keratan sulfate[2] (MPS VIII), integration of the signals in the α- and β-anomeric regions can give a rough estimate of the relative proportions of each glycosaminoglycan present.

Assignments may also be made in the *N*-acetyl region (see Table I), since for each glycosaminoglycan the *N*-acetyl group has a different chemical environment. Integration of the signals in this region will not give quantitative results, however, as the degree of *N*-acetylation may vary widely.

The spectra from patients A and B showed signals in the α region indicating the presence of heparan sulfate. Comparison of the α and β regions with the standard heparan sulfate indicated that only heparan sulfate (and no dermatan sulfate) was present. Signals in the *N*-acetyl region were consistent with this. Diagnosis of MPS II A, San Filippo A disease was made.

The spectrum from patient C was essentially the same as those from patients A and B, except for an additional signal in the *N*-acetyl region. The patient was diagnosed enzymically as having MPS III B, San Filippo B disease, where the missing enzyme is the *N*-acetyl-α-D-glucosaminidase after *N*-acetylation[10]. The two *N*-acetyl signals were consistent with there being two different *N*-acetyl groups in the molecule at this stage of degradation.

The spectrum from patient D showed signals in the α region, but their relative intensities were smaller than in the previous cases, suggesting that dermatan sulfate was also present. This was borne out by the signals in the *N*-acetyl region. Diagnosis of MPS 1, Hurler disease, was made.

Spectra from patients E and F lacked signals in the α region. In the β region, the spectra showed great similarities to those from keratan sulfate and chondroitin 6-sulfate, and in the *N*-acetyl region two distinct signals were seen, in agreement. Diagnosis of Morquio disease was made.

Finally, although a mixture of oligosaccharides was present, the spectrum for patient G gave sharp signals in both the α and β regions, and a single signal in the *N*-acetyl region. The data were in full agreement with published spectra[11] for mannosidosis.

EXPERIMENTAL

Glycosaminoglycans were precipitated, from at least 50 mL of urine, with cetylpyridinium chloride (CPC) and then eluted from Dowex AG 1-X2 (Cl^-) columns (2.5 × 20 cm; 200–400 mesh) with 2M NaCl. The eluant was dialyzed and then lyophilized. The mixture of mannosidosis oligosaccharides was isolated from desalted urine by fractionation on a column (1 × 100 cm) of Bio-Gel P-2 (100–200

mesh) eluted with water. Neutral samples were prepared for ^{1}H-n.m.r. analysis by exchanging three times with 99.7% D_2O and then dissolving in 99.9% D_2O. Spectra (32 or 128 scans) were recorded with a Brucker 400 MHz instrument operating at 368 K, or at 298 K employing a water-null technique, to shift or to remove the residual HOD peak.

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