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Amylose conformational transitions in binary DMSO/water mixtures

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The effects on amylose conformation of percentage water in dimethyl sulfoxide (DMSO)/water mixtures were measured by following changes in specific optical rotation, limiting viscosity number, and ^{13}C-NMR chemical shifts. The temperature dependence of specific optical rotation showed differences in amylose conformation at four chosen ratios of dimethyl sulfoxide/water. An amylose conformational change was also deduced from ^{13}C-NMR chemical shift data. Changes in limiting viscosity of amylose in different proportions of DMSO/water, and the effect of tetramethylurea on the specific rotation of amylose, indicate that intramolecular hydrogen bonding decreases with increased water content. 66.6% DMSO appears to be a crossover concentration, below which the helical conformation is progressively lost as water is added. When water content is over 60%, transition to a conformation which allows iodine complexation to take place is complete. A transition of amylose conformation from helix to loose helix to random coil with increasing water content was deduced from the experimental results. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Water, dimethyl sulfoxide (DMSO) and water–DMSO mixtures have been used widely as dispersing and solubilizing agents for starch and starch components. The conformational behaviour of amylose in these solvents is still a matter of some controversy. For the conformation of amylose in aqueous solution, Banks and Greenwood (1968, 1971; 1972), and Dintzis and Tobin (1969); Dintzis and Tobin (1978) suggested a random coil from experimental results based on optical rotation, limiting viscosity, light scattering and sedimentation measurements. This random coil model was considered as a combination of loose helical sections (each containing about 100 anhydroglucose units) with portions of random coil, the latter conferring flexibility on the molecule as a whole. When amylose forms a complex, the loose helix is forced into a tighter helical conformation by the complexing agent, which enters the central cavity, and the hydrodynamic volume of amylose decreases. The above interpretation (Senior and Hamori, 1973) is in agreement with kinetic studies on the amylose–iodine reaction (Tompson and Hamori, 1971).

The predominant conformation of amylose in DMSO appears to be tightly helical. The NMR spectra of amylose in DMSO-d_6 at increasing temperatures show differential upfield migration of the hydroxyl proton signals. This is considered to be evidence for intramolecular hydrogen bonding which is deemed necessary to form a helical structure (St-Jacques et al., 1976). Early work based on intrinsic viscosity $[\eta]$ and weight-average molecular weight (M_w) values led to differing conclusions: from a random coil conformation (Everett and Foster, 1959; Banks and Greenwood, 1968) to a helical conformation (Cowie, 1961; Fujii et al., 1973). These differences in interpretation have probably arisen because the weak dependence of these parameters on molecular weight in some amyloses has not always been recognised (Nakanishi et al., 1993).

As the water level is increased, the ^{13}C spectra of amylose in DMSO/water mixtures show greater downfield shifts of the signals for C-1 and C-4 than for other carbon atoms. This was considered to reflect the changes in the degree of compactness of the helical structures (Jane and Robyt, 1985).

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The difference in amylose conformation in DMSO vs. water, and the dependence of amylose–iodine complex formation on water content in DMSO/water mixtures (Peng and Perlin, 1987) suggest that there might be a transition and/or equilibrium of conformations occurring.

The conformational transitions are temperature-sensitive, especially for the helical structure. The conformational changes reflect the changes in the torsion angles of the glycosidic linkages, which have a close relationship to specific optical rotation (Rees, 1970), limiting viscosity (Everett and Foster, 1959) and chemical shift in ^{13}C-NMR (St-Jacques et al., 1976; Bock et al., 1986). Should amylose conformations over a range of DMSO/water mixtures be similar, values of the above parameters should be little affected. On the other hand, the degree of intramolecular hydrogen bonding in amylose over a range of DMSO/water mixtures could be expected to determine the properties of helices, e.g. from tight to loose. The aspect of tightness was examined via the effect of tetramethylurea on the specific optical rotation and limiting viscosity of amylose in DMSO/water mixtures.

EXPERIMENTAL

Preparation of amylose solutions

Amylose (2 g), (Koch-light Co.) purified by butanol complexation (Adkins and Greenwood, 1969), was stirred in DMSO (100 ml) at 80°C until the solution became clear. After centrifugation (5000 × g), the supernatant liquid was treated with ethanol (3 vol.) and recentrifuged. The precipitate was washed with ethanol, acetone and ether and dried under vacuum. Amylose solution was prepared for optical rotation and viscosity analysis by slurrying the sample (1.5 g) in DMSO (40 ml) and heating at 80°C for 16 h. After cooling, the sample solution was kept in a desiccator prior to analysis. Solutions for ratios of DMSO/water were prepared by diluting the above solution with distilled water.

Measurement of optical rotation

Optical rotations were measured at the sodium D line (589 nm) in a JASCO DIP-1000 polarimeter fitted with an automatic digital readout using a 1 ml thermostatted, quartz cell (path length, 10 cm). The cell temperature was raised from 25 to 70°C at 1°C min^{-1} by a Julabo VC-5 circulating waterbath and Model PRG-1 programmer. Cooling to room temperature was achieved by slow cold water circulation. Measurement of the optical rotation vs. temperature was carried out in the following solvents: anhydrous DMSO, 66.6% DMSO (DMSO/water=2:1), 50% DMSO (DMSO/water = 1:1) and 33.3% DMSO (DMSO/water = 1:2). The dimethyl sulfoxide (AJAX) was used directly from the same bottle which was kept tightly sealed in a vacuum desiccator. The average (±0.001°) of three recordings was taken. All readings were adjusted for the cell blank which contained the appropriate solvent at the corresponding temperature. The refractive indices of mixed solvents at different temperatures were measured with a Bellingham–Stanley Limited refractometer with Julabo VC-5 circulating waterbath. The theoretically calibrated values of specific rotation in mixed solvents at different temperature were calculated according to the equation:

$$(\alpha)_{T_2} = (\alpha)_{T_1}[(\bar{n}_{T_1}^2 + 2)/(\bar{n}_{T_1}^2 + 2)]/(\rho_{T_2}/\rho_{T_1})$$

where ρ_{T_2} and ρ_{T_1} are the mixed solvent density at temperatures T_2 and T_1. $(\alpha)_{T_2}$ and $(\alpha)_{T_1}$ are the optical rotations of the solution at T_2 and T_1. $\bar{n}_{T_2}$ and $\bar{n}_{T_1}$ are mixed solvent refractive indices at T_2 and T_1.

Determination of limiting viscosity

The viscosity number of amylose in DMSO or the mixed solvent was determined in a Cannon-Ubbelohde type capillary viscometer (BS/U size C). Solvent or solution (8 ml) was added to the viscometer in a water bath and equilibrated for 5 min. The average value of three determinations was taken. The variation over several readings was not greater than 0.1 s. Control experiments for all solvents were carried out. The amylose concentration dependence of the flow times was determined by subsequent dilutions with solvent. The flow time and amylose concentration data were converted to viscosity number $(\eta_r - 1)/C$, where η_r (relative viscosity) is the ratio of flow time of the solution to that of the corresponding solvent. The limiting viscosity number $[\eta]$ was obtained from the experimental data according to the Huggins relationship $\eta_{sp}/C = [\eta] + K_1[\eta]^2C$.

^{13}C-NMR spectra of amylose solutions

Carbon-13 NMR spectra were obtained on a Bruker WM-300 spectrometer, using the VTEXP.AU program. Amylose solutions as placed in a 10 mm sample tube containing a capillary of the external standard dioxane. During data acquisition, proton resonances were broad-band decoupled. Chemical shifts were measured by reference to external dioxane at 66.5 ppm. DMSO-d$_6$ (20%) was used for field-frequency lock. Temperature was set at 30°C (303 K), 40°C (313 K), 50°C (323 K), 60°C (333 K) and 70°C (343 K) respectively. Number of scans was 20 000. The main instrument settings used were: pulse width, 3 μs; pre-acquisition delay, 58.8 μs; dwell time, 45 μs. Data points (16 K) were zero filled to 32 K. Sweep width, 15 000 Hz. Line broadened 5 Hz and Fourier transformed. Chemical shift is reported in ppm. Samples were held 15 min at each temperature before data acquisition was started to ensure temperature equilibrium and to obtain reproducible results. Assignment of the carbon resonances was done by comparison with literature values (Jane and Robyt, 1985).

Influence of water on the conformation of amylose

The system contained amylose in DMSO (1 ml) + water (0.5–2 ml) + DMSO to make the total volume 3 ml. The mixed solution was stirred quickly, maintained at room temperature to attain equilibrium (5 min) and the specific optical rotations and ^{13}C-NMR spectra were recorded.

Influence of water on the formation of the blue amylose–iodine complex

A system containing amylose solution in DMSO (0.5 ml) + 0.5 ml 90% DMSO containing 6×10^{-3} M iodine + distilled water (6.5–9 ml) + DMSO to make the total volume 10 ml was prepared, and kept at room temperature for 1 h for colour development. The maximum absorption wavelength (λ_{max}) and absorbance (ϵ_{max}) of each starch–iodine complex was recorded using an Hitachi Vis/UV spectrophotometer between 400 and 800 nm.

Influence of water on the formation of amylose–butanol complex

The system consisted of amylose solution (1 ml) + DMSO (0–2 ml) + 10% butanol (1 ml) + water to make the total volume to 4 ml. Solutions were kept for 5 min before optical rotations were recorded as described above.

Influence of tetramethylurea on the conformation of amylose and dextran in DMSO solution

This was investigated by recording the specific optical rotation at 589 nm in the presence of tetramethylurea (10–50%). A typical system contained amylose (1 ml), tetramethylurea (0–1.5 ml) + DMSO to make the total volume 3 ml. The system was equilibrated at 30°C for 20 min. The specific optical rotation was recorded at 589 nm. The influence of tetramethylurea on dextran conformation was studied similarly.

RESULTS AND DISCUSSION

Solubility behaviour of amylose in dimethyl sulfoxide

Amylose conformational transitions were induced by a heating and cooling cycle, and followed by polarimeter. It was important to ensure that the sample had completely dissolved. Amylose in DMSO was kept at 80°C for 16 h. The solution was transparent and after centrifugation, no precipitate appeared. The retention times and profiles of these samples in HPLC were not affected by this treatment, so depolymerization of amylose was considered to be unlikely. If the sample concentration was greater than 3%, after cooling to room temperature, due to aggregation of amylose, some gel-like precipitate appeared with extended time. It was thus necessary to keep the concentration of starch as low as possible, within the limits of optical rotation sensitivity. A 1–2% concentration of amylose was found to be most suitable for optical rotation analysis. The optical rotation of amylose dissolved in DMSO in this way remained stable and reproducible.

The effect of water content on amylose conformation

Variation of the specific rotation of amylose as a function of temperature was studied. The wavelength of the sodium D line (589 nm) was used since it is far from any absorption band, ensuring that the measurement of optical rotation is insensitive to secondary perturbation such as solvent interaction and inter-residue hydrogen bonding (Rees, 1970). Fig. 1 shows that there are different degrees of change in the value of specific rotation for amylose over four concentrations of DMSO (33.3–100%). The choice of 66.6% DMSO as one of the concentrations was deliberate. The discontinuity in optical rotation observed by Dintzis and Tobin (1969) occurs at about 66% DMSO.

Compared with calculated specific rotation curves, which were compensated for changes in solvent refractive index with increased temperature, there are also some differences. These results suggest that the change in specific rotation with increasing temperature is not the result of changes in solvent refractive index, but mainly the result of a change in conformation. Dintzis and Tobin showed that their observed discontinuity in the compensated specific rotation of starch components in a range of water/DMSO mixtures was a property of the polymer, i.e, it was not shared by oligomers that have an α-(1 → 4)-glycosidic linkage. They concluded that the discontinuity indicated symmetry changes about amylose chain segments. Optical rotation is very sensitive to conformation, especially to linkage conformation which is influenced by structural change and by attractions and repulsions such as Van der Waals interactions, dipolar interactions and inter-residue hydrogen bonding (Rees, 1970). In 100% DMSO, the observed specific rotation is close to the calculated value, showing that the conformation of amylose in anhydrous DMSO is almost independent of temperature between 25 and 70°C. This result is in agreement with those of Dintzis and Tobin (1969). The observed value of the specific rotation in 66.6% DMSO shows the greatest difference from the calculated value (Fig. 1(b)). The experimental curve is close to sigmoidal, which is indicative of a conformational transition (Rees et al., 1983; Cheetham and Mashimba, 1991). With an increase in the water content the difference between observed value and calculated value was again small (Fig. 1(c) and 1(d)). All the changes implied in Fig. 1 were reversible, i.e. the cooling curve was essentially the same as the heating curve. No hysteresis occurred. Considering the overall results of specific rotation changes we tentatively conclude that amylose in 100% DMSO is helical rather than random coil. With the addition of water, the interaction between amylose and DMSO is reduced progressively, leading to the conformational

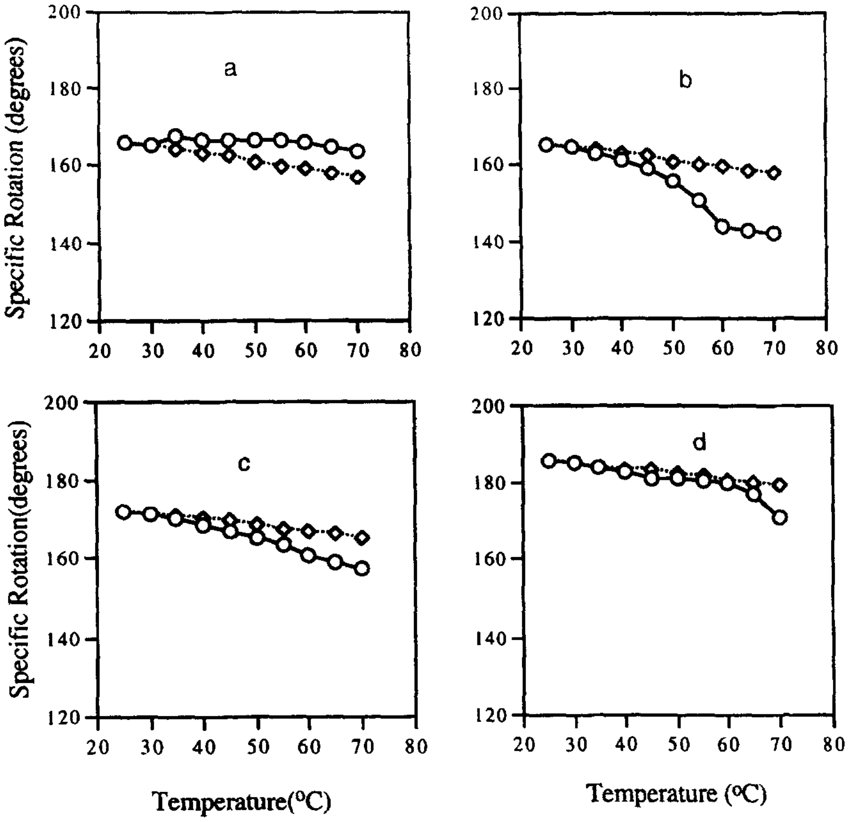

Fig. 1. Specific optical rotation of amylose in DMSO/water mixtures. (a) 100% DMSO; (b) 66.6% DMSO; (c) 50% DMSO; (d) 33.3% DMSO. (O) Observed value, (◇) calculated value.

transition of amylose from tight helical to loose helical to disordered coil.

Fig. 2 shows the change in limiting viscosity with change in DMSO level. There are two linear portions of the curve, which intersect at ~65% DMSO. The discontinuity in specific rotation observed by Dintzis and Tobin occurs at 66% DMSO. The maximum interaction between water and DMSO also occurs at this level, with the formation of the hydrate DMSO·2H_2O. It might be expected that at DMSO/water ratios above that required for complete hydrate formation, the solvation of amylose is predominantly by DMSO, and after complete hydrate formation, predominantly by water, i.e. of the equilibria

$$\text{DMSO} + \text{HO} - \text{amylose} \leftrightarrow \text{DMSO}\cdot\text{HOamylose}$$

and

$$\text{DMSO} + 2\text{HOH} \leftrightarrow \text{DMSO}\cdot 2\text{H}_2\text{O}$$

the latter has the greater equilibrium constant. Thus 66% DMSO constitutes a true 'crossover point', and this is reflected in detectable changes in the physical properties of amylose in solution. There is a linear decrease in limiting viscosity between 100% and 66% DMSO. Probably the breaking of intramolecular hydrogen bonds between OH2 and OH3′ is involved as the tight amylose helix is progressively converted to a looser one by the DMSO/water competition for hydrogen-bonding sites (Casu et al., 1966; Peng

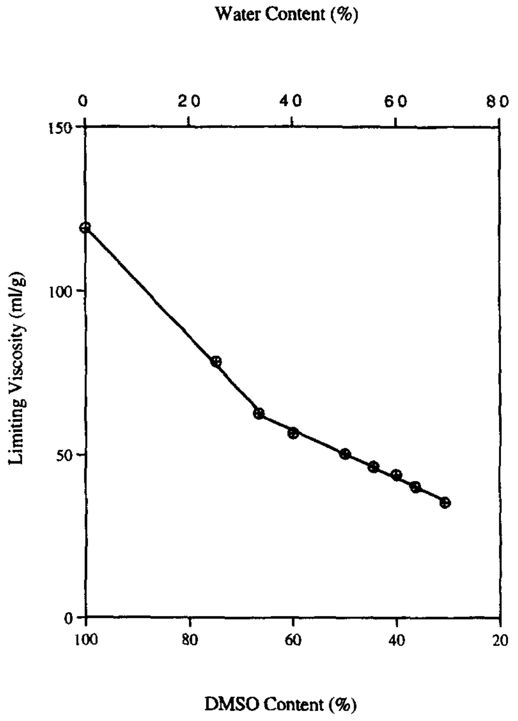

Fig. 2. The effect of water content on limiting viscosity of amylose in DMSO/water at 30°C.

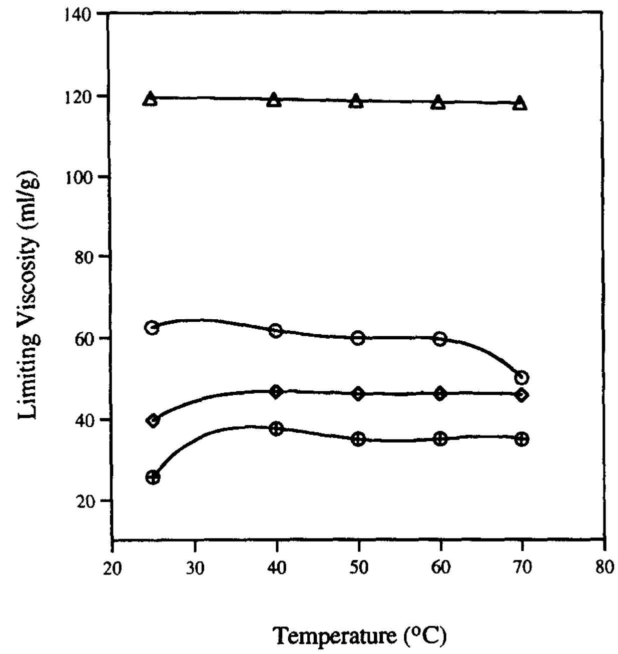

Fig. 3. The effects of temperature on limiting viscosities of amylose in different proportions of DMSO/water. (⊕) 33% DMSO; (◇) 50% DMSO; (○) 66% DMSO; (△) 100% DMSO.

and Perlin, 1987). Below 60% DMSO, the loose helix conformation gradually moves toward the random conformation. This is consistent with the optical rotation behaviour (Fig. 1).

Fig. 3 shows the effect of temperature on the limiting viscosity of amylose in DMSO/water mixtures. There is essentially no change between 25°C and 70°C in DMSO alone, indicating that the proposed tight helical amylose conformation is maintained at the latter temperature (compare with the optical rotation behaviour over the same temperature range (Fig. 1(a))). At 66% DMSO and below, possible conformational changes are indicated but the data probably demonstrate that most of the change has already occurred at the 66% level. These results are consistent with Erlander's conclusion that the helical conformation of amylose is less stable in water than in 80% to 100% DMSO solutions (Erlander and Tobin, 1968).

The effect of water on amylose complex formation

When amylose forms complexes with iodine or some organic solvent such as n-butanol, the water content is an important factor. Only when the water content is over 60% (Fig. 4) can the blue amylose–iodine complex be detected by absorbance at 600 nm. With further increase in water content up to 90%, the maximum absorption wavelength of the complex and the absorbance value also increase (Figs. 4 and 5). For formation of the amylose–butanol complex, the minimum water content is also ≈ 60–66%. The specific optical rotation of amylose in DMSO/butanol is almost constant when the water content is below 66%, even when butanol concentration is 15% (Fig. 6). This indicates that the presence of water is essential in some way to the formation of amylose– butanol complex. In this study, at water levels greater than 66%, the specific rotation of amylose–butanol solution fell rapidly but inconsistently, due to formation of insoluble amylose/butanol particles.

The effect of tetramethylurea on the optical rotation of amylose

Tetramethylurea is a DMSO- and water-miscible compound

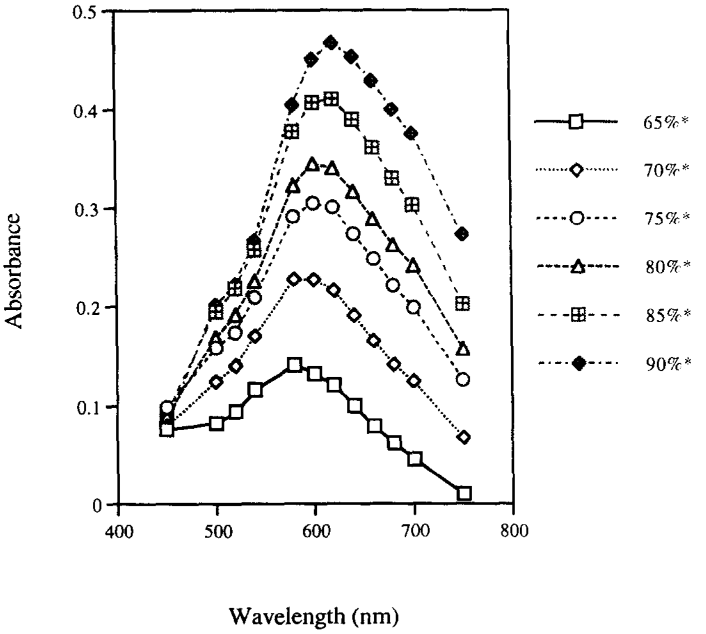

Fig. 4. The effects of water content on absorption maximum wavelength and absorption of amylose–iodine complex. *, water content (%v/v).

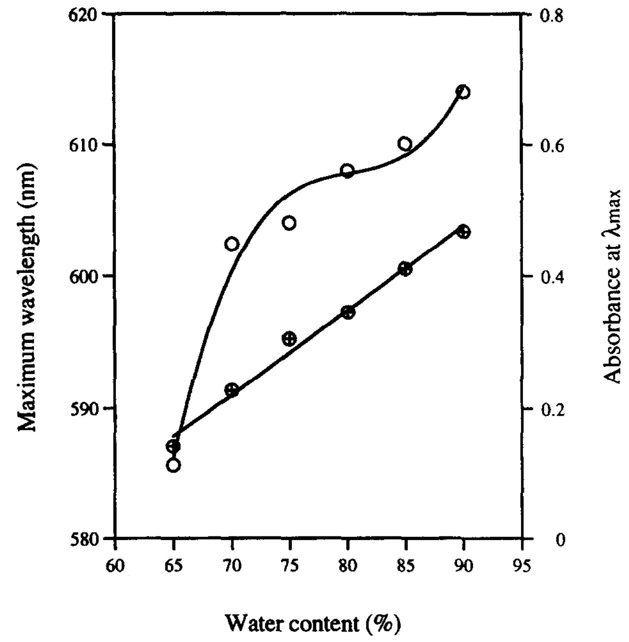

Fig. 5. The effect of water content on the absorption maximum wavelength of amylose–iodine complexes. (○) λ_{max}; (⊕) ϵ_{max}.

Table 1. The effect of tetramethylurea on the specific optical rotation of amylose and dextran

Sample	Tetramethylurea concentration			
	0	16.7%	33.3%	50%
Amylose in 100%DMSO	+ 166.0	+ 158.2	+ 155.1	+ 150.7
Amylose in 83%DMSO	+ 167.7	+ 159.0	+ 150.7	+ 146.9
Amylose in 66% DMSO	+ 169.1	+ 164.5	+ 160.8	-
Amylose in 50%DMSO	+ 172.1	+ 170.3	-	-
Dextran in 100%DMSO	+ 189.9	+ 189.9	+ 189.8	-
Dextran in 83%DMSO	+ 191.8	+ 191.6	+ 191.6	-
Dextran in 66%DMSO	+ 192.3	+ 192.3	+ 192.3	-
Dextran in 50%DMSO	+ 193.1	+ 193.1	-	-

which can disrupt hydrogen bonding. The effect of tetramethylurea on amylose in different concentrations of DMSO (Table 1), was to cause a decrease in specific rotation. The effect decreases with increasing water content. These results imply that intramolecular hydrogen bonding plays an important role in amylose conformation in DMSO. With addition of more water the number of intramolecular hydrogen bonds decreases, gradually being replaced by amylose–water intermolecular bonds. The existence of H-bonds in DMSO suggests that a helix, or at least a coil containing significant helical segments, is maintained by amylose.

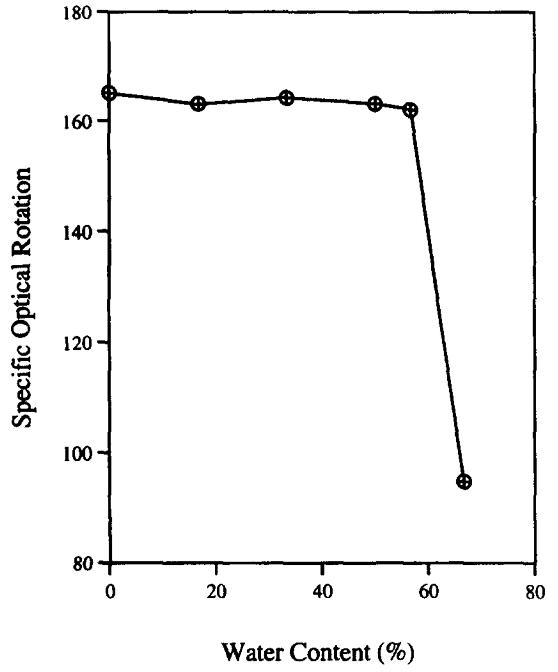

Fig. 6. The effect of water content in DMSO/water mixtures on the specific optical rotation of the amylose–butanol complex.

In contrast, the conformation of dextran T-500, an α-(1-6)-linked-D-glucan of bacterial origin, which is a typical random coil (Rees et al., 1983), was not affected by tetramethylurea. The addition of different concentrations of tetramethylurea to dextran solution made no difference to the specific rotation (Table 1). This implies that the decrease in specific optical rotation of amylose solution after addition of tetramethylurea was due to conformation changes destroying some structure (maintained largely by hydrogen bonding) rather than general solvent effects. It further confirmed the conformation of dextran as a random coil. At concentrations of tetramethylurea above about 50%, the dextran solution became turbid.

^{13}C-NMR spectra of amylose solution

In order to avoid possible effects of mixed solvent on an internal standard, all NMR chemical shifts were determined relative to dioxane which was used as the external standard (66.5 ppm).

Fig. 7 shows that ^{13}C chemical shifts of carbons of amylose were affected by the concentration of DMSO. Chemical shifts of all carbons moved down-field with the increase in concentration of DMSO, C-1 and particularly C-4 being shifted to the greatest extent, as previously observed (Jane and Robyt, 1985). Such large movements in resonances of carbons involved in glycosidic linkages have been interpreted as reflecting conformation changes across those linkage (Perlin, 1976). Dextran, which does not form helical complexes with DMSO, has the same extent of downfield

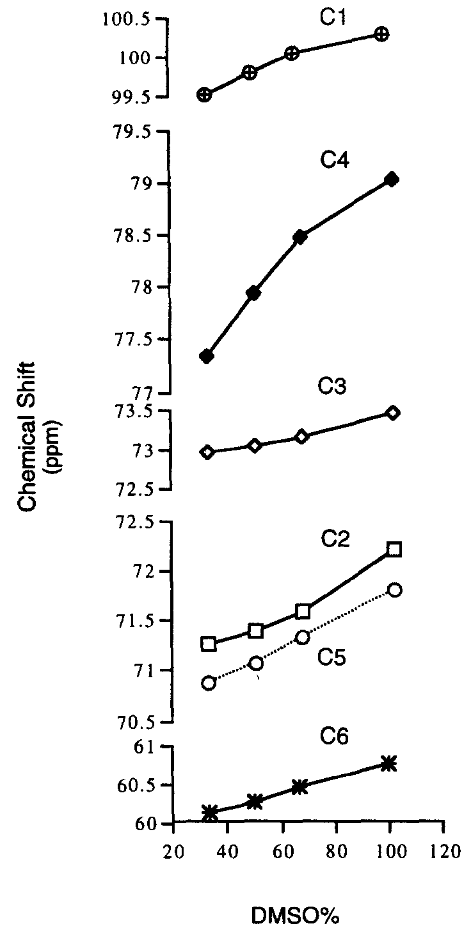

Fig. 7. ^{13}C chemical shifts of amylose in DMSO/water mixtures.

shifts for each carbon resonance (Jane and Robyt, 1985). It is noteworthy that changes in C-1 and C-4 chemical shifts of amylose with DMSO concentration were not linear, as inflection points in these curves can be seen. This suggests an optimal state for conformational transition at these points. Thus, when the concentration of DMSO is decreased from 100% to 66.6%, the amylose conformation presumably transits from helix to loose helix. Further increases in water content lead to a steady change of conformation probably from loose helix to nearly random coil at ≈ 33.3% DMSO. This is consistent with the results of the changes of specific rotation and limiting viscosity of amylose in different DMSO /water mixtures with increasing temperature. The extent of downfield shift of C-4 is much greater than that of C-1. This suggests that the change in the glycosidic angle ψ (C_1–O_1–C'_4–H'_4) may be greater than that in the ϕ angle (H_1–C_1–O_1–C'_4).

Fig. 8 shows the effect of temperature on the ^{13}C chemical shifts of amylose resonances. The general trend is for a small downfield movement, of approximately the same magnitude, for all resonances. The exception is the resonance of C-1, which moves slightly upfield, at DMSO levels of 66% and less. We are unsure how to interpret this result, but the small slopes of the C-1 plots compared with those of the C-4 plots are consistent with larger changes in angle ψ than in angle ϕ, as suggested above.

CONCLUSIONS

(1) Amylose conformation over a range of DMSO/water mixtures is variable, as shown by optical rotation, limiting viscosities, and ^{13}C-NMR measurements. In 100% DMSO, there is almost constant specific optical rotation over a large temperature range, high limiting viscosity, and similar temperature dependence for each carbon resonance in ^{13}C- NMR. In 66% DMSO, a sigmoidal optical rotation vs. temperature curve, a kink in the limiting viscosity plot, and a temperature dependence of chemical shifts for C-1 and C-4 resonances which is different from those of other carbons, are observed.

When the water content is increased above 33%, the temperature dependence of specific optical rotation and the limiting viscosity decreases gradually and remains almost constant at 66.6% water. At about this concentration solubility problems prevented further studies. These results indicate that amylose undergoes conformational transitions probably from tight helix-loose helix-random coil in DMSO/water mixtures over a range of 0–66% water.

(2) Only when the water content is over 60% can amylose form a complex with iodine or butanol. When the water content is less than 60%, no absorption in the range 400–800 nm can be detected. Above about 60% water, the λ_{max} of absorption shifts progressively to longer wavelengths, and ϵ_{max} increases. Sufficient water is essential to allow the formation of the conformation which favours amylose–iodine and amylose–butanol complex formation.

(3) Addition of tetramethylurea decreases the specific optical rotation of amylose in DMSO, but does not change the specific optical rotation of dextran, which is known not to form a helical complex. These results suggest that amylose is capable of forming a number of cooperative intramolecular hydrogen bonds in DMSO, a major factor in maintaining a helical conformation.

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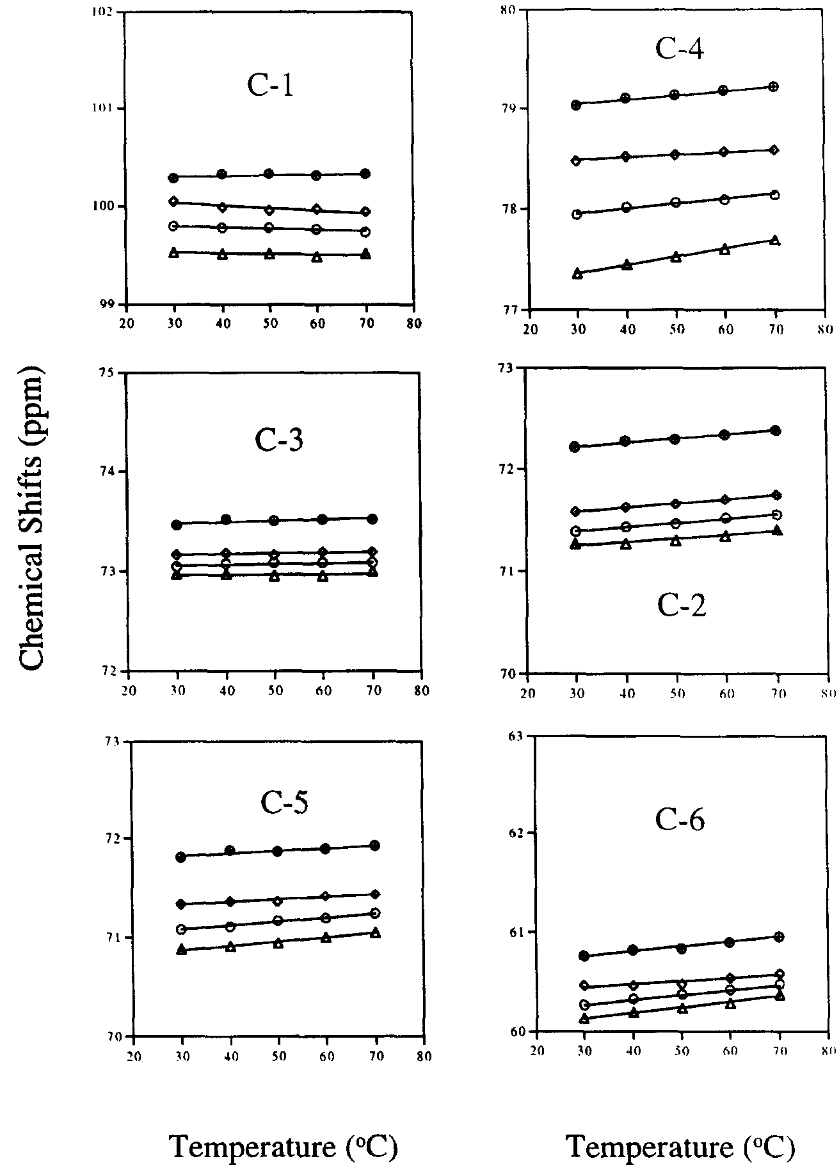

Fig. 8. The chemical shifts of amylose in DMSO/water mixtures vs. temperature. (⊕) 100% DMSO; (◇) 66.6% DMSO; (○) 50% DMSO; (△) 33.3% DMSO.

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