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Cross-Polarization Magic Angle Spinning Carbon-13 Nuclear Magnetic Resonance Study of Linear Dextran Crystallized from Poly(ethylene glycol)–Water Solution at a High Temperature

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ABSTRACT: The solid-state structure of a synthetic linear dextran, which was crystallized at 160 °C from a mixture of water and poly(ethylene glycol), has been investigated by CP/MAS ^{13}C NMR spectroscopy. The CP/MAS ^{13}C NMR resonances narrow upon absorbing water without any change in chemical shifts, suggesting that some distortion of the molecular chains, which is produced by drying, may be relaxed upon the sorption of water. Each resonance line of wet dextran contains two components with ^{13}C spin–lattice relaxation times, T_{1C}, of 170–203 and 8.0–11.4 s, which correspond to the crystalline and noncrystalline components, respectively. When the difference in T_{1C} is used, separate spectra of the crystalline and noncrystalline components have been obtained. The line-shape analysis shows that the crystalline spectrum consists of six doublets with a 1:1 ratio corresponding to six carbon sites. These six doublets may originate from two different electronic environments produced by two different packing arrangements. The degree of crystallinity is approximately estimated to be 46% by the line-shape analysis, and the lamellar thickness is determined as 60 Å from SAXS. From these values the crystalline and noncrystalline thicknesses are assumed to be of the orders of 28 and 16 Å, respectively.

Introduction

Native dextran (α-1,6-glucan) is produced, for example, from saccharoses by *Leuconostoc mesenteroides*,[1] but it is not readily crystallizable because of the presence of branches. In contrast, linear dextran can be chemically synthesized by the polymerization of 1,6-dianhydro-2,3,4-tri-*O*-benzyl-β-D-glucopyranose following the debenzylation.[2] Chanzy et al.[3,4] have found that such a linear dextran can be crystallized from dilute solution in the form of lamellar single crystals, provided low molecular weight fractions are used. In addition, linear dextran exhibits two polymorphs; a "low-temperature" polymorph obtained below 100 °C and a "high-temperature" polymorph crystallized above 120 °C.[4-6] The principal difference between these polymorphs is that the high-temperature polymorph is anhydrous while the low-temperature crystalline form is hydrated.

In this paper we have investigated the solid-state structure of linear dextran, which was crystallized at 160 °C from dilute solution according to the previous method,[4] by cross-polarization (CP)/magic angle spinning (MAS) ^{13}C NMR spectroscopy. The NMR analysis is based on the methods previously studied for the solid-state structure of α-1,4- and β-1,4-glucans such as amylose[7,8] and cellulose.[9-14] The resolution enhancement of the resonance lines is made by measuring the NMR spectra in the presence of water. Then the contributions of the crystalline and noncrystalline components in the spectra are separately recorded by using the difference in ^{13}C spin–lattice relaxation times (T_{1C}) between the two components. On the basis of these spectra and the T_{1C} values, we discuss the crystalline and noncrystalline structures of the dextran sample.

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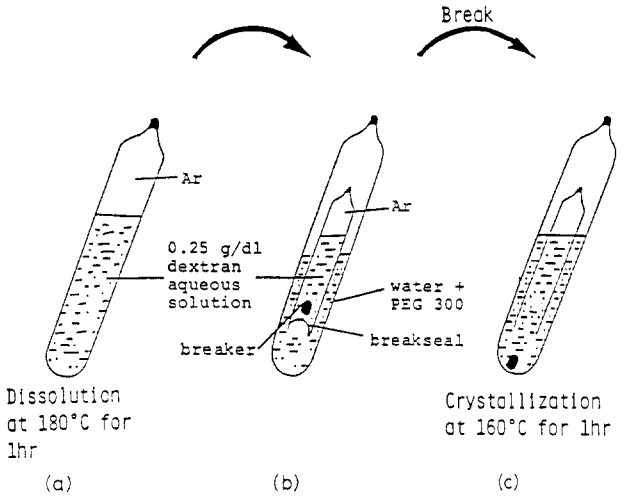

Figure 1. Crystallization procedures for the dextran sample with a high-temperature polymorph.

Experimental Section

Sample. The linear dextran was prepared by cationic polymerization of 1,6-dianhydro-2,3,4-tri-*O*-benzyl-β-D-glucopyranose in methylene chloride at −78 °C followed by debenzylation of the product.[2] The number average molecular weight of the resultant polymer was determined to be 13 000 by GPC. The crystallization of the dextran was carried out by the procedure developed by Chanzy et al.[4] The basic steps are shown in Figure 1. A 0.25% aqueous solution of dextran was sealed in a glass tube under an argon atmosphere and heated at 180 °C for 1 h in an autoclave (Figure 1a). The solution was cooled and filtered for purification. The filtered solution was again sealed in an ampule with a break-seal. This ampule was further sealed in a larger glass tube together with an appropriate amount of aqueous solution containing 90% poly(ethylene glycol) (PEG; M_n = 300). The composition of PEG of the solution was chosen so that the crystallization of the dextran was performed in PEG–water of 80/20 (v/v) (Figure 1b). This larger glass tube was placed inside an autoclave immersed in an oil bath at 160 °C. The autoclave was shaken in order to break the seal and to mix the dextran solution with the outer PEG solution. The subsequent crystallization was conducted at 160 °C for 1 h (Figure 1c). The resulting dextran crystals were collected, washed by successive centrifugations in methanol, and dried at 50 °C in vacuo for 2–3 days. A wet sample was obtained by exposure to an atmosphere with a relative humidity of 95%. After conditioning, the sample was packed in a MAS rotor and allowed to equilibrate in the same atmosphere for a few more days.

NMR Measurements. CP/MAS ^{13}C NMR spectra were measured at room temperature on a JEOL JNM-FX200 spectrometer operating under a static magnetic field of 4.7 T. ^1H and ^{13}C radio frequency field strengths, $\gamma B_1/2\pi$, were 69.4 kHz for the CP process, while the ^1H dipolar decoupling field was reduced to 54 kHz. The contact time was 1 ms, and the recycle time after collection of the FID was 10 s. A MAS rotor with an O-ring seal[7,11] was used for both dry and wet samples, and the MAS rate was 3.1–3.4 kHz. ^{13}C chemical shifts relative to tetramethylsilane were determined by using the peak at 32.89 ppm of a small chip of crystalline linear polyethylene inserted as an internal standard.

X-ray Diffraction. X-ray diffraction patterns for the dry and wet samples sealed in capillary tubes were recorded on a JEOL DX-GE-2PX instrument using nickel-filtered Cu Kα radiation. The long period of the dextran samples was determined by small-angle X-ray scattering measurements using a Kratky compact camera.

Results and Discussion

Figure 2 shows the wide-angle X-ray diffraction patterns of the dry and wet dextrans. Both samples display the same set of *d*-spacings, but the diffraction pattern of the wet sample is somewhat sharper than that of the dry dextran, indicating a greater level of order in the crystalline regions of the former. The similarity of the observed value of *d*-spacing to the previous results[4] indicates that the crystal structure of the sample prepared in this study is identical with the high-temperature polymorph found by Chanzy et al.[4]

Figure 3 shows 50-MHz CP/MAS ^{13}C NMR spectra obtained for the dry and wet dextrans together with a stick-type scalar-decoupled ^{13}C NMR spectrum of the same dextran in solution. It is clear that water enhances the resolution of the resonance lines, although it induces no change in chemical shifts as shown in Table I. This observation is in good accord with the case of cellulose.[11-13] Therefore, the sharpening of the X-ray diffraction pattern and the narrowing of NMR spectrum for the wet dextran suggest that some distortion of the molecular chains, produced upon drying, may be relaxed upon the sorption of water.

The assignments of C1, C2, C3, and C4 carbons were made based on the corresponding solution spectrum.[15] As already reported[9] in the case of cellulose samples, resonance lines of C1, C4, and C6 carbons shift downfield by 2.3–9.6 ppm in the solid state compared to those in the solution state. Such large downfield shifts are mainly attributed to the different conformations about the β-1,4-glycosidic linkage and the exocyclic C5–C6 bond in which these carbons are involved: The chemical shifts of the carbon sites C1, C4, and C6 are correlated to the torsion angles ϕ and ψ about the β-1,4-glycosidic linkage and χ about the C5–C6 bond, respectively. In contrast, effects of packing and hydrogen bonding may produce only 0.2–2.4 ppm downfield or upfield shift for the conventional carbohydrates.[9] On the basis of these results, the chemical shifts of C1, C5, and C6 carbons of dextran may be correlated to three torsion angles ϕ, ψ, and ω about the α-1,6-glycosidic linkage, respectively, as shown in Figure 3. The chemical shift of C1, C5, and C6 carbons may therefore shift to some extent in the solid state compared to the solution state, depending on the different conformations. We believe that the chemical shift of the C5 carbon is larger than that of the C6 carbon in accord with the solution spectrum, though this assignment should be reexamined in future. The C1 resonance consists of one doublet and a very broad peak, which may be ascribed to the crystalline and noncrystalline components, respectively. In order to confirm this, we have measured the ^{13}C spin–lattice relaxation time, T_{1C}, by Torchia's pulse sequence.[16]

Figure 4 shows the semilogarithmic decay curves of the respective peak intensities in the ^{13}C spin–lattice relaxation spectrum for the wet dextran. Each decay curve contains two components with different T_{1C} values, 170–203 and 8.9–11.4 s, which are assigned to the crystalline and noncrystalline components, respectively. In Table II the ^{13}C spin–lattice relaxation time, T_{1C}, are compiled for the carbons of the dry and wet samples. The T_{1C}'s of the crystalline component of the wet sample are 20–30% shorter than those of the dry sample, suggesting somewhat higher molecular mobility in the wet state. Such increase in molecular mobility may also induce the ordering of the crystalline region and lead to the narrowing of each resonance line. There is also significant change in the T_{1C}'s of the noncrystalline component of the wet dextran. This also suggests that water alters the molecular mobility of the noncrystalline chains, but the rubberlike

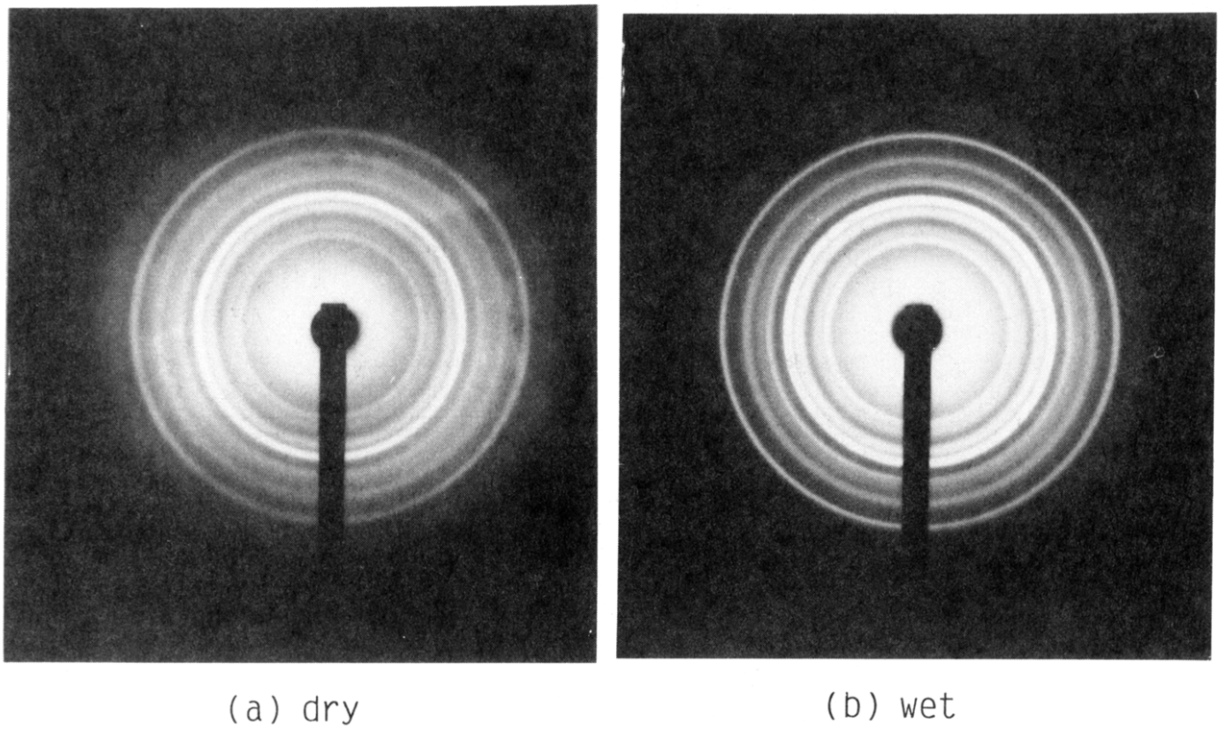

Figure 2. Wide-angle X-ray diffraction patterns of dry (a) and wet (b) dextrans.

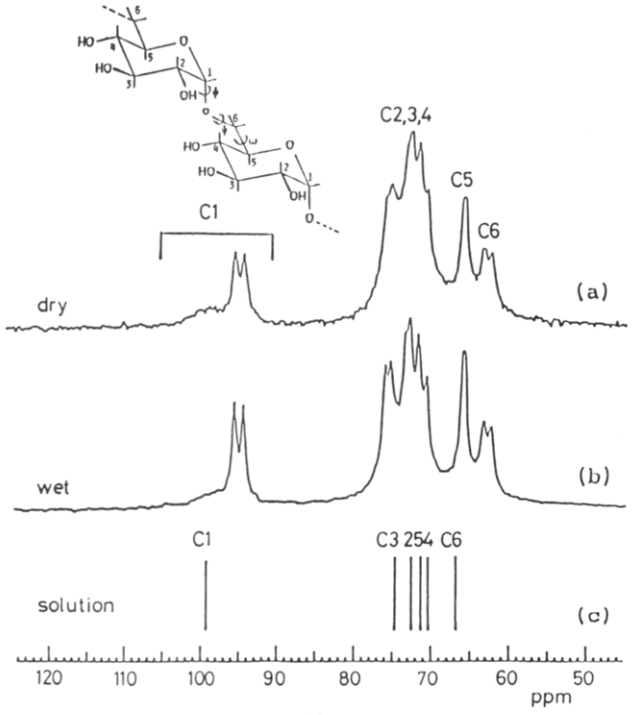

Figure 3. 50-MHz CP/MAS ^{13}C NMR spectra of dry (a) and wet (b) dextrans and stick-type scalar-decoupled ^{13}C NMR spectrum of dextran in D_2O solution (c).

component with much higher mobility seems not to appear under such an experimental condition. We have used the large differences in T_{1C} between the crystalline and noncrystalline components to obtain separate spectra of these components.

Figure 5a shows the spectrum of the crystalline component of the wet dextran recorded by Torchia's pulse sequence by setting the delay time, t, for the longitudinal relaxation in the CPT1 experiment[16] to 100 s. Here the t value is more than 5 times the T_{1C}'s of the noncrystalline components of dry and wet dextrans, and then

Table I
^{13}C Chemical Shifts of the Dry and Wet Dextran Samples

	chemical shifts, ppm			
sample	C1	C2, 3, 4	C5	C6
dry	95.02	75.27, 72.59, 71.08	65.20	62.57
	93.80	74.63, 72.10, 69.96		61.79
wet[a]	94.90	75.27, 72.64, 71.13	65.29	62.67
	93.71	74.68, 72.10, 69.96	65.00	61.69

[a] Conditioned in an atmosphere of RH 95%.

the contribution from the noncrystalline component disappears under this condition. It seems that the crystalline spectrum consists of six doublets corresponding to the six carbon species, although the doublet of the C5 carbon resonance is not clear in this figure.[17] Therefore, each resonance was resolved into two Lorentzians using a nonlinear least-squares method by a computer. As shown in Figure 5b, the composite curve is in good accord with the observed curve for each carbon. Hence, it is concluded that the crystalline spectrum is composed of six doublets, and each doublet is described in terms of two Lorentzians with almost equal line widths and intensities.

According to the crystal structure of dextran determined through a combined electron and X-ray diffraction analysis and stereochemical model refinement,[5] two antiparallel packed molecular chains pass through the unit cell, and the asymmetric unit contains two glucopyranose residues A and B. The conformational angles ϕ, ψ and ω, of the A and B residues are different from each other, and moreover the residues A and B have different packing arrangements. These features lead to two different environments for the carbons, and thus the resonance lines for the respective carbons become doublets.

Figure 6c shows the spectrum of the noncrystalline component, which was obtained by subtracting the spectrum of the crystalline component (Figure 6b) from the whole spectrum (Figure 6a). The glass transition temperature of the dextran, obtained by partial acid hydrolysis of native dextran, is 140 °C.[1] Accordingly, the broader

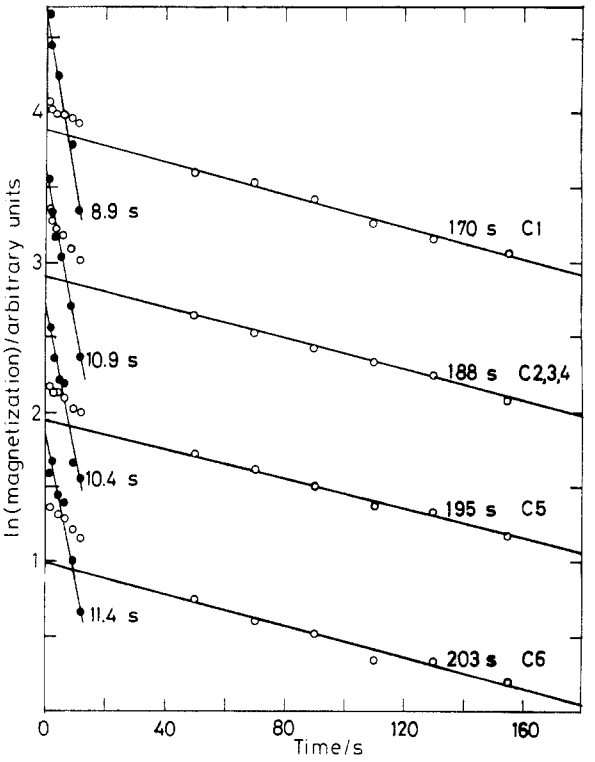

Figure 4. ^{13}C longitudinal decays for wet dextran. Each curve was shifted arbitrarily along the ordinate.

Table II
^{13}C Spin-Lattice Relaxation Times T_{1C} of the Respective Carbons of the Dextran Samples

sample		T_1, s			
		C1	C2, 3, 4	C5	C6
dry	C[a]	246, 243	224, 200, 213	254	210
	N[b]	12.5, 11.7	11.6, 14.8, 18.7	7.6	14.7
wet	C[a]	170, 186	184, 188, 178, 182	195	203, 188
	N[b]	8.9, 10.1	8.0, 10.9, 11.2, 9.7	10.4	11.4, 9.9

[a] Crystalline component. [b] Noncrystalline component.

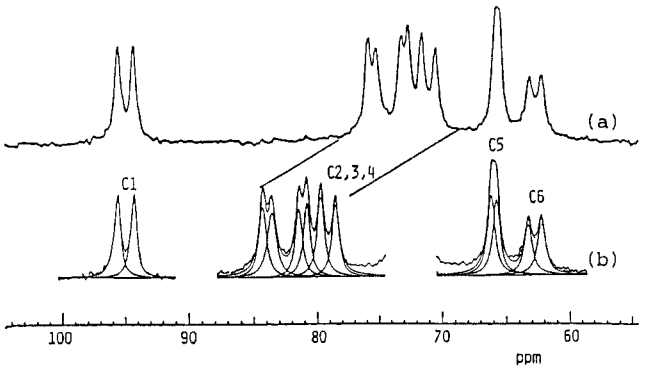

Figure 5. 50-MHz CP/MAS ^{13}C NMR spectrum of the crystalline component of wet dextran (a) and the results of lineshape analysis by the least-squares method (b).

lines of the noncrystalline component arise from a distribution of conformations resulting from the low molecular mobility as in the case for regenerated cellulose.[11-13]

It is well-known that the CP technique used to enhance the signal intensities may make the determination of the degree of crystallinity difficult for semicrystalline polymers such as polyethylene, because the CP efficiencies significantly differ between the crystalline and noncrystalline regions and thus their signal intensities are not proportional to their compositions. In a previous report,[10] we analyzed the CP process of different cellulose samples in detail and discussed the conditions necessary to obtain signal intensities proportional to the number of carbons. In the case of dry dextran sample, however, a significant difference in the proton spin-lattice relaxation time $T_{1\rho}^{H}$ in the rotating frame exists between the crystalline and noncrystalline components of the C1 carbon. Hence, it is questionable to apply the same analysis for the dry dextran as in the case of cotton cellulose. However, in order to obtain the spectrum exactly reflecting the contribution from all structural components by a 45° single pulse sequence, it is necessary that the recycle time is more than 3 times longer than T_{1C} after the collection of the FID. This is very time-consuming for the dextran sample, because the T_{1C} values are as long as 200–254 s. Therefore, we approximately estimate the degree of crystallinity by comparing the integrated intensities of the crystalline and noncrystalline components of the C1 resonance line in the CP/MAS ^{13}C NMR spectrum. Since the CP efficiency is usually higher for the crystalline region than for the noncrystalline region, the value thus obtained may be the maximum value of the crystallinity. The estimated degree of crystallinity is 46%.

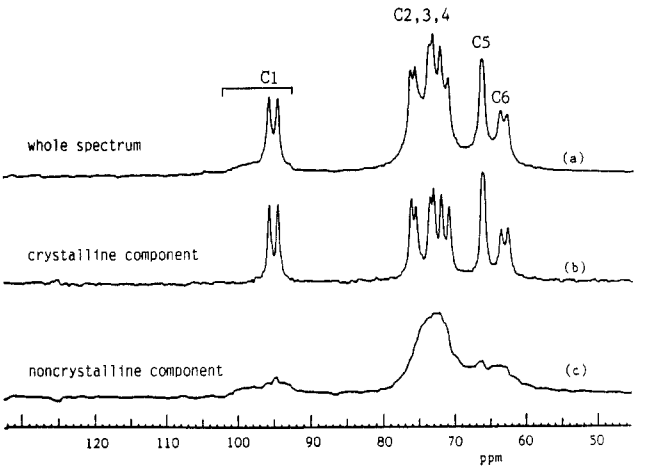

Figure 6. 50-MHz CP/MAS ^{13}C NMR spectra of wet dextran: (a) the whole spectrum, (b) the crystalline component, (c) the noncrystalline component.

Figure 7 shows an electron micrograph of the dextran sample. The lamellae radiate from the nucleation center of the spherulite. The thickness of the lamellae is estimated to be 70 Å, which corresponds well to the value obtained by Chanzy et al.[4] This value is also in good accord with the long period (60 Å) determined by small-angle X-ray scattering. Figure 8 shows a schematic structure model of dextran molecules in a lamellar crystallite. This model is drawn in analogy with the model for polyethylene single crystals that are composed of a lamellar crystalline phase and a noncrystalline overlayer.[7] The crystalline and noncrystalline thicknesses are estimated to be 28 and 16 Å, respectively, using the degree of crystallinity and the long period. Since the virtual bond length of a glucosidic residue determined by X-ray diffraction was 4.47 Å,[5] each loop of the noncrystalline chain may be composed of about seven glucosidic residues, provided the dextran chains are perpendicular to the plane of the lamellar crystals.

Conclusions

1. The solid-state structure of a synthetic linear dextran has been investigated by CP/MAS ^{13}C NMR spectroscopy. The synthetic dextran was prepared by cationic polymerization and crystallized at 160 °C from a mixture of water and poly(ethylene glycol).

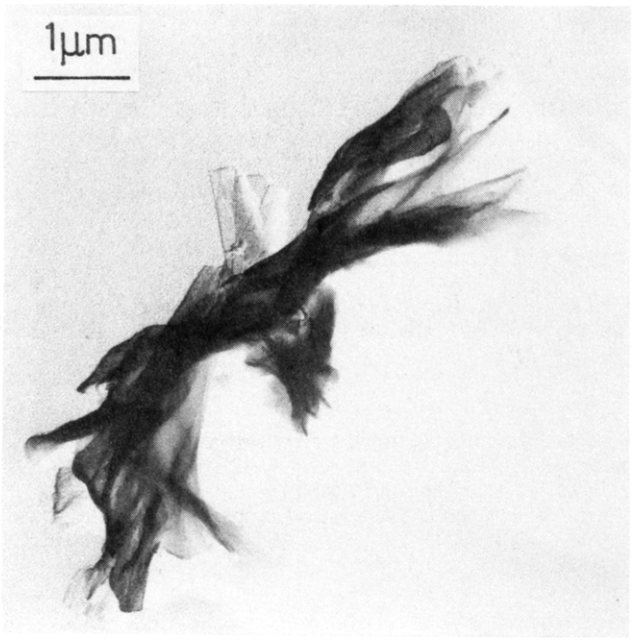

Figure 7. Electron micrograph of the dextran sample.

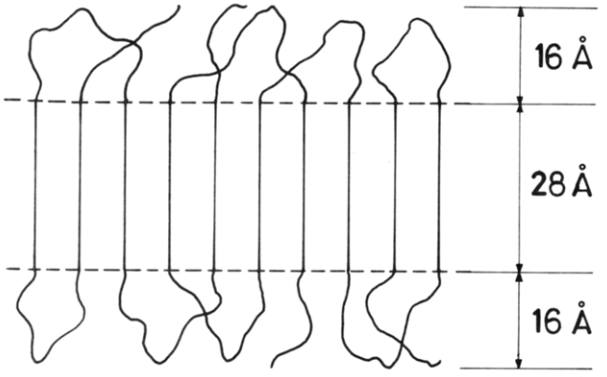

Figure 8. Schematic structure model of dextran molecules in a lamellar crystallite.

2. The resolution enhancement of the resonance lines is induced by the addition of water.

3. The contributions of the crystalline and noncrystalline components in the CP/MAS ^{13}C NMR spectrum are separately recorded by using the difference in T_{1C}'s between the two components.

4. The line-shape analyses show that the crystalline spectrum is composed of six doublets corresponding to the six carbon sites. These six doublets may originate from two different electronic environments produced by two different packing arrangements.

5. The crystalline and noncrystalline thicknesses are approximately estimated to be of the order of 28 and 16 Å, respectively, using the degree of crystallinity (46%) and the long period (60 Å).

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Registry No. Dextran, 9004-54-0.