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Note

^{13}C-C.p.–m.a.s. n.m.r. studies of frozen solutions of (1→4)-α-D-glucans as a probe of the range of conformations of glycosidic linkages: the conformations of cyclomaltohexaose and amylopectin in aqueous solution

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The conformations adopted by carbohydrate chains in solution are likely to be important determinants of the functional properties of, for example, the side chains of glycoproteins[1–4] and polysaccharides of industrial importance[5]. The major variable in the conformation of carbohydrate chains is the glycosidic linkage[5], and many studies have shown that n.m.r. spectroscopy is a powerful probe of the conformation of glycosidic linkages through measurements of, for example, inter-residue n.O.e.'s[2,4] and ^{13}C–^{1}H coupling constants[7,8]. From such studies, time-averaged conformational parameters can be deduced. However, calculations of conformational energies[5,9] suggest that many types of glycosidic linkage can adopt a wide range of conformations in solution, so that average conformations may have no physical meaning unless information is available concerning the range of conformations present[10]. It has been shown[11–13] that ^{13}C-c.p.–m.a.s. n.m.r. chemical shift data for (1→4)-α-D-glucans in the solid state are sensitive to the torsion angles (ϕ and ψ) which define[5] the conformation of glycosidic linkages. Such variations of chemical shifts with the conformation of glycosidic linkages have been suggested to be a general phenomenon in both solid[14] and solution[15] states. We now report ^{13}C-c.p.–m.a.s. n.m.r. data for frozen solutions of (1→4)-α-D-glucans, which demonstrate that useful information can be derived concerning the range of conformations adopted by the glycosidic linkages.

Fig. 1 shows ^{13}C-n.m.r. spectra of cyclomaltohexaose (α-cyclodextrin, α-CD, **1**), a cyclic hexamer of (1→4)-linked α-D-glucose. The single-pulse excitation ^{13}C-n.m.r. spectrum (Fig. 1a) of a solution at 30° contains 6 sharp resonances corresponding to C-1/6. On freezing this solution, broad spectral features are apparent in the ^{13}C-c.p.–m.a.s. spectrum (Fig. 1b) with resolved resonances showing variations in chemical shifts, *i.e.*, C-6 (58–66 p.p.m.) > C-4 (79–85 p.p.m.) > C-1

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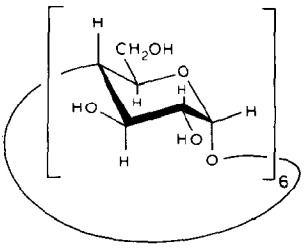

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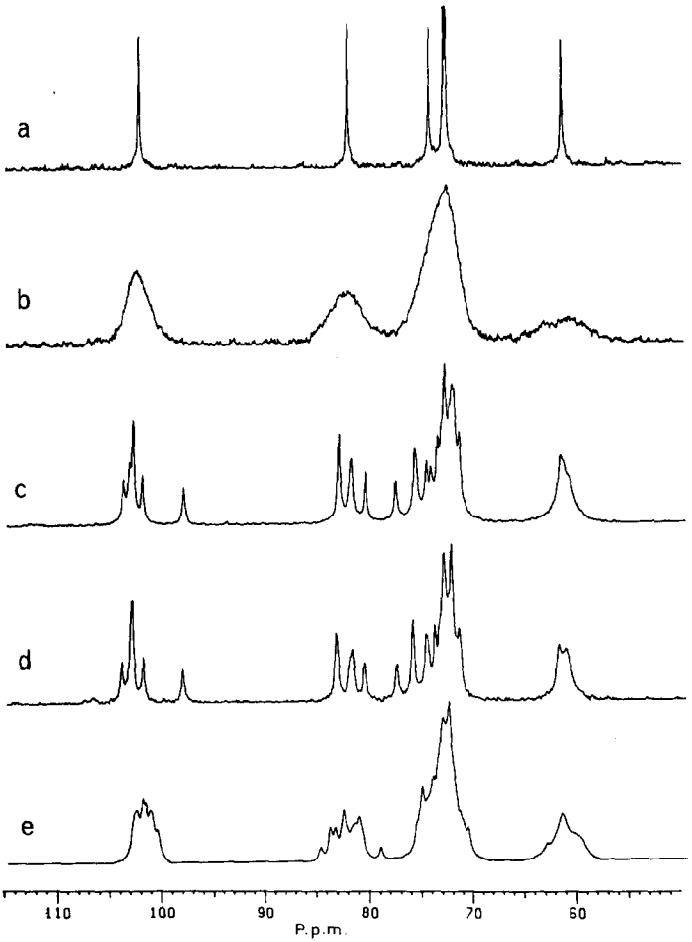

Fig. 1. (*a*) Single-pulse excitation ^{13}C-n.m.r. spectrum of an aqueous 20% solution of α-CD at 30°, (*b*) a ^{13}C-c.p.–m.a.s. spectrum of the same solution at −40°, (*c*) a ^{13}C-c.p.–m.a.s. spectrum at 30° of α-CD hexahydrate type I[13], (*d*) the same sample at −40°, (*e*) a ^{13}C-c.p.–m.a.s. spectrum at 30° of α-CD re-crystallised from 1.2M $BaCl_2$.

(100–104 p.p.m.). Average chemical shifts of the resolved signals are similar for frozen solutions and at 30°, suggesting that the broadening of the spectral features on freezing is due to the trapping of a range of conformations. This inference is consistent with the broadest signal being due to the most conformationally mobile site (C-6). In contrast, and as would be expected, the widths of signals for the crystalline hexahydrate of α-CD are similar at 30° and −40° (Fig. 1c,d). However, numerous minor (<0.5 p.p.m.) differences in chemical shifts are observed for the crystalline α-CD at the two temperatures (Fig. 1c,d), possibly reflecting different networks of hydrogen bonds.

The c.p.–m.a.s. spectrum (Fig. 1c) of the crystalline hexahydrate of α-CD contains two signals (98.1 and 77.7 p.p.m.) which have been assigned[16] to C-1 and C-4 adjacent to a single, conformationally strained glycosidic linkage in the macrocycle[17]. There are no signals at either of these chemical shifts in frozen solutions (Fig. 1b: integration suggests that the resonance for C-4 lies in the range 79–85 p.p.m.). This observation suggests that the conformation of the glycosidic linkage characterised by the resonances at 98.1 and 77.7 p.p.m. is not present to a significant extent in solution and, therefore, that the structure of the hexahydrate[17] in the crystal is not maintained in aqueous solution. Although the hexahydrate polymorph studied (type I[18]) is produced preferentially during re-crystallisation from water[18], the presence of 1.2M $BaCl_2$ leads to a different crystalline form[19] composed of α-CD molecules with an open, near-symmetrical structure and lacking any strained glycosidic linkages[19]. The ^{13}C-c.p.–m.a.s. spectrum (Fig. 1e) of α-CD crystallised from 1.2M $BaCl_2$ contains no signals at 98.1 and 77.7 p.p.m., but contains signals whose ranges of chemical shifts are similar to those observed for frozen aqueous solutions (Fig. 1b). These results strongly suggest that α-CD adopts an open, near-symmetrical structure in aqueous solution similar to that[19] obtained on crystallisation from 1.2M $BaCl_2$. Our observations appear to rule out a proposed "induced-fit" mechanism[17] for the formation of inclusion complexes of α-CD in aqueous solution, which is based on the relief of conformational strain by complexation.

We have studied also solutions of the (1→4)-α-D-glucans, amylose and amylopectin. Fig. 2a shows the single-pulse excitation ^{13}C-n.m.r. spectrum of an aqueous solution of amylopectin at 30°, and Fig. 2b shows the ^{13}C-c.p.–m.a.s. spectrum of the same solution at −40°. The range of chemical shifts in the latter spectrum is similar to that found in the spectra of amorphous solid (1→4)-α-D-glucans[20] (Fig. 2c) and is particularly large (~12 p.p.m.) for the C-1 signal which is considered to be sensitive to conformational features[12,13]. The C-1 spectrum for amorphous (1→4)-α-D-glucans can be modelled[13] by assuming that all energetically allowed conformations are present. To a first approximation, this approach appears to be true also for aqueous solutions of amylopectin (Fig. 2). Different chemical shifts are observed for the resonances of C-4 at 30° (78.6 p.p.m., Fig. 2a) and −40° (82–83 p.p.m., Fig. 2b) for aqueous solutions of amylopectin, suggesting either a (reversible) conformational change or, more likely, that the chemical shift of the

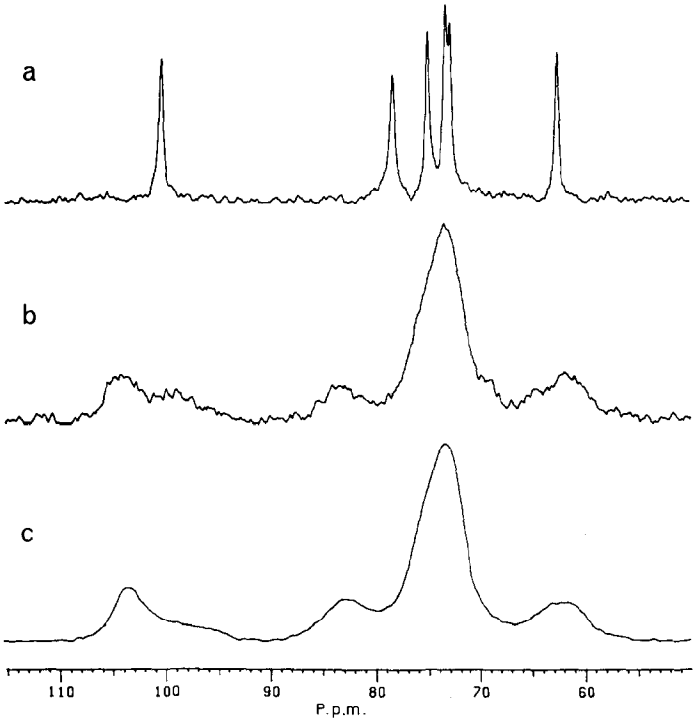

Fig. 2. (*a*) A single-pulse excitation ^{13}C-n.m.r. spectrum at 30° of an aqueous 10% solution of amylopectin lightly degraded by acid to improve solubility, (*b*) a ^{13}C-c.p.–m.a.s. n.m.r. spectrum of the same solution at −40° [on re-heating to 30°, a spectrum identical to that in (*a*) was obtained], (*c*) a ^{13}C-c.p.–m.a.s. n.m.r. spectrum of solid amorphous (1→4)-α-D-glucan[15] at 30° (essentially the same spectrum was observed at −40°).

C-4 resonances may not be determined solely by conformational effects. Indeed, no single simple relationship between these chemicals shifts and conformational features was found for a range of solid (1→4)-α-D-glucans[13]. Frozen solutions of amylose in methyl sulphoxide, which are thought to involve ordered helical structures[21], show a range of chemical shifts of 2–3 p.p.m. for the C-1 resonances consistent with a more defined conformation and chemical shift values similar to those observed for solid single-helical (V-type) amylose structures[13].

The above results suggest that ^{13}C-c.p.–m.a.s. n.m.r. spectroscopy can provide an approach to the characterisation of the conformational features of carbohydrates in frozen solutions and, by implication, of the range of conformations adopted in free solution. Extension of the methodology to systems for which solid-state model structures are not available awaits further progress in the determination of conformation/chemical shift relationships[12,13].

EXPERIMENTAL

All spectra were recorded on a Bruker CXP-300 instrument operating at 75.46 MHz. Chemical shifts are referenced to Me_4Si *via* the low-field resonance of solid adamantane (38.6 p.p.m.) in a ^{13}C-c.p.–m.a.s. spectrum recorded immediately prior to the sample of interest. Spectra of the same samples at different temperatures were fully reversible on freeze–thaw cycling.

REFERENCES

1 S. W. HOMANS, R. A. DWEK, AND T. W. RADEMACHER, *Biochemistry*, 26 (1987) 6571–6578.
2 J. R. BRISSON AND J. P. CARVER, *Can. J. Chem.*, 61 (1983) 1067–1078.
3 H. PAULSEN, T. PETERS, V. SINNWELL, AND M. HEUME, *Carbohydr. Res.*, 156 (1986) 87–106.
4 K. BOCK, J. ARNARP, AND J. LOENNGREN, *Eur. J. Biochem.*, 129 (1982) 171–178.
5 D. A. REES, E. R. MORRIS, D. THOM, AND J. K. MADDEN, in G. O. ASPINALL (Ed.), *The Polysaccharides*, Vol. 1, Academic Press, Orlando, 1982, pp. 195–290.
6 H. THOGERSEN, R. U. LEMIEUX, K. BOCK, AND B. MEYER, *Can. J. Chem.*, 60 (1982) 44–57.
7 R. U. LEMIEUX AND S. KOTO, *Tetrahedron*, 30 (1974) 1933–1944.
8 A. PARFONDRY, N. CYR, AND A. S. PERLIN, *Carbohydr. Res.*, 59 (1977) 299–309.
9 D. A. BRANT, *Q. Rev. Biophys.*, 9 (1976) 527–596.
10 O. JARDETZKY, *Biochim. Biophys. Acta*, 621 (1980) 227–232; D. A. CUMMING AND J. P. CARVER, *Biochemistry*, 26 (1987) 6664–6676.
11 Y. INOUE, T. OKUDA, AND R. CHUJO, *Carbohydr. Res.*, 141 (1985) 179–190.
12 R. P. VEREGIN, C. A. FYFE, R. H. MARCHESSAULT, AND M. G. TAYLOR, *Carbohydr. Res.*, 160 (1987) 41–56.
13 M. J. GIDLEY AND S. M. BOCIEK, *J. Am. Chem. Soc.*, 110 (1988) 3820–3829.
14 H. SAITO, *Magn. Reson. Chem.*, 24 (1986) 835–852.
15 K. BOCK, A. BRIGNOLE, AND B. W. SIGURSKJOLD, *J. Chem. Soc., Perkin Trans. 2*, (1986) 1711–1713.
16 M. J. GIDLEY AND S. M. BOCIEK, *J. Chem. Soc., Chem. Commun.*, (1986) 1223–1226.
17 P. C. MANOR AND W. SAENGER, *J. Am. Chem. Soc.*, 96 (1974) 3630–3639.
18 K. LINDNER AND W. SAENGER, *Acta Crystallogr., Sect. B*, 38 (1982) 203–210.
19 K. K. CHACKO AND W. SAENGER, *J. Am. Chem. Soc.*, 103 (1980) 1708–1715.
20 M. J. GIDLEY AND S. M. BOCIEK, *J. Am. Chem. Soc.*, 107 (1985) 7040–7044.
21 M. ST-JACQUES, P. R. SUNDARAJAN, K. J. TAYLOR, AND R. H. MARCHESSAULT, *J. Am. Chem. Soc.*, 98 (1976) 4386–4391.