BIOPOLYMERS VOL. 12, 1203-1221 (1973)

The Viscosity Behavior and Conformations of Low-Molecular-Weight Poly-γ-benzyl-L-glutamate in Various Solvents

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Synopsis

Reduced viscosity and infrared spectra of low-molecular-weight poly-γ-benzyl-L-glutamate (which was prepared by polymerization of the *N*-carboxyanhydride with *n*-hexylamine initiation at [A]/[I] 3, 4, and 8) have been measured in various organic solvents. Infrared spectra indicate that the polypeptide molecules consist of a series of residues of two forms, the solvated σ-form and the hydrogen-bonded β-form, and relative abundance of the two forms depends on solvent species and polypeptide concentration. An approximate method is developed for estimating the content of β-structure from a single spectrum of dissolved polypeptide. The reduced viscosity of some solutions is scarcely dependent on polypeptide concentration, in which a single conformation is predominantly kept over the concentration range. In the other solutions the reduced viscosity displays a strong concentration dependence or some anomalous behavior. The observed viscosity behavior has been attributed to the changes in size and shape of aggregates, which are determined by the number of hydrogen bonds in the aggregate. This unusual behavior is exhibited by solutions of the polypeptides which have a moderate content of β-structure at a finite concentration. Both the content of β-structure and the extent of association increase in the following solvents, ranked in order of effectiveness: dimethylformamide, trifluoroethanol < trimethyl phosphate < chloroform < dioxane < ethylene dichloride < ethylene dibromide. Infrared spectra suggest that the conformation of the polypeptide in dichloroacetic acid differs from either the σ- or the β-conformation.

INTRODUCTION

Low-molecular-weight poly-γ-benzyl-L-glutamate cannot exist in the α-helical conformation either in the solid state or in the helical solvents.[1-3] Infrared spectroscopy has shown that molecules of those polypeptides consist of a series of residues in either of two forms, designated as the σ- and β-forms, respectively, when they are dissolved in chloroform, dioxane, or ethylene dichloride. Those two forms of residue can be discriminated by the location of amide I band on infrared spectra: the σ-form has an absorption around 1675 to 1660 cm^{-1}, while the β-form has absorptions at 1630 and 1695 cm^{-1}, characteristic of hydrogen-bonded β-structure of antiparallel chains. Relative abundance of the two forms depends on solvent species as well as on molecular weight and concentration of the polypeptide. The

β-form is more predominant in the polypeptide of higher molecular weights and at higher concentrations, and it is more stable in ethylene dichloride than in dioxane.[4,5]

Vapor-pressure osmometry has shown that polypeptide molecules are associated in dioxane and ethylene dichloride, but are isolated from one another at infinite dilution. The average size of aggregates increases with increasing concentration, and the association occurs more extensively in ethylene dichloride than in dioxane. The number of residues within aggregates does not generally agree with the number of hydrogen bonds, and thus the associated molecules do not always consist of residues of the β-form alone but include residues of the σ-form as well.[5]

To elucidate the mode of association of the polypeptide molecules in more detail, we have measured reduced viscosity of dilute solutions, which have low-molecular-weight poly-γ-benzyl-L-glutamate dissolved in various organic solvents. Observed viscosity behavior strongly depends on solution and is considered to reflect different modes of association. We have also measured infrared spectra of the polypeptides in these solvents and determined the content of β-structure, which will define the number of hydrogen bonds participating in the association. The observed viscosity behavior is correlated with the content of β-structure. The solvents examined are dichloroacetic acid (DCA), dimethylformamide (DMF), trifluoroethanol (TFE), trimethyl phosphate (TMP), chloroform (CHL), dioxane (DIOX), ethylene dichloride (EDC), and ethylene dibromide (EDB).

EXPERIMENTAL

Materials

Three samples of low-molecular-weight poly-γ-benzyl-L-glutamate were the same as previously used.[5] A fraction soluble in formic acid was isolated from the polypeptide prepared at A/I 4 and 8, respectively. All the three samples were found to have average molecular weights approximately corresponding to the A/I values (the molar ratios of *N*-carboxyanhydride to *n*-hexylamine initiator used in preparing the polypeptides). The number-average molecular weight was 760 for A/I 3, 910 for A/I 4, and 2000 for A/I 8 polymers, as determined by the vapor-pressure osmometry.

A sample of high-molecular-weight poly-γ-benzyl-L-glutamate was prepared by polymerizing the *N*-carboxyanhydride with sodium methoxide at A/I 47. Its weight-average molecular weight was estimated to be more than 90,000 from the intrinsic viscosity of dichloroacetic acid solution.[6,7] From the location of amide I band of infrared spectra at 1652 or 1653 cm^{-1}, its conformation was identified to be the α-helix, both in film cast from chloroform solution and in solutions dissolved in dioxane and in ethylene dichloride.

Trifluoroethanol was purchased from Aldrich Chemical Co., Ltd. All the other solvents were redistilled before use. Dichloroacetic acid, chloroform, dioxane, and ethylene dichloride were purified as before.[5] Trimethyl

phosphate was distilled *in vacuo* (48–52°C/1 mm Hg). Ethylene dibromide was dried over anhydrous K_2CO_3 and redistilled (131°C). Dimethylformamide was dried over anhydrous Na_2CO_3 and vacuum distilled.

Measurements

Infrared spectra were recorded on a Jasco DS-402 G Spectrophotometer at 25°C, as previously described.[5] The procedure for optical density determinations of a dissolved polymer, has also been described.[5] The residue extinction coefficient, ϵ, was calculated by using residue molecular weight, which was assigned a value of the formula molecular weight divided by the A/I value so as to distribute the contributions of *n*-hexylimino and other terminal groups evenly over all the residues.

Reduced viscosity of dilute solutions was measured in capillary viscometers of the Ubbelohde type which had large bulbs capable of performing tenfold dilution. A viscometer with a suitable capillary bore was chosen for each solvent, in such a way to give flow times longer than 80 sec. Values of reduced viscosity given below are calculated from ratios of flow times for solution and solvent, without any other corrections applied. Temperature was regulated at 25 ± 0.01°C in most experiments, and the effect of temperature on viscosity was examined at 37 and 50°C, both within ±0.01°C. As will be stated later, the flow time of some solutions changed for a period after they were prepared, and in such cases the reduced viscosity at equilibrium or near-equilibrium was recorded.

RESULTS

Infrared Spectra and Conformations of the Polypeptides

The conformations of the polypeptides in all the solvents except in dichloroacetic acid and dimethylformamide can be determined by infrared spectral measurements. Some of the typical spectra of dissolved polypeptides in ethylene dichloride and in dioxane have already been published in a previous paper.[5] The spectra observed in trifluoroethanol have also been presented.[8]

The amide I bands of the polypeptides lie either at 1660 to 1675 cm^{-1} or at 1695 and 1630 cm^{-1}, or at both. The former band is assigned to the residues of σ-form, while the latter is to the residues in the β-structure of antiparallel chains. The two bands for the β-form are designated as $\tilde{\nu}(0,\pi)$ and $\tilde{\nu}(\pi,0)$, respectively.

Table I summarizes the location and relative intensity of amide I bands of the polypeptides dissolved in various solvents, all at about 1 g/dl. Table II gives the location and intensity of the ester carbonyl bands, which also reflect some interaction with the solvent and seem to have some correlation with the backbone conformation of polypeptides. Those solvents can be roughly classified into three groups. In trifluoroethanol and trimethyl phosphate all the polypeptides are in the σ-conformation, while in the two

TABLE I
Amide I Bands of Poly-γ-benzyl-L-glutamate in Various Solvents[a,b]

	[A]/[I] 3			[A]/[I] 4			[A]/[I] 8		
	σ	$\tilde{\nu}(0,\pi)$	$\tilde{\nu}(\pi,0)$	σ	$\tilde{\nu}(0,\pi)$	$\tilde{\nu}(\pi,0)$	σ	$\tilde{\nu}(0,\pi)$	$\tilde{\nu}(\pi,0)$
TFE	1670(s)	—	—	1666(s)	—	—	1663(s)	—	—
TMP	1675(s)	—	—	1675(s)	—	—	1668(s)	—	—
CHL	1668(s)	—	—	1668(s)	—	1629(sh)	1660(m)	1695(w)	1626(s)
DIOX	1676(s)	—	—	1674(s)	—	1631(w)	1672(w)	1696(w)	1627(s)[c]
EDC	1675(s)	—	1639(sh)	1667(m)	—	1632(m)	1658(w)	1696(w)	1627(s)
EDB	1675(m)	—	1637(m)	1668(w)	1700(sh)	1633(s)	1652(sh)[d]	1696(w)	1626(s)

[a] Polypeptide concentration, 0.89–1.04 g/dl.

[b] Wave number in cm^{-1}; Intensity: (s), strong; (m), medium; (w), weak; (sh), shoulder.

[c] An additional band, 1652(w), probably due to the α-helix, was observed.

[d] The band, 1652(sh), was assigned to the σ-form, since a band at 1662 cm^{-1} was isolated if the amide I bands were decomposed into three components.

TABLE II
Ester Carbonyl Bands of Poly-γ-benzyl-L-glutamate in Various Solvents[a,b]

	[A]/[I] 3		[A]/[I] 4		[A]/[I] 8	
	β	σ	β	σ	β	σ
TFE	—	1716(s)	—	1717(s)	—	1718(s)
TMP	1738(s)	1708(s)	1738(s)	1708(s)	1738(s)	—
CHL	—	1717(s)	1727(s)		1735(s)	—
DIOX	1739(s)	1720(s)	1739(s)	1720(sh)	1737(s)	—
EDC	1736(s)	1720(sh)	1736(s)	—	1736(s)	—
EDB	1735(s)	—	1735(s)	—	1736(s)	—

[a] Concentration, 0.89–1.04 g/dl.
[b] Wave number in cm^{-1}; Intensity: (s), strong; (sh), shoulder.

ethylene dihalides the β-conformation is considerably stabilized. Chloroform and dioxane are the intermediate group.

Close examination of Tables I and II permits us to distinguish the solvents of each group further. The ester carbonyl band shifts to a lower frequency in trifluoroethanol than in trimethyl phosphate, and such a shift is considered to be characteristic of the σ-conformation.[5,8] Chloroform and dioxane may also be distinguished from each other in the same respect. Evidently, ethylene dibromide favors the β-conformation more than ethylene dichloride.

As can be seen in Table I, the β-conformation is generally more stable in solution as the molecular weight is higher. It is noted further that the amide I band, $\bar{\nu}(\pi,0)$, for the β-form shifts towards lower frequencies as the molecular weight is higher, probably owing to the increasing strength of hydrogen bonding. The amide I band for the σ-form also shifts to lower frequencies for higher molecular weights.

Reduced Viscosity of the Polypeptide Solutions

Viscosity behavior of the polypeptide solutions can be roughly classified into two or three types. Normally, the reduced viscosity, η_{sp}/c, of polymer solution is expressed as a linear function of concentration, c, by

$$\frac{\eta_{sp}}{c} = [\eta] + k'[\eta]^2 c \tag{1}$$

where $[\eta]$ is the intrinsic viscosity and k' is the Huggins' constant. Table III includes values of those parameters observed for the solutions which display normal reduced viscosity. For the other solutions the reduced viscosity increases with concentration more strongly than normally or it has a maximum value at a certain concentration, as will be seen later. Table III also indicates the ways the viscosity anomaly appears in those solutions.

In dichloroacetic acid, dimethylformamide, and trimethyl phosphate, the reduced viscosity is normal and scarcely dependent on polypeptide concentration. Values of $[\eta]$ and k' are given in Table III.

TABLE III
Reduced Viscosity and Content of β-Structure for Poly-γ-benzyl-L-glutamate in Various Solvents[a]

	[A]/[I] 3			[A]/[I] 4			[A]/[I] 8			[A]/[I] 47	
	$[\eta]$ (dl.g^{-1})	k'	f_β	$[\eta]$ (dl.g^{-1})	k'	f_β	$[\eta]$ (dl.g^{-1})	k'	f_β	$[\eta]$ (dl.g^{-1})	k'
DCA	0.061	$\simeq$0	—	0.062	$\simeq$0	—	0.069	$\simeq$0	—	0.568	0.279
DMF	0.033	$\simeq$0	—	0.034	$\simeq$0	—	0.035	$\simeq$0	—	0.710	0.595
TMP			0			0	0.044	$\simeq$0	0		
CHL	0.034	1.0	0	0.036	2.4	0.06	anomal.(max.)		0.55	1.70	1.82
DIOX	0.035	4.3	0	0.038	4.2	0.06	anomal.(max.)		0.55	4.60	4.58
EDC	anomal.(high)		0.08	anomal.(high)		0.28	anomal.(max.)		0.65	0.940	1.92
EDB	anomal.(high)		0.25	anomal.(max.)		0.57	0.445	1.26	0.75	4.20	1.12

[a] Values of f_β are for solutions containing 0.89–1.04 g/dl of polypeptides.

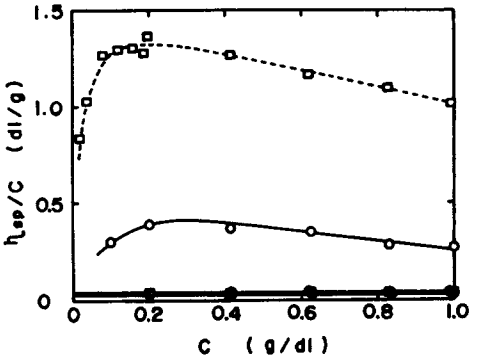

Fig. 1. Reduced viscosity of poly-γ-benzyl-L-glutamate in chloroform and dioxane at 25°C. Circles, CHL; squares, DIOX. ⓘ □□, A/I 3; ⊙ ⊡, A/I 4; ○ □, A/I 8.

Figure 1 shows reduced viscosity of the polypeptides in chloroform and dioxane. The viscosity of the A/I 3 and 4 polymers is seen to be normal, but the Huggins' constant is slightly larger than that found in dimethylformamide and trimethyl phosphate, while the intrinsic viscosity is low. On the other hand, the A/I 8 polymer behaves quite anomalously in both chloroform and dioxane. With increasing concentration, the reduced viscosity sharply increases, reaches a maximum, and then gradually decreases. The reduced viscosity observed in dioxane is generally about three times as large as that in chloroform, and its values in those solvents are too high to correspond to the molecular weight of polypeptide.

Similar but stronger anomaly can be observed in reduced viscosity of almost all the polypeptides in the two ethylene dihalides, as shown in Figure 2. In ethylene dichloride the A/I 3 polymer has a low value of intrinsic

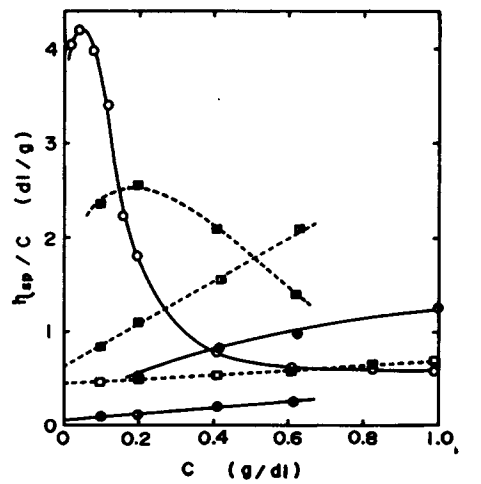

Fig. 2. Reduced viscosity of poly-γ-benzyl-L-glutamate in ethylene dichloride and ethylene dibromide at 25°C. Circles, EDC; squares, EDB. ⓘ □□, A/I 3; ⊙ ⊡, A/I 4; ○ □, A/I 8.

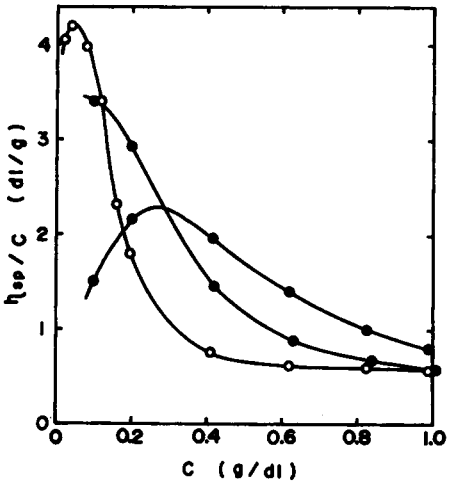

Fig. 3. Effect of temperature on reduced viscosity of the A/I 8 polymer in ethylene dichloride. O, 25°; ⊙, 37°; ⊕, 50°.

viscosity but the Huggins' constant is tremendously large, about 120. The A/I 4 polymer shows a reduced viscosity curve with an upward curvature, and the A/I 8 polymer has a sharp maximum value of reduced viscosity as high as about 4.2 dl/g at a low concentration. Over the concentration range, values of reduced viscosity for the three polypeptides are very high, even as compared with those observed in chloroform and dioxane.

In ethylene dibromide the viscosity anomaly is very strong even for the A/I 3 and 4 polymers. The viscosity behavior observed for a polymer in ethylene dibromide seems to correspond to that for a polymer of higher A/I in ethylene dichloride. That is, the A/I 3 and 4 polymers in ethylene dibromide behave like the A/I 4 and 8 polymers in ethylene dichloride, respectively. However, the reduced viscosity of the A/I 8 polymer in ethylene dibromide is even smaller than that of the A/I 3 and 4 polymers in the same solvent. It displays near-normal behavior, with still large values of intrinsic viscosity and Huggins' constant.

Figure 3 illustrates the effect of temperature on reduced viscosity of the A/I 8 polymer in ethylene dichloride. It is clear that the viscosity anomaly is partially reduced by elevation of temperature but still persists even at 50°C.

DISCUSSION

Estimation of the Content of β-Structure

In the previous paper[5] we have proposed a method of estimating the content of β-structure for the polypeptides in solution. If the residues constituting a polymer in solution are either in the β-form or in the σ-form, the content of β-structure, f_β, which is defined as the weight (mole) fraction of residues of the β-form, can be estimated as follows. If the observed residue

extinction coefficients of amide I bands, $\tilde{\nu}_\beta$ and $\tilde{\nu}_\sigma$, are represented by $\epsilon_{\tilde{\nu}_\beta}$ and $\epsilon_{\tilde{\nu}_\sigma}$, respectively, a linear relation

$$\frac{\epsilon_{\tilde{\nu}_\beta} - \epsilon_{\tilde{\nu}_\beta}{}^{\sigma}}{\epsilon_{\tilde{\nu}_\beta}{}^{\beta} - \epsilon_{\tilde{\nu}_\beta}{}^{\sigma}} + \frac{\epsilon_{\tilde{\nu}_\sigma} - \epsilon_{\tilde{\nu}_\sigma}{}^{\beta}}{\epsilon_{\tilde{\nu}_\sigma}{}^{\sigma} - \epsilon_{\tilde{\nu}_\sigma}{}^{\beta}} = 1 \tag{2}$$

should hold for a series of spectra measured for different polymers at different concentrations in a single solvent, or in different compositions of mixed solvents at a fixed polymer concentration, where $\epsilon_{\tilde{\nu}_\beta}{}^{\beta}$ and $\nu_{\tilde{\nu}_\sigma}{}^{\sigma}$ are the residue extinction coefficients of pure β-form at $\tilde{\nu}_\beta$ and of pure σ-form at $\tilde{\nu}_\sigma$, respectively. The effects of tail of the other band, $\epsilon_{\tilde{\nu}_\beta}{}^{\sigma}$ aud $\epsilon_{\tilde{\nu}_\sigma}{}^{\beta}$, may be estimated as previously described.[5] The peak positions, $\tilde{\nu}_\beta$ and $\tilde{\nu}_\sigma$, are also determined for each polymer from its spectra at the highest and the lowest contents of β-structure, respectively. If relevant data are available sufficient to reproduce the linear relation represented by Eq. (2), values of $\epsilon_{\tilde{\nu}_\beta}{}^{\beta}$ and $\epsilon_{\tilde{\nu}_\sigma}{}^{\sigma}$ can be readily obtained by extrapolations, and the content of β-structure can be calculated by

$$f_\beta = \frac{\epsilon_{\tilde{\nu}_\beta} - \epsilon_{\tilde{\nu}_\beta}{}^{\sigma}}{\epsilon_{\tilde{\nu}_\beta}{}^{\beta} - \epsilon_{\tilde{\nu}_\beta}{}^{\sigma}} \tag{3}$$

This procedure was successfully applied for solutions of polypeptides at different concentrations in two solvents, ethylene dichloride and dioxane.[5] The same procedure was also applied for solutions of the A/I 8 polymer dissolved in trimethyl phosphate–ethylene dibromide mixtures at a fixed concentration, which will be described later. Values obtained as the residue extinction coefficients of the pure β- and σ-forms in those three solvents are tabulated in Table IV.

TABLE IV
Residue Extinction Coefficients of the Amide I Bands for the Pure σ- and β-forms

	DIOX	EDC	TMP–EDB[a]
$\epsilon_{\tilde{\nu}_\sigma}{}^{\sigma}$	360	450	405
$\epsilon_{\tilde{\nu}_\beta}{}^{\beta}$	870	910	805

[a] For the A/I 8 polymer at 0.62 g/dl.

Since the application of the above method requires to measure a series of spectra, we have developed an approximate method for estimating the content of β-structure from a single spectrum. As can be seen from Table IV, the residue extinction coefficient of the β-form is about twice as large as that of the σ-form in the three solvent system, and then it may be assumed that a relation

$$\epsilon_{\tilde{\nu}_\beta}{}^{\beta} = 2\epsilon_{\tilde{\nu}_\sigma}{}^{\sigma} \tag{4}$$

holds for all the solvents examined here. If the amide I band of an observed spectrum is decomposed into two symmetrical components at $\tilde{\nu}_\beta$ and $\tilde{\nu}_\sigma$, their residue extinction coefficients are directly related with the content of β-structure by

$$\begin{aligned} \epsilon_{\tilde{\nu}_\beta}' &= f_\beta \epsilon_{\tilde{\nu}_\beta}^{\beta} \\ \epsilon_{\tilde{\nu}_\sigma}' &= (1 - f_\beta)\epsilon_{\tilde{\nu}_\sigma}^{\sigma} \end{aligned} \tag{5}$$

Eliminating $\epsilon_{\tilde{\nu}_\beta}^{\beta}$ and $\epsilon_{\tilde{\nu}_\sigma}^{\sigma}$ from Eqs. (5) by means of Eq. (4), the content of β-structure is obtained as

$$f_\beta = \frac{\epsilon_{\tilde{\nu}_\beta}'}{\epsilon_{\tilde{\nu}_\beta}' + 2\epsilon_{\tilde{\nu}_\sigma}'} \tag{6}$$

By using Eq. (6) the content of β-structure was estimated for the three polymers in various solvents at about 1 g/dl. and the results are given in Table III. Those values were found to agree, within 0.03, with those obtained by Eq. (3), for the systems in dioxane, ethylene dichloride, and trimethyl phosphate–ethylene dibromide mixtures.

Viscosity Behavior and the Content of β-Structure

In considering its conformational effect on high-molecular-weight polypeptides and its strong hydrogen bonding capacity,[6,9-12] dichloroacetic acid would differ from the other solvents in its interaction with the low-molecular-weight polypeptides. The conformation of the polypeptide in this solvent will be separately discussed in the last part of this paper.

As can be seen from Table III as well as from Table I, trifluoroethanol and trimethyl phosphate stabilize the σ-conformation of the polypeptides the best, and probably dimethylformamide would be a solvent of the same nature. It can be reasonably assumed that there occurs no conformational change upon dilution (to less than 1 g/dl) but the polypeptides remain to be in the σ-conformation over the concentration range of viscosity measurements. The normal viscosity behavior and the low values of reduced viscosity indicate the presence of a single and molecularly dispersed conformation of polypeptide, which is identified with the σ-conformation.

The A/I 3 polymer will also have the same conformation in chloroform and dioxane, but slightly large concentration dependence of reduced viscosity suggests that stronger interaction is operative between polypeptide molecules in those solvents.

On the other hand, the reduced viscosity of all the solutions which display anomalous viscosity behavior is generally high and clearly indicates the formation of aggregates of polypeptides. It can also be seen from Table III that in those solutions the polypeptide molecules consist of residues of both σ- and β-forms at a finite concentration (about 1 g/dl). Their content of β-structure ranges approximately from 5 to 65%. For the polypeptides with f_β from 5 to 30%, the reduced viscosity is high and increases with con-

centration very strongly, while for the polypeptides with f_β higher than about 30% the reduced viscosity has a maximum value at a certain concentration. However, the viscosity of solution, in which the polypeptide has f_β less than about 5%, is normal, as in the cases of the A/I 4 polymer in chloroform and dioxane. Similarly, it seems likely that a solution of polypeptide with f_β higher than about 65% does not display anomalous viscosity, as in the case of the A/I 8 polymer in ethylene dibromide. Since the content of β-structure defines the number of hydrogen bonds participating in the association, the observed viscosity behavior must be related with the mode of association or the structure of aggregates, largely determined by the hydrogen bonding.

Values of reduced viscosity given in Figure 1 clearly indicate that the association occurs more extensively in dioxane than in chloroform. Comparison of those values given in Figures 1 and 2 also suggests that the association is generally much stronger in ethylene dichloride than in dioxane, which is the result consistent with that previously obtained by direct determination of molecular weights.[5] In ethylene dibromide the viscosity behavior does not follow the order of the molecular weight of polypeptide sample, but it is clear that extensive association should be present.

Before giving an explanation for different viscosity behavior in relation to the structure of aggregates, it will be relevant to comment on the content of β-structure. As was previously demonstrated,[5] the content of β-structure of the polypeptides would vary with concentration in different ways, depending on solvent species and molecular weight of polypeptides. Thus the specification of f_β at a single concentration would not suffice to correlate the conformation with the state of association. For example, the A/I 4 polymer in dioxane is normal in viscosity behavior, while the reduced viscosity of the A/I 3 polymer in ethylene dichloride is anomalous, in spite of both having almost equal contents of β-structure at 1 g/dl.

Previously,[5] we have also shown that the A/I 8 polymer can form the intramolecularly hydrogen-bonded β-structure, both in dioxane and in ethylene dichloride. In the other solvents in which the A/I 8 polymer can form the β-conformation, the same structure would be able to form, at least, at low concentrations. With increasing concentration, conversion of the intramolecular to the intermolecular β-conformation occurs, whereby each molecule would be subject to unfolding and extension of its polypeptide chain. However, the chain is so short that any configurational change of a molecule would not bring about such a large viscosity change as was observed, especially, as in the anomalous viscosity. Furthermore, the A/I 3 and 4 polymers, which cannot accommodate with the intramolecularly folded β-structure, also exhibit anomalous viscosity behavior in some solvents. Thus the observed anomaly in viscosity should not be concerned with the change in shape of single molecules, but it must be connected with the whole structural change of aggregates.

In this connection, it should be pointed out that the mode of association is not directly related with the content of β-structure, which measures total

number of hydrogen bonds, but is determined by some fraction of it, which participates in the intermolecular cross-linking.

The Structure of Aggregates

Generally, the intrinsic viscosity of polymer solution is considered to represent the effective hydrodynamic volume of a solute molecule or particle, and the Huggins' constant is taken as a measure of polymer–solvent interaction. The A/I 47 polymer is α-helical in all the solvents that dissolve it,[3,6,13] except for in dichloroacetic acid, and the Huggins' constant in such solutions has been taken as an indication of association of the helical molecules.[6] Table III shows that the Huggins' constant of the polypeptides in a given solvent has a roughly common value, irrespective of molecular weight of polypeptide, i.e., of polypeptide conformation, as far as the normal viscosity is observed. We may then assess the concentration dependence of reduced viscosity for a solution having anomalous behavior, which it would have if it were normal, by using the value of Huggins' constant observed for a solution of other polypeptide in the same solvent but having normal viscosity. The concentration dependence calculated in this way was found to be generally small as compared with that exhibited in anomalous reduced viscosity.

To understand the anomalous behavior of reduced viscosity, we may then regard the reduced viscosity at any concentration as a measure of effective hydrodynamic volume of solute particle at that concentration in solution. In those solutions in which the viscosity behavior is largely anomalous, polypeptide molecules have a finite content of β-structure, at least, at 1 g/dl. The content of β-structure increases with polypeptide concentration in different ways, depending on solvent and molecular weight of polypeptide, and it determines the number of hydrogen bonds which defines the mode of association of aggregates. The intermolecular hydrogen bonding has two effects on the structure of aggregates. One is to promote the association of polypeptide molecules and thus to make aggregates larger. As far as the number of hydrogen bonds is not large, the polypeptide molecules in an aggregate are cross-linked together by a small number of hydrogen bonds at the residues of β-form and the aggregates would have a large effective volume. With increase in number of hydrogen bonds, however, cross-linkages between molecules in an aggregate would also form more extensively, instead of incorporating other molecules into it. This has another effect: it makes the shape of an aggregate more compact and thus reduces the effective volume of aggregates. As the polypeptide concentration is increased, those two effects compete with each other to such an extent as determined by the interaction of polypeptide with solvent, and the resulting change in effective hydrodynamic volume of aggregates, accompanying with concentration change, is revealed in the reduced viscosity. Thus the reduced viscosity increases with concentration if the aggregates grow larger, but, conversely, it decreases with increasing concentration, when hydrogen bonding prevails to contract the aggregates.

When the content of β-structure is sufficiently high, the molecules in an aggregate are closely held together by many hydrogen bonds, and a near-normal viscosity is observed. The A/I 8 polymer in ethylene dibromide has f_β amounting to 64% even at 0.1 g/dl and is considered to form rather compact aggregates. In this solution, the Huggins' constant would reflect the extent of interaction between almost-completed sheet-like structures or, possibly, of their further association through hydrogen bonding and side-chain interactions.

It is interesting to observe that stable foams can be readily produced on ethylene dibromide solutions of the A/I 8 polymer. We may imagine that this can be attributed to the formation of rigid network structures on surface, which may be identified with the hydrogen-bonded sheet-like structures.

Furthermore, it should be noted that the A/I 8 polymer is soluble in ethylene dibromide at room temperature, only up to about 0.8 g/dl. The solubility becomes increased at higher temperatures, where the number of hydrogen bonds would be reduced. This indicates that the polypeptide with contents of β-structure possibly higher than 75% would not be soluble nor stable in solution.

The effect of temperature on the reduced viscosity as given in Figure 3 can be interpreted on the basis of the above considerations. Blout and Asadourian[2] have observed that elevation of temperature decreases the intensity of the 1630 cm^{-1} band of an A/I 4 polymer in dioxane. We may similarly expect that the β-form is partly converted into the σ-form when the A/I 8 polymer is heated in ethylene dichloride. We have also observed that the average size of aggregates in this solution is slightly decreased at higher temperatures.[5] The observed temperature effect on the reduced viscosity indicates that, at low concentrations, the decrease in number of hydrogen bonds is effective to depress the association and thus to lower the reduced viscosity, while at high concentrations thermal effect is rather strong to prevent the aggregates from contracting and makes the viscosity higher.

A Series of Solvents Effective for the Association and the β-Structure Formation of the Polypeptides

From all the above considerations as well as the comparison of values of $[\eta]$ and f_β given in Table III, we may conclude that the association of low-molecular-weight poly-γ-benzyl-L-glutamate proceeds in various solvents in the same order as the formation of β-structure is promoted, and the following series of solvents can be constructed:

$$\text{DMF, TFE} < \text{TMP} < \text{CHL} < \text{DIOX} < \text{EDC} < \text{EDB}$$

It should be pointed out that the polypeptide–solvent interaction as revealed in k' is scarcely dependent on polypeptide conformation, and, therefore, it reduces to the residue–solvent interaction.

We can notice that the series of solvents for the association of low-molecular-weight poly-γ-benzyl-L-glutamate does not follow that for the associa-

tion of α-helices of high-molecular-weight poly-γ-benzyl-L-glutamate.[6,14-16] This can be clearly seen by comparison of the values of $[\eta]$ given in Table III.

The Conformation and Viscosity of the A/I 8 Polymer in TMP–EDB Mixtures

Since trimethyl phosphate favors the σ-form and ethylene dibromide stabilizes the β-form the best, it can be expected that polypeptide conformations with different contents of β-structure should be realized in mixtures of those solvents, and consequently, the polypeptide solutions will display different viscosity behavior as surveyed above in the series of pure solvents, when the solvent composition is varied. Examination of such solutions would give a support for our explanation on the occurrence of anomalous viscosity behavior.

Figure 4 shows infrared spectra of the A/I 8 polymer in the mixed solvents at a fixed concentration, 0.62 g/dl. It is clear that the polypeptide conformation varies with solvent composition and the content of β-structure gradually increases with increasing content of ethylene dibromide.

Figure 5 shows reduced viscosity of the same polymer in the mixed solvents as a function of concentration. In the pure solvents, the reduced viscosity is normal, but in the intermediate solvent compositions examined it is anomalous. In the solvent mixture containing 40% ethylene dibromide, the viscosity is anomalous, showing strong concentration dependence. With further increase in content of ethylene dibromide, the concentration dependence of reduced viscosity is stronger. In a solvent containing 90% ethylene dibromide, the reduced viscosity curve has a maximum at a certain concentration, as was observed for the A/I 8 polymer in ethylene dichloride and others.

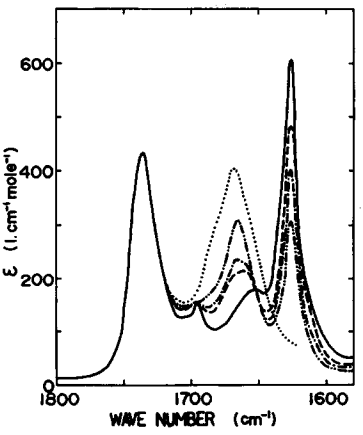

Fig. 4. Infrared spectra of the A/I 8 polymer in trimethyl phosphate–ethylene dibromide mixtures. Solvent composition (volume % of EDB): ·····, 0; —·—·—·, 60; —·—·, 80; - - - - -, 90; ——, 100.

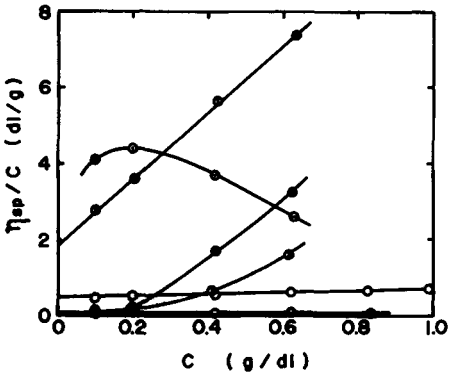

Fig. 5. Reduced viscosity of the A/I 8 polymer in trimethyl phosphate–ethylene dibromide mixtures at 25°C. Solvent composition (volume % of EDB): ◒, 0; ◑, 40; ⊕, 60; ⓘ, 80; ⊙, 90; ○, 100.

From an analysis of infrared spectra as given before, it was demonstrated that the residue extinction coefficients for the A/I 8 polymer in the mixed solvents obey the linear relation represented by Eq. (2). Extrapolations of the linear relation gave values of the residue extinction coefficients for the pure β- and σ-forms, as listed in Table IV. By using those values, the content of β-structure was estimated by Eq. (3) for each spectrum.

In Figure 6 are shown the content of β-structure at 0.62 g/dl and the reduced viscosity at three concentrations, both as functions of solvent composition. It is remarkable to observe that the σ–β transition of the polypeptide is very gradual against the solvent composition. The reduced viscosity increases at the intermediate compositions of mixed solvents, in which the polypeptide has moderate contents of β-structure. These results are con-

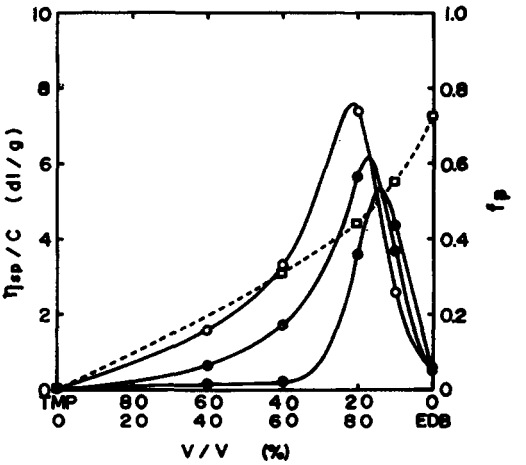

Fig. 6. The content of β-structure and reduced viscosities as functions of solvent composition. The A/I 8 polymer in TMP–EDB mixtures. □, f_β at 0.62 g/dl; η_{sp}/c: ○, at 0.62 g/dl; ⊙, at 0.41 g/dl; ⓘ, at 0.20 g/dl.

sistent with those deduced before and can be interpreted on the basis of changes in size and shape of aggregates. If the viscosity maxima at different concentrations, as given in Figure 6, correspond to an identical content of β-structure, ethylene dibromide must be further added with decreasing concentration, to have a similar viscosity maximum.

Examination of Other Factors Influence on Reduced Viscosity

In the viscosity measurements of some solutions, flow time changed for some period after the solution was prepared either by dissolving the solid polypeptide into solvent or by diluting a more concentrated solution in a viscometer. Those solutions were found to display anomalous viscosity behavior in their equilibrium state. Those were the solutions of the A/I 3 and 4 polymers dissolved in ethylene dichloride or ethylene dibromide, and the A/I 8 polymer dissolved in dioxane, ethylene dichloride, or trimethyl phosphate–ethylene dibromide mixtures containing 40–90% of ethylene dibromide. The change of flow time continued for a few hours to a few days, depending on the solution, before an equilibrium was attained, and its direction was specific for the particular solution. When the reduced viscosity obtained at equilibrium decreased upon dilution, the flow time decreased for a period after dilution and approached an equilibrium value. However, when the reduced viscosity at equilibrium increased upon dilution, as did at a concentration higher than that where the maximum reduced viscosity appeared, the flow time increased monotonically with time and approached a constant value. The magnitude of change in viscosity value was also various. For the A/I 8 polymer the reduced viscosity increased by about 20% of the equilibrium value, during 24 hours in dioxane and 3 hours in ethylene dichloride, both at the highest concentrations examined. Those were the largest change observed, and for the other solutions the change of viscosity was less. The time dependence of reduced viscosity must be related with the structural change of aggregates caused by the conformational change.

In general, high-molecular-weight polymers in solution display non-Newtonian viscosity behavior and their reduced viscosity decreases with increasing rate of shear or shearing stress. The shear dependence of reduced viscosity has been investigated for helical poly-γ-benzyl-L-glutamate[17,18] and for randomly coiled polymers.[17,19,20] It is found that such behavior is generally small for random coil polymers, as far as the molecular weight of polymer is not very large (less than 10^6) and the viscosity coefficient of solvent is not high.

When the content of β-structure of the polypeptide is moderate, which display anomalous concentration dependence of reduced viscosity, the aggregates formed in solution would have a structure, more or less, hydrodynamically similar to that of random coil. They could exhibit non-Newtonian viscosity. We have actually used viscometers, in which the rate of shear is, for example, of the order of 100 sec for ethylene dichloride. Taking

account of relatively low rates of shear and small sizes of aggregates, however, the effect of shear on the reduced viscosity could be safely ignored. Even if it were corrected, the viscosity anomaly would merely become more outstanding. Then all the viscosity behavior observed may be attributed to the changes in size and shape of aggregates accompanying with conformational change of polypeptide.

In the previous work,[5] we have observed that the number-average molecular weights of polypeptides are, at most, a few thousands either in ethylene dichloride or in dioxane. Those values of molecular weight would be too small to accommodate with the most values observed for reduced viscosity. However, the number-average molecular weight is rather insensitive to the presence of large aggregates, while the reduced viscosity would be more sensitive to their presence. Then the observed viscosity behavior can be regarded as largely contributed by those large aggregates present. Actually preliminary observations on sedimentation equilibria in dioxane solutions have definitely shown the formation of small amounts of large aggregates, besides of small aggregates. In most of the other solvents, in which the association of polypeptides is more extensive, the polypeptides would contain higher amounts of large aggregates.

Finally it will be pertinent to refer to the effect of polydispersity of the polypeptides samples on viscosity behavior. Since those samples have not been prepared by a stepwise synthesis, they cannot be always strictly monodisperse, although they would be fairly homogeneous in molecular weight. The interaction of polymer with solvent has been attributed to the interaction between residue and solvent and is thus indifferent to molecular weight of polypeptide. The reduced viscosity would then simply stand for an average hydrodynamic volume of those complex aggregates formed from molecules of different chain lengths. The polydispersity of the original polypeptide samples would not influence on our explanation for the anomalous viscosity behavior.

Conformational Changes of the A/I 8 Polymer in EDB–DCA Mixtures

To elucidate the conformation of the low-molecular-weight polypeptides in dichloroacetic acid as well as the stability of the β-structure in ethylene dibromide, the conformations of the A/I 8 polymer were examined in mixtures of ethylene dibromide and dichloroacetic acid, by means of infrared spectral measurements.

Infrared spectra of the A/I 8 polymer in the mixed solvents with relatively low contents of dichloroacetic acid are shown in Figure 7. The polypeptide concentration was fixed around 0.6 g/dl. In pure ethylene dibromide the amide I band at 1626 cm^{-1} is very strong, indicating the prominence of the β-conformation, but, with increasing dichloroacetic acid content, the spectra change in a complex way. The amide I band at 1626 cm^{-1} becomes weak, indicating the disruption of the β-conformation, and, at 1% dichloroacetic acid content, the amide I band appears only at 1661 cm^{-1}.

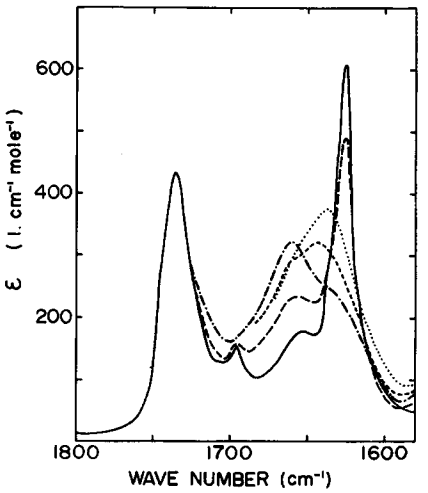

Fig. 7. Infrared spectra of the A/I 8 polymer in ethylene dibromide–dichloroacetic acid mixtures. Concentration, 0.61 g/dl. Solvent composition (volume % of DCA): ———, 0; — — —, 0.4; —·—·—, 1.0; - - -, 4.0; ·····, 10.0.

Thus the polypeptide conformation has been converted sharply from a nearly perfect β-structure to the σ-conformation. However, further increase in dichloroacetic acid content influences on the stability of the σ-conformation, and converts it into a conformation having the amide I band at 1638 cm^{-1}. This new conformation is complete at 10% dichloroacetic acid content and probably persists even in pure dichloroacetic acid. It is interesting to note that the same frequency of amide I band has been observed in very-low-molecular-weight poly-γ-benzyl-L-glutamate in the solid state, which is designated as the γ-peptide.[5,21]

It has been observed that the β-conformation of high-molecular-weight polypeptides, poly-*O*-benzyl-L-serine[11] and poly-*S*-carbobenzoxymethyl-L-cysteine,[12] formed in chloroform containing very low amounts of dichloroacetic acid is subject to a sharp transition to the random coil when dichloroacetic acid is further added. The random coil polypeptide is characterized by the amide I band at 1658 cm^{-1}.[12] Thus the σ-conformation and the new conformation with the amide I band at 1638 cm^{-1} are both considered to be characteristic of the low-molecular-weight polypeptides alone.

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Received August 1, 1972
Revised January 22, 1973