CHEMISTRY LETTERS, pp. 713-716, 1981.

^{13}C CHEMICAL SHIFTS OF SOLID (1→4)-α-D-GLUCANS BY CROSS POLARIZATION/MAGIC ANGLE SPINNING (CP/MAS) NMR SPECTROSCOPY. CONFORMATION-DEPENDENT ^{13}C CHEMICAL SHIFT AS A REFERENCE IN DETERMINING CONFORMATION IN AQUEOUS SOLUTION

Hazime SAITÔ* and Ryoko TABETA
Biophysics Division, National Cancer Center Research Institute, Tsukiji 5-chome, Chuo-ku, Tokyo 104

^{13}C chemical shifts of linear and cyclic amyloses in solid state were measured by cross polarization/magic angle spinning (CP/MAS) NMR spectroscopy. It is found that rapid conformational isomerism occurs in aqueous solution for amylose and its linear oligomer with lifetime $<10^{-3}$s with reference to the conformation-dependent ^{13}C chemical shifts obtained in solid state.

Previously, we have demonstrated that ^{13}C chemical shifts of backbone-carbons are very sensitive to conformational changes of biopolymers such as polypeptides,[1] polynucleotides[2] and polysaccharides.[3,4] Such a sensitivity, however, is in many instances obscured by the presence of rapid time-averaging of chemical shifts from different conformers caused by conformational isomerism, if lifetime of a certain fixed conformer is short compared with NMR time scale. Thus, it is essential to have a knowledge whether ^{13}C chemical shifts under consideration are time-averaged or not for the application of ^{13}C NMR spectroscopy to the conformational problems. In principle, this can be done by observing conformation-dependent ^{13}C chemical shift as a reference if an appropriate compound with known fixed conformation is available.[1-4] Such a procedure, however, cannot always be achieved for many cases, especially for oligo- and polysaccharides to be studied in this paper. An alternative but more general approach is to compare the ^{13}C chemical shifts in solution with those in solid state[5] when conformation of the latter is known by X ray diffraction or other.

Here we demonstrate the latter approach to reveal the conformation-dependent ^{13}C chemical shift of (1→4)-α-D-glucans (amyloses) as a reference in probing conformation in aqueous solution. Typically, amylose in anhydrous crystalline form is known to adopt left-handed six-fold helix (V form).[6] Conformation-behavior in aqueous solution, however, is a matter of dispute: tight helical,[7] extended helical[8] and random-coiled structures[9] have been presented. In addition, it was previously pointed out[10] that substantial upfield displacements of the ^{13}C chemical shifts (of C-1 and C-4 signals) in neutral aqueous solution of amylose with respect to those of cycloamyloses could be ascribed to the presence of the conformational isomerism in the former.

Amyloses with DP's 25 and 100 are products of Hayashibara Biochemicals (EX-1 and 111, respectively). Cycloamyloses and maltotriose were purchased from Seikagaku Kogyo, Tokyo. Mostly, powder samples as received were used for the CP/MAS experiment.[11] "V" amylose was prepared by a procedure of crystallization with n-butyl alcohol followed by drying at 40° for 24 h.[12] Amyloses in aqueous solution (20 mg/ml) were prepared by dissolving the powder samples to D_2O at pH 13 followed by neutralization to pH 7. 75.46 MHz ^{13}C CP/MAS and ordinary high resolution NMR spectra were recorded by a Bruker CXP-300 spectrometer with and without a CP/MAS accessary, respectively.

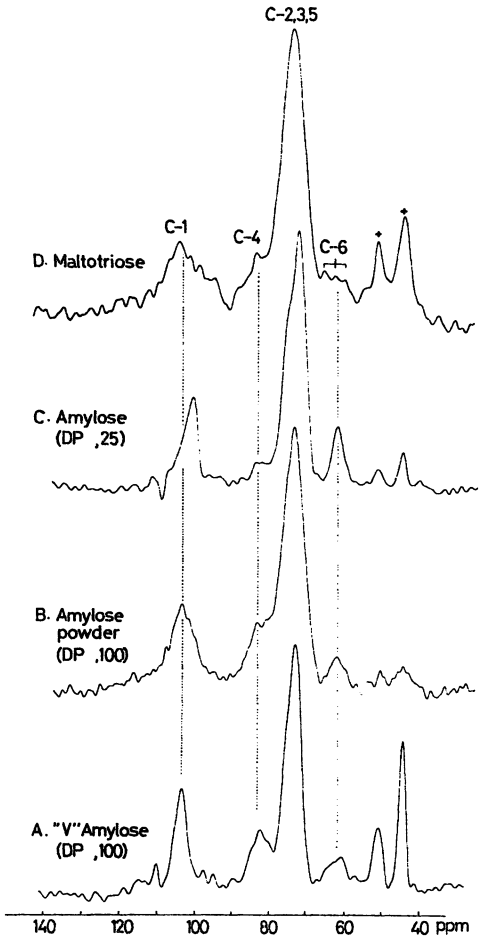

Figure 1. 75.46 MHz ^{13}C CP/MAS NMR spectra of amylose and its oligomer in solid state. A."V" amylose(DP 100), B.Amylose powder(DP 100), C.Amylose (DP 25), and D.Maltotriose. Peaks designated by + are from the rotor.

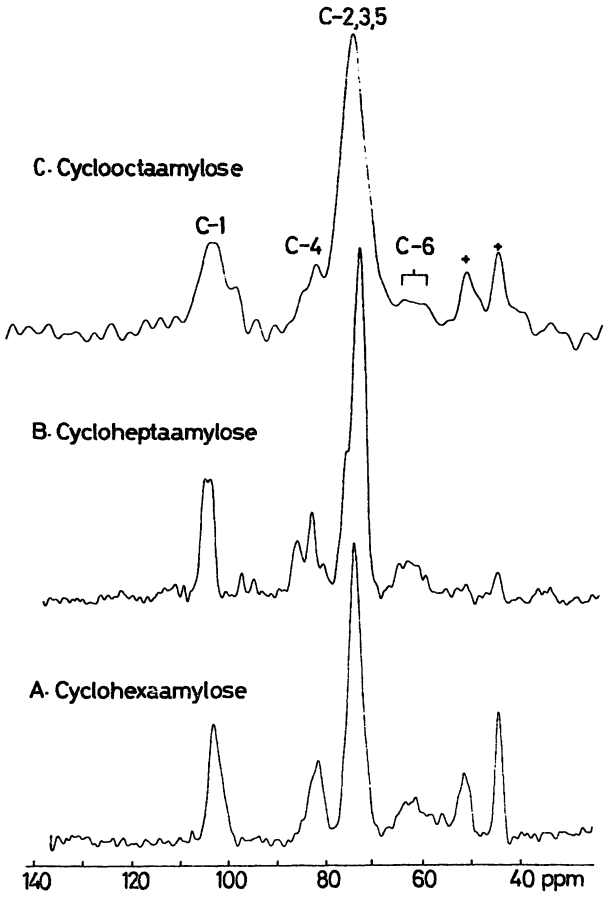

Figure 2. 75.46 MHz ^{13}C CP/MAS NMR spectra of cycloamyloses in solid state. A. Cyclohexaamylose, B. Cycloheptaamylose, and C. Cyclooctaamylose. Spectral condition for Figs.1 and 2 is the same as that of ref. 5.

It appears from Figure 1 that the ^{13}C signals of C-1, C-4 and C-6 of amylose and its oligomer are well resolved but the C-2, C-3 and C-5 signals are overlapped because of increased linewidths compared with those in aqueous solution (Table 1). Nevertheless, the extent of peak separation is excellent for the present purpose, because ^{13}C signals from carbon at the glucosidic linkages (C-1 and C-4) are mainly sensitive to conformational changes. For the high molecular weight amylose (DP 100), there appears very little change of the ^{13}C chemical shifts between amylose powder (as received) and "V" amylose, except for the substantial narrowing in the linewidths in the latter. Thus, the improved crystallinity in the present "V" amylose sample seems significantly to contribute to the narrowing of the linewidths. As pointed out previously,[5] ^{13}C chemical shift is sensitive to the intramolecular contribution such as conformation of molecular chains rather than that of packing of molecules to the crystalline portion. For this reason, major conformer in the amylose powder is not strongly deviated from that of the "V" amylose as viewed from the similarity of the chemical shift positions, although a significant dispersion of the chemical shifts due to the superposition of conformers slightly deviated from the "V" amylose structure is mainly responsible for the increased linewidths in the amylose powder.

Another interesting feature is that the C-1 ^{13}C chemical shift of the lower molecular weight amylose (DP 25) is shifted upfield by amount of 3 ppm as compared with that of the high molecular

Table 1. Comparison of ^{13}C chemical shifts of (1→4)-α-D-glucans in solid state with those in aqueous solution (pH 7)(ppm from TMS; ± 0.5 ppm for solid samples)†**

	Linear glucans						Cyclic glucans					
	Amylose(DP 100)		Amylose(DP 25)		Maltotriose		Cyclohexa-amylose		Cyclohepta-amylose		Cycloocta-amylose	
	solid	soln.	solid	soln.	solid	soln.	solid	soln.	solid	soln.	solid	soln.
C-1	102.6	100.3	99.5	100.3	102.9	99.8	101.5	102.5	102.8 104.2	102.5	101.2 103.2	102.4
C-4	82.4	77.9	82.9†† ~78 *	77.9	82.8	77.0	81.2	81.9	82.0 85.2	81.8	82.2	81.2
C-3		74.2		74.2		73.4		74.0		73.8		73.6
C-2	73.2	72.4	72.1	72.4	73.7	72.9	72.5	72.7	72.5	72.7	72.9	73.0
C-5		72.0		72.0		71.8		72.4		72.5		72.5
C-6	62.4	61.4	61.8	61.4	59.5 62.1 65.2	60.5	60.5 63.4	61.2	58.8 64.4	61.0	58.6 62.3	61.0

† Assignment of peaks based on refs. 11 and 13. †† Peak-intensity decreased to~50%.
* Displaced peak at the shoulder of C-2,3,5 signal. ** The slight difference of the ^{13}C chemical shifts observed in aqueous solution with those from ref. 11 is due to the magetic susceptibility effect in the iron magnet system.

weight amylose (Table 1). In addition, the peak intensity of the C-4 signal in this case is substantially decreased (~50%) compared with that of the high molecular weight amylose. It is likely that the suppressed peak-intensity of the C-4 signal is caused by the upfield displacement (~5 ppm) resulting in superposition upon the intense C-2,3,5 signals as manifested from the enhanced shoulder peak and consistent with the concomitant upfield displacement of the C-1 signal mentioned above. Interestingly, the ^{13}C chemical shifts of the C-1 and C-4 (superimposed on the shoulder of the C-2,3,5 signals) of low molecular weight amylose in solid state are very close to those obtained in aqueous solution (pH 7, see Table 1). In contrast, maltotriose is found to give rise to the ^{13}C chemical shifts of the C-1 and C-4 similar to those of the high molecular weight amylose in solid state. Again, the ^{13}C chemical shifts in aqueous solution are substantially deviated from those observed in solid state. These findings strongly suggest that the ^{13}C chemical shifts of maltotriose in aqueous solution and low molecular weight amylose both in solid and solution states are time-averaged by rapid conformational isomerism, resulting in the conformational behavior like random-coil. The presence of the conformational isomerism in solid state of low molecular weight glucan is consistent with our previous finding on (1→3)-β-D-glucans.[5] On the contrary, the ^{13}C chemical shifts of high and low molecular weight amyloses are identical in aqueous solution (Table 1). Therefore, the substantial differences of the ^{13}C chemical shifts (C-1 and C-4) of the high molecular weight amylose between solid and solution states should be ascribed to the rapid conformational isomerism in aqueous solution. Nevertheless, it was shown[14] on the basis of circular dichroism spectra that amylose and its oligomers adopt similar helical conformer in aqueous solution to that of the V form. Obviously, such a discrepancy arises from differences in time-scale of observation between NMR and optical methods. On the basis of the conformation-dependent ^{13}C chemical shifts and well-known formula of chemical exchange process,[15] the lifetime of the helical conformer in aqueous solution is estimated as shorter than 10^{-3}s.

Figure 2 shows ^{13}C NMR spectra of a series of solid cycloamyloses. As expected from the similarity of the conformational angles[16] with those of the V amylose, the ^{13}C chemical shifts of the C-1 and C-4 of cyclohexaamylose are very similar to those of the high molecular weight amylose in solid state. Unexpectedly, however, those ^{13}C signals of cyclohepta- and cyclooctaamylose are

splitted into at least two sets of signals. We found that in aqueous solution these compounds gave only six resolved signals and no substantial difference of the chemical shifts was observed between solution and solid states, in contrast to the case of the linear (1→4)-α-D-glucans as mentioned above. Accordingly, such a splitting of peaks should be ascribed to the presence of at least two kinds of different conformers in solid state. This may be explained in terms of the presence of distorted glucosidic linkages due to less symmetrical nature of "empty" cycloamyloses, as pointed out by Saenger and coworkers for cyclohexaamylose.[17]

Interestingly, another class of conformation-dependent ^{13}C chemical shift is also seen as obvious splittings of the C-6 signals caused by several different orientations of the hydroxymethyl groups with respect to the C(5)-O(5) and C(5)-C(4) bonds in maltotriose, "V" amylose and cycloamyloses (Figs. 1 and 2). As expected, the magnitude of the maximum displacement of the ^{13}C shifts is almost the same as that of the carbons at the glucosidic linkages. More detailed account of these conformation-dependent ^{13}C chemical shifts will be described elsewhere together with the effect of formation of inclusion complex.

In conclusion, the ^{13}C chemical shifts of amylose and its oligomer in aqueous solution are modulated by rapid conformational isomerism around the glucosidic linkage with reference to the conformation-dependent ^{13}C chemical shifts obtained from the solid-state ^{13}C NMR measurements.

References

1) H. Saitô, T. Ohki, M. Kodama, and C. Nagata, Biopolymers, 17, 2587 (1978) and references therein.
2) M. Miwa, H. Saitô, H. Sakura, N. Saikawa, F. Watanabe, T. Matsushima, and T. Sugimura, Nucleic Acids Res., 4, 3997 (1977).
3) H. Saitô, T. Ohki, and T. Sasaki, Biochemistry, 16, 908 (1977).
4) H. Saitô, in "Solution Properties of Polysaccharides", ed. by D. A. Brant, ACS Symposium Series, No. 150, American Chemical Society, 1981, Chapter 10 (in press).
5) H. Saitô, R. Tabeta, and T. Harada, Chem. Lett., 1981, 571.
6) W.T. Winter, and A. Sarko, Biopolymers, 13, 1447 (1974).
7) J. Szejtli, Starke, 23, 295 (1971).
8) M. B. Senior, and E. Hamori, Biopolymers, 12, 65 (1973).
9) W. Banks, and C. T. Greenwood, Makromol. Chem., 67, 49 (1963).
10) P. Colson, H. J. Jennings, and I. C. P. Smith, J. Am. Chem. Soc., 96, 8681 (1974).
11) J. Schaefer, and E. O. Stejskal, in "Topics in Carbon-13 NMR Spectroscopy", ed. by G. C. Levy, Wiley-Intersciences, 1979, Vol. 3, 283.
12) J. J. Cael, J. L. Koenig, and J. Blackwell, Carbohydr. Res., 29, 123 (1973).
13) A. Heyraud, M. Rinaudo, M. Vignon, and M. Vincendon, Biopolymers, 18, 167 (1979).
14) D. G. Lewis, and W. C. Johnson, Jr., Biopolymers, 17, 1439 (1978).
15) J. A. Pople, W. G. Schneider, and H. J. Bernstein, "High-resolution Nuclear Magnetic Resonance", McGraw-Hill Book Company, Inc., 1959, Chapter 10.
16) A. Hybl, R. E. Rundle, and D. E. Williams, J. Am. Chem. Soc., 87, 2779 (1965).
17) P. C. Manor, and W. Saenger, Nature, 237, 392 (1972); P. C. Manor and W. Saenger, J. Am. Chem. Soc., 96, 3630 (1974); W. Saenger, M. Noltemeyer, P. C. Manor, B. Hingerty, and B. Klar, Bioorg. Chem., 5, 187 (1976).

(Received April 4, 1981)