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Preliminary communication

Structural studies of *Klebsiella* capsular polysaccharides by using natural-abundance carbon-13 n.m.r. spectroscopy

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We have previously delineated[1,2] the diagnostic potential of proton nuclear magnetic resonance (n.m.r.) spectroscopy as a method for determining the number of monosaccharide residues in the repeating unit of a polysaccharide, the configuration at these anomeric centers, the proportion of substituent groups such as acetyl or 1-carboxyethylidene, and the presence of deoxy sugars. These methods, now used routinely by us and others[3], have evolved from studies on the capsular polysaccharides of *Klebsiella*, polymers that are characterized as heteroglycans possessing highly regular structures. For this reason, these polysaccharides are admirably suited to examination by natural-abundance ^{13}C-n.m.r. spectroscopy. We now report several important technical features of this method that are based on our experiences with the polysaccharides obtained from *Klebsiella* serotypes K5, K36, and K70.

Few applications of ^{13}C-n.m.r. spectroscopy to polysaccharides have been reported, but these include, for example, studies on glucans[4], mannans[5], hyaluronic acid[6], and meningococcal antigens[7]. Whereas these polymers have mono- or di-saccharide repeating units, those from *Klebsiella* are composed of three to six sugar residues, some of which may carry acetyl or 1-carboxyethylidene substituents.

The first ^{13}C-n.m.r. spectrum of a carbohydrate system obtained at 90.5 MHz is presented here, and the application of ^{1}J C-1–H-1 couplings[8] for determining anomeric linkages in polysaccharides is demonstrated.

In contrast to ^{1}H-n.m.r. spectra of the intact polymers, which must[1] be recorded at ~95° to obtain adequate resolution, well resolved ^{13}C-n.m.r. spectra can be secured at ambient probe-temperatures (~35°). The spectra of native K-36 polysaccharide measured at 90.5 MHz and at 20 MHz illustrate this point (see Fig. 1).

Although the increased spectral dispersion obtained at higher fields reveals much

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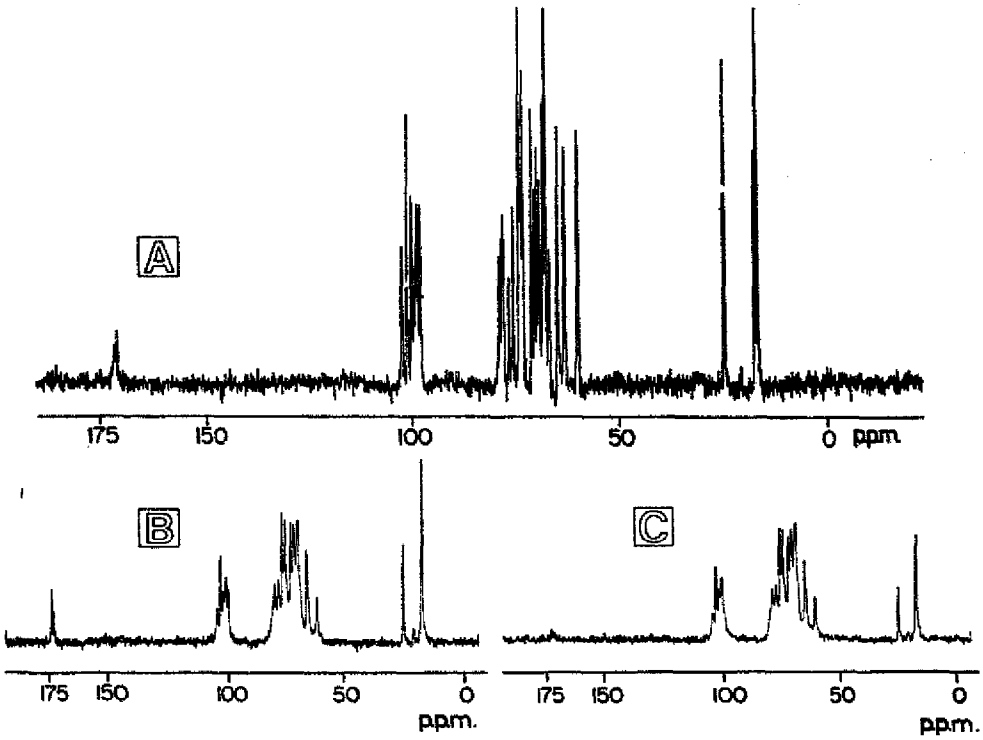

Fig. 1. Natural-abundance carbon-13 n.m.r. spectra of *Klebsiella* K-36 capsular polysaccharide in D_2O. [A , measured at 90.5 MHz (50,000 transients, spectral width 20 kHz, using a 1% (w/v) solution. B and C, measured at 20 MHz (50,000 transients, spectral width 4 kHz, zero pulse-delay, using a 2.5% (w/v) solution. The spectrum in B was measured with an acquisition time of 1.0 sec, and hence required ~14 h of machine time; that in C used an acquisition time of 0.2 sec and only ~3 h of machine time. The frequency scale of each of the spectra is in p.p.m. from tetramethylsilane, and all spectra were measured with complete proton-decoupling.]

additional fine structure, even spectra measured at 20 MHz show many characteristic resonances, sufficient in most cases for the routine examination of these polysaccharide antigens. For example, the spectrum in Fig. 1B shows the presence of two types of carbonyl groups[8], one hexose residue having a free CH_2OH group[8], one unit of acetal-linked pyruvic acid, and three units of rhamnose[8]. Comparison of this spectrum with that of acid-treated polymer (autohydrolysis at pH 2.2, overnight at 95°) confirmed the assignments of the pyruvate-acetal resonances; the hydrolysis also resulted in a shift to high field of the resonance at 66.9 p.p.m., which can thus be assigned to the CH_2OH group originally associated with the acetal.

The resonances in the anomeric region of the 90.5-MHz spectrum confirm that the polysaccharide contains six different anomeric linkages, and these can be assigned tentatively to α- and β-configurations by their chemical shifts. The ^1J, C-1–H-1 couplings can be measured by using the gated decoupling technique[9]; it is known[10] that α-glycosides give a coupling of ~170 Hz, whereas β-glycosides give a coupling of ~160 Hz, and the couplings

for K-36 accord with those values. Further confirmatory evidence for the assignments of the anomeric resonances and of those of the other ring-carbon atoms comes from comparisons with the shifts for the oligosaccharides obtained by standard[2] degradation procedures and for synthetic, model compounds. Full details are not given here, but shift comparisons between the latter sets of reference compounds are generally within ±0.2 p.p.m whereas the agreement between those reference data and those for the intact polysaccharide is often less good (±0.4 p.p.m.), nevertheless, it is important that, so far, we have encountered very few major mismatches. The resonances in the region 78–82 p.p.m. merit particular attention, because they come from those ring-carbon atoms that are involved in the interglycosidic linkages.

A final technical point merits comment. Because the intact polysaccharides have high molecular weights and form viscous solutions, their spin–lattice relaxation rates are very fast, e.g., ~20,000 $msec^{-1}$ for the ring-carbon atoms of K-36 in a 1% solution in D_2O. Hence, it is neither necessary, nor appropriate, to sample such spectra by use of long acquisition times, nor is any pulse delay needed; as a result, adequate spectra can be obtained with a substantial saving of time[11], as is illustrated in Fig. 1, the conditions used for the spectrum in Fig. 1B would normally be used for an oligosaccharide. One risk associated with this procedure is that the resonances of any carbon atoms that do have high relaxation-rates may be partially, or completely, saturated in such an experiment.

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