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Conformation of Poly(*S*-benzyl-L-cysteine) in Solution

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Conformation of poly(*S*-benzyl-L-cysteine) was investigated by measurements of optical rotatory dispersion, infrared absorption and viscosity in dichloroacetic acid (DCA)–chloroform mixtures. Transition from random coil to the β-structure was confirmed to occur when DCA content of the solvent was reduced below 40% (v/v). Both dispersion parameters a_0 and b_0 increased as the polymers were transformed into the β-structure. Viscosity of the polymer solution changed only slightly with accompanying the transition. This was interpreted in terms of the formation of intramolecular β-structure. In the region where the β-structure was dominant both optical rotatory parameters and viscosity were independent of solvent composition. Such behavior has been rarely observed in the β-coil transition of other polypeptides. The β-structure of poly(*S*-benzyl-L-cysteine) was shown to be more stable than that of poly(*O*-benzyl-L-serine) as referred to respective random coil conformations.

Early studies on the β-structure in solution were mostly carried out in organic solvent systems for the purpose of detecting the β-structure in solution for the polypeptides which had been confirmed to be in this conformation in solid state. Short chain poly(γ-benzyl-L-glutamate)[1–5] and polymers of the derivatives of

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serine,[6–8] cysteine,[9–12] and threonine[13] have been shown experimentally to assume the β-structure in solution under suitable conditions (solvent composition, polymer concentration, temperature, *etc.*).

Those studies were also aimed at deducing experimentally the optical rotatory properties of the β-structure. It was commonly found that the increase in dextrorotation due to the formation of the β-structure as compared with the random coil conformation.[1–3,5–13] However, different results have been obtained about the Moffitt-Yang parameter b_0.[14,15] The parameter increased in many cases,[3,6–8,10–13] but it remained constant in others.[9,12] The extent of increase in b_0 was not unique, being fairly large in some cases[3,7] and small in others.[6,8,10–12] The different behavior of the change in b_0 has not been clarified so far.

With recent development in the preparation of protolytic polypeptides capable of forming the β-structure,[16–18] it has become possible to make various approaches to a study of the β-structure in solution. Nevertheless, the importance of its study in organic media is by no means reduced, since the β-structure in both organic solvents and aqueous media can not be assumed *a priori* to be identical. This is partly supported by the significant role assigned to the hydrophobic interaction in the β-structure in aqueous media.[19]

In the present study, the β-random coil transition of poly(*S*-benzyl-L-cysteine) was investigated in a mixed solvent system of dichloroacetic acid (DCA)–chloroform. The polymer was chosen because it has a less polar side chain than that of other polypeptides forming the β-structure,[1–13] and also because a comparison of the β-structures of serine and cysteine derivatives will be best attained, when polymers of their benzyl esters are compared.

The conformation of the present polypeptide in solid state was previously shown to be of β-structure.[20,21]

Experimental

Materials. *S*-Benzyl-L-cysteine was prepared according to the literatures.[22,23] *S*-Benzyl-*N*-carboxy-L-cysteine anhydride was synthesized as usual. Mp 109—110 °C. Polymerization was carried out in dichloromethane using sodium methoxide as an initiator. Polymer solutions were prepared by dissolving a weighed amount of the polymer into a mixed solvent prepared in advance.

Optical Rotatory Dispersion (ORD). The rotatory dispersion was measured on a JASCO ORD/UV 5 spectropolarimeter using a 1 cm cell at 25 °C. The wavelength region examined was 300 to 550 nm. Polymer concentration c was about 0.5 g/dl unless otherwise stated. The results were analyzed in terms of the Moffitt-Yang equation,[14,15]

$$
\begin{aligned}
[m'] &= [3/(n^2+2)][\alpha](M_0/100) \\
&= a_0\lambda_0^2/(\lambda^2-\lambda_0^2) + b_0\lambda_0^4/(\lambda^2-\lambda_0^2)^2
\end{aligned}
$$

Additivity of the term $[3/(n^2+2)]$ was assumed for different solvent compositions.

Viscosity. Viscosity was measured at 25.0 °C using Ubbelohde type viscometers. Flow times of the solvents were larger than 100 s. Reduced viscosity was measured in the concentration range below 1 g/dl and plotted against c. Intrinsic viscosity $[\eta]$ and Huggins' constant k' were obtained from the intercept and slope of the plot, where k' was defined as $(1/[\eta]^2)\mathrm{d}(\eta_{sp}/c)/\mathrm{d}c$.

Infrared Spectra. Infrared spectra were recorded on a JASCO DS 402-G spectrophotometer at 25 °C. For determination of the spectra in solution, a cell with potassium bromide windows was used.

Results and Discussion

Solubility and Its Change with Time. Solubility of the sample D1119 in both DCA and chloroform was very low (less than 0.5 g/dl at 25 °C), but a mixture of the two liquids was found to serve as a solvent. Solutions with polymer concentration of about 0.5 g/dl were obtained when the DCA content was 4—80% (v/v). However, the solubility was found to change considerably after storage of the sample for about a year in a desiccator over P_2O_5. The range of DCA content giving solutions of the aged sample to the same concentration was reduced to 15—80%. Various measurements were then made on the aged sample. Almost the same results were obtained but quantitative agreement was not attained for some properties. The aged sample was designated D1119A and treated as a different sample.

Low solubility in DCA has been reported on the β-structure of polypeptides with nonpolar side chains, such as polyglycine I, poly-L-alanine, and poly-L-valine.[24] Thus similar behavior found in the present polymer might be attributed to the relatively nonpolar side chains involved.

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Conformation of the Polymer in Solid State. Infrared spectra of the polymer in solid films cast from DCA–chloroform solutions showed amide I band at 1629 and 1694 cm^{-1} and amide II band at 1520 cm^{-1}. Thus the β-structure with antiparallel arrangement of adjacent chains can be assigned to the conformation of the polymer in solid state, which is consistent with previous results.[20,21]

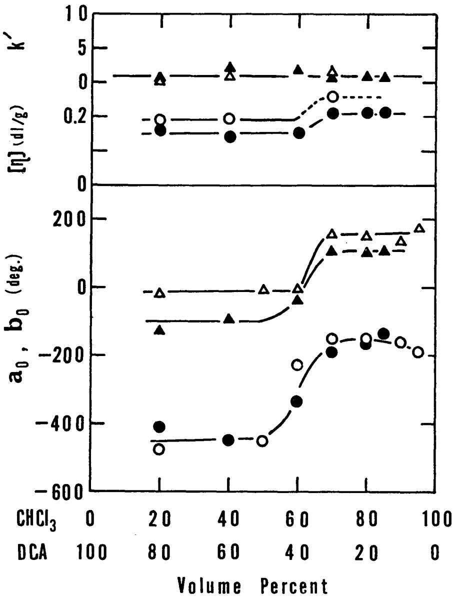

Fig. 1. Solution properties as a function of solvent composition. Polymer concentration: 0.5 g/dl. 25 °C. Circles: a_0 and intrinsic viscosity $[\eta]$. Triangles: b_0 and Huggins' constant k'. Open marks refer to D1119 and filled ones to D1119A.

Conformation of the Polymer in DCA–Chloroform Mixtures. The optical rotations of all polymer solutions were levorotatory everywhere in the wavelength region examined. The values of a_0 and b_0 are plotted in Fig. 1 against solvent composition. In the solvents of high DCA content (greater than about 50%) the dispersion is characterized by constant values of b_0 which are about zero for D1119 and −100° for D1119A. On further reduction of DCA content, b_0 begins to increase up to about 150 and 100°, respectively, indicating a conformational transition. The transition seems to be complete when DCA content reaches 30% or less, since no further change in b_0 is observed. Another parameter a_0 is also shown to increase around this region (DCA 30—50%) and to reach an almost constant value. A difference in the two samples is not detectable in the case of a_0.

Infrared spectra of the sample D1119 in solutions of low DCA content are given in Fig. 2. The difference spectra were obtained by subtracting the spectra of solvent from those of the solution. The amide I band splits into two peaks, one at 1630 cm^{-1} and the other at 1695 cm^{-1}. The results demonstrate that the conformation of the polymer in the solution is the β-structure with antiparallel arrangement of adjacent chains. Thus we can reasonably interpret the rotatory behavior in terms of the transition between random coil and β-structure.

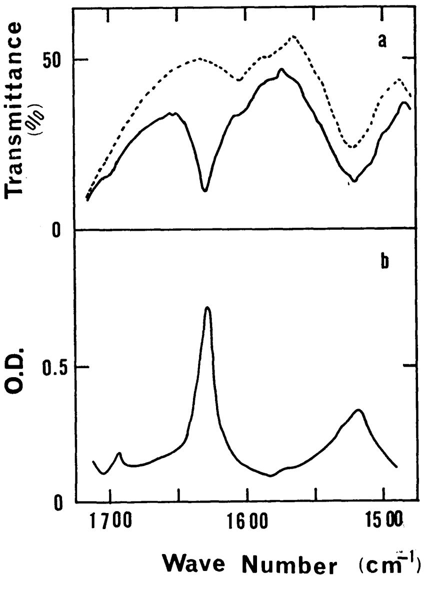

Fig. 2. Infrared spectra of the polymer solution. Sample: D1119. Solvent composition: DCA 5%-CHCl$_3$ 95% (v/v). Polymer concentration: 1 g/dl. Cell length: 200 μ.
(a) Spectra of the solution (solid line). Spectra of the solvent (broken line).
(b) Difference spectra of the polymer in solution.

Values of both intrinsic viscosity and Huggins' constant are also shown in Fig. 1. In the composition range where rotatory property changes sharply (DCA 30—40%), a slight but definite change in intrinsic viscosity occurs. Since the conformational transition from random coil to β-structure is confirmed to occur in this solvent composition range, the observed change in viscosity can be regarded to correspond to the transition. Contrary to the drastic changes in rotatory property, which is considered to reflect both the conformational change and the effect of polarity of the environment of chromophores, viscosity shows no such large change accompanying the transition. Since viscosity represents over-all shape and dimension of polymer chains and the effect of association as well, the results obtained strongly suggests that the β-structure of the present polymer is formed intramolecularly and that the association of polymers does not play a significant role in the formation of the β-structure. Huggins' constant also shows no appreciable change accompanying the transition, which has been known to be a measure of interparticle hydrodynamic interaction. It should be noted that changes in both intrinsic viscosity and Huggins' constant are many times larger than those obtained in the present study if association of polymers is essential for the formation of the β-structure.[10]

We can obtain the change in b_0 accompanying the transition to be about 150° (from 0 to +150°) or 200° (from −100 to +100°). This large change in b_0 is similar to that of poly(*O*-benzyl-L-serine),[7] these polypeptides differing from others in this respect. On the other hand, the increase in a_0 accompanying the transition is about 300° (from −450 to −150°), which is smaller than any other reported values for the coil to β transition. Generally, two contributions to a_0

should be taken into account, one from conformation-dependent optical activity and the other from environmental effect for chromophores. Since more peptide groups are considered to be solvated with DCA in the intramolecular β-structure than in the intermolecular one, the observed small increase in a_0 may be a support for the intramolecular β-structure of the present polymer.

Effect of Polymer Concentration. Effect of polymer concentration was examined using the sample E1119 at three values of c, 0.490, 0.802, and 1.173 g/dl, in the solvent of DCA content 5%. The values of b_0 were found to be constant (180—190°) and those of a_0 to slightly decrease with concentration. That rotatory properties are independent of polymer concentration also supports the assignment of the intramolecular β-structure.

Stability of the β-Structure. Comparison of the stability of the β-structure of the present polymer with that of poly(*O*-benzyl-L-serine)[7] becomes possible since temperature and solvent system (DCA–chloroform) are identical. If we assume that the β-structures of both polypeptides are molecularly dispersed, we can compare the stability in a quantitative manner, following the reasoning by Auer and Doty.[25] The composition where the midpoint of transition is located is about 10% DCA in the case of poly(*O*-benzyl-L-serine). This is considerably smaller than the value of about 35% DCA in the present polymer. Hence the difference in stability amounts to $2.303\,RT \log (35/10)$.[25] This demonstrates that the relative stability of the β-structure as referred to the random coil is larger by about 760 cal per mole of residue than that of the corresponding serine polymer. Since the chain length (as judged from intrinsic viscosity in DCA) and concentration of the two polymers were almost the same in the two experiments, the observed difference would be explained in terms of the difference between sulfur atom and oxygen atom involved in the respective side chains. It should be noted that association of polymers was not necessarily ruled out in the formation of the β-structure of poly(*O*-benzyl-L-serine), because viscosity was not measured.[7] Thus, if association was significant for the β-structure of poly(*O*-benzyl-L-serine), the estimated figure for the difference in stability becomes meaningless.

It should be emphasized that in the present system both optical rotatory parameters and viscosity were independent of solvent composition when the β-structure became dominant. Such behavior has been rarely reported for the β-coil transition.

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