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The β-Conformation and Association of Low-Molecular-Weight Poly-γ-benzyl-L-glutamate in Solutions

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Synopsis

Three samples of poly-γ-benzyl-L-glutamate have been prepared from γ-benzyl-N-carboxy-L-glutamate anhydride with *n*-hexylamine initiation at anhydride-to-initiator molar ratios, [A]/[I], of 3, 4, and 8, and their conformation and association in ethylene dichloride and dioxane have been investigated by means of infrared spectra and vapor-pressure osmometry. Two conformations, σ- and β-forms, are present in those solvents, and the content of β-form increases with increasing A/I value and concentration. At infinite dilution molecular association is absent, but the number-average molecular weight increases with concentration, markedly in ethylene dichloride and, to a lesser extent, in dioxane. The fraction of residues involved in associated molecules have been estimated as a function of concentration. Combination of the content of β-structure with the fraction of association leads to the following results. The A/I 3 and 4 polymers form intermolecularly hydrogen-bonded aggregates, in which hydrogen-bonded residues are in the β-structure. The A/I 8 polymer has the intramolecularly hydrogen-bonded β-structure at very low concentrations, but it also forms intermolecularly hydrogen-bonded aggregates at high concentrations.

INTRODUCTION

As early as 1954 when they first reported the α-helical poly-γ-benzyl-L-glutamate in solutions, Doty and his co-workers[1] noted that the low-molecular-weight poly-γ-benzyl-L-glutamate cannot assume the α-helix but exists in the associated β-structure in chloroform and dioxane. Blout and Asadourian[2] pursued the problem further, and they found that the conformation of the polypeptides in solid film is dependent on the anhydride-to-initiator molar ratio used in preparing the polypeptides when the ratio is lower than 50 with *n*-hexylamine initiation. They also reported that these polypeptides change their conformation in chloroform and dioxane, depending on concentration, and the β-form is more stable at higher concentrations. All these results were mainly derived from the infrared spectral measurements, but Yang and Doty[3] observed the concentration dependence of optical rotatory dispersion for the same polypeptide in chloroform. Wada, Tsuboi, and Konishi[4] combined those two methods and studied the problem in a more systematic way, in order to obtain the Moffitt parameters for the β-structure in solutions.

In the present work infrared spectroscopy and vapor-pressure osmometry are applied to the low-molecular-weight poly-γ-benzyl-L-glutamate in ethylene dichloride and dioxane to elucidate the relation of its conformation with the associated state in those solvents. Polymerization of γ-benzyl-L-glutamate *N*-carboxyanhydride was initiated by *n*-hexylamine at the anhydride-to-initiator molar ratios, [A]/[I], 3, 4, and 8, to obtain the polypeptides of corresponding degrees of polymerization.

Since one of the current interests in polypeptide β-structure in solution lies in whether it is associated intermolecularly or not, the aim of the present work is directed to investigate this point. Most of the polypeptide β-structure so far found in solutions is either soluble only in mixed organic solvent systems, or in partially ionized in aqueous solvents, and its solution necessarily forms a multi-component system, for which thermodynamic or equilibrium measurements can derive uniquely definite results with some difficulty. In this sense, the β-structure formed by low-molecular-weight poly-γ-benzyl-L-glutamate in ethylene dichloride or dioxane is more suitable to be examined by those methods, although its formation is dependent on polypeptide concentration.

EXPERIMENTAL

Materials

γ-Benzyl-*N*-carboxy-L-glutamate anhydride prepared by the method of Blout and Karlson[5] was polymerized in dioxane by using *n*-hexylamine as an initiator at A/I 3, 4, and 8. Polymerization proceeded for several days, and the polymers were isolated by pouring the solution into petroleum ether. The A/I 4 and 8 polymers were further fractionated by treatment with 50 times the amount of 98% formic acid.[2] After the filtrate was evaporated up *in vacuo*, the residues were dissolved in dioxane and reprecipitated by pouring into water. The polymers were dried *in vacuo* at 50°C. Results of their elemental analysis and assumed chemical formula are given in Table I. Values of "residue" molecular weight, also listed in Table I, were obtained by dividing the formula molecular weight by the A/I value, and they are used for the calculation of residue extinction coefficient below.

n-Hexylamine was redistilled *in vacuo* (42–44°C, 29 mm Hg). Dichloroacetic acid was also vacuum redistilled. Ethylene dichloride and chloroform were dried over K_2CO_3 and CaH_2, respectively, and dioxane was refluxed with Na. All of them were redistilled immediately before use.

Measurements

Infrared spectra were recorded on a Jasco DS-402 G Spectrophotometer at 25°C over the frequency region from 1800 to 1450 cm^{-1} with an expanded frequency scale (6 cm/100 cm^{-1}). Solid films were cast from chloroform solutions on KBr plates. Solution spectra were measured in three cells of fixed path length, having CaF_2, KBr, and NaCl windows,

TABLE I
Elemental Analysis, Chemical Formula, and Residue Molecular Weight

Code number	[A]/[I]	Elemental analysis (%) Found C	Found H	Found N	Calculated C	Calculated H	Calculated N	Chemical formula	Residue molecular weight
K203	3	64.04	7.16	8.31	64.44	7.19	7.39	$C_6H_{13}NH(C_{12}H_{13}O_3N)_3H$	253.0
K204	4	64.10	6.80	7.79	64.47	6.81	6.84	$C_6H_{13}NH(C_{12}H_{13}O_3N)_4H \cdot HCO_2H$	252.1
K208	8	63.74	6.36	7.15	65.04	6.43	6.64	$C_6H_{13}NH(C_{12}H_{13}O_3N)_8H \cdot HCO_2H$	237.6

respectively. Path length was 0.05 mm for concentration range, 0.6 to 8 g/dl^{-}, 0.5 mm for 0.1 to 0.6 g/dl, and 1 mm for lower concentrations. Reproducibility of spectrum was always better than 0.3% in transmittance scale, for both solution and solvent. The optical density of dissolved polymer was obtained from the ratio of transmittance of solvent to that of solution at a given frequency, both measured in the same cell. The residue extinction coefficient, ϵ, was calculated by dividing the optical density by path length and residue molecular weight given in Table I.

Vapor-pressure osmometry was performed on a Hewlett-Packard 302 B Vapor Pressure Osmometer with fixed thermostats at 25, 37, and 50°C, by using a nonaqueous probe, 18572. The output imbalance voltage, V, of thermistor bridge is related with the difference in chemical potential of solvent, in the presence and absence of solute, which, in turn, is inversely proportional to the apparent number-average molecular weight, $\bar{M}_{n,\text{app}}$

$$\frac{V}{c} = K_{\text{calib}} \frac{1}{\bar{M}_{n,\text{app}}} \tag{1}$$

where K_{calib} is the calibration constant to be determined by the aid of a standard material for each solvent. The apparent number-average molecular weight at concentration, c (g/ml), is generally given by

$$\frac{1}{\bar{M}_{n,\text{app}}} = \frac{1}{\bar{M}_n} + Bc \tag{2}$$

where $\bar{M}_n$ is the number-average molecular weight of solute and B is the second virial coefficient. Values of K_{calib} were determined by two standard materials, benzil and sucrose octaacetate, both in ethylene dichloride and dioxane at each temperature. The values determined by the two materials at infinite dilution were consistent with each other within 4%, for each solvent at each temperature. Steady state between drops of solvent and solution was attained within 10 minutes, and reading of the output voltage was taken at 10 minutes after a solution drop was attached to the thermistor bead.

All the solutions were prepared on a weight basis, but the concentration of solution is expressed by c in the unit of g/dl by correcting the density of solution.

RESULTS

Conformation of the Polypeptides in the Solid State

Infrared spectra of the three polypeptides in the solid film are shown in Figure 1. Polypeptide conformation can be assigned to each polypeptide from the location and intensity of the characteristic amide I band. The A/I 3 polymer is associated with a 1643 cm^{-1} band and a weak 1704 cm^{-1} band, and the former can be ascribed to the γ-peptide, according to Idelson

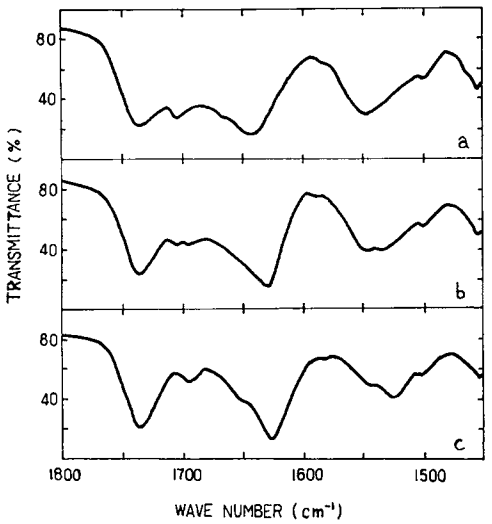

Fig. 1. Infrared spectra of poly-γ-benzyl-L-glutamate in solid film. (*a*) A/I 3; (*b*) A/I 4; (*c*) A/I 8.

and Blout.[6] The A/I 4 polymer is exclusively in the β-structure of antiparallel chain arrangement, as evidenced by the 1631 and the weak 1695 cm^{-1} bands.[7] The highest-molecular-weight polymer, A/I 8, is also in the β-structure of antiparallel chain arrangement, but it contains a small amount of the α-helical structure characterized by the 1652 cm^{-1} band. These results are in agreement with those previously reported.[2,6]

Infrared Spectra of the Polypeptides in Solutions

Figure 2 shows the infrared spectra of the three polypeptides in ethylene dichloride at a fixed concentration. The spectra are strongly dependent on the A/I value or the degree of polymerization, as in the solid state. The amide I band around 1630 and 1670 cm^{-1} can be ascribed to the β-form and the σ-form, respectively. The β-form is clearly of antiparallel chains type, since the 1630 cm^{-1} band is always accompanied by a weak 1696 cm^{-1} band.[7] The σ-form, designated after Blout and Asadourian,[2] has never been clearly defined but would be a conformation in solution, characteristic of low-molecular-weight polypeptides. In dioxane similar spectra are observed, but the 1630 cm^{-1} band is weaker, as seen in Figure 3. The β-form is more stable for the higher A/I polymers in both solvents.

Figure 4 shows the infrared spectra of the A/I 4 polymer at different concentrations in ethylene dichloride. The spectra are strongly dependent on the concentration. The relative change of intensities of the two amide I bands indicates that the β-form is more stable at higher concentrations, while it is converted into the σ-form upon dilution. Similar results are obtained for the A/I 4 polymer in dioxane, but the equilibrium shifts towards favoring the σ-form. These observations are in accord with

those previously published.[2,4] For higher A/I polymers, marked conformational change is observed to occur at lower concentration range. Figure 5 illustrates the concentration dependence of spectra for the A/I 8 polymer in ethylene dichloride.

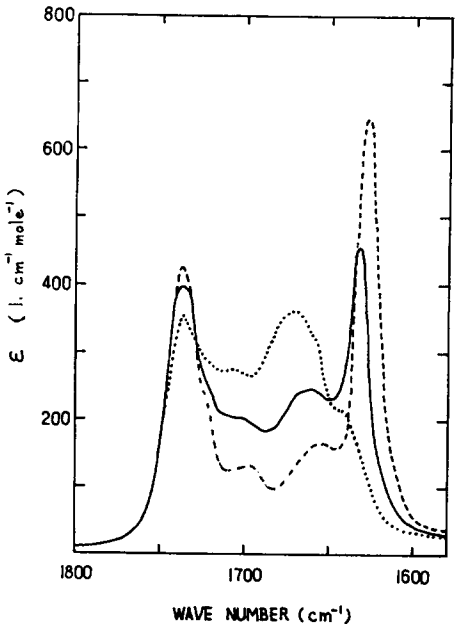

Fig. 2. Infrared spectra of poly-γ-benzyl-L-glutamate in ethylene dichloride. ·····, A/I 3, 3.83 g/dl; ——, A/I 4, 3.93 g/dl; - - -, A/I 8, 3.78 g/dl.

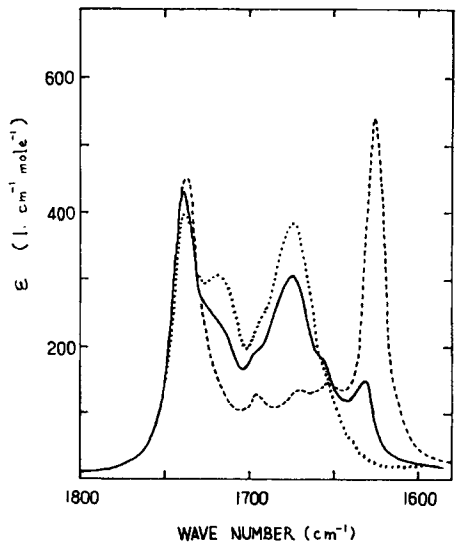

Fig. 3. Infrared spectra of poly-γ-benzyl-L-glutamate in dioxane. ·····, A/I 3, 3.89 g/dl; ——, A/I 4, 3.66 g/dl; - - -, A/I 8, 3.71 g/dl.

Table II gives the exact positions of the amide I bands for these three polypeptides in the two solvents. Frequencies of both bands shift to lower as the A/I value is higher. Furthermore, the ester carbonyl band shifts from 1736 cm^{-1} to 1720 cm^{-1}, when the σ-form is predominant.

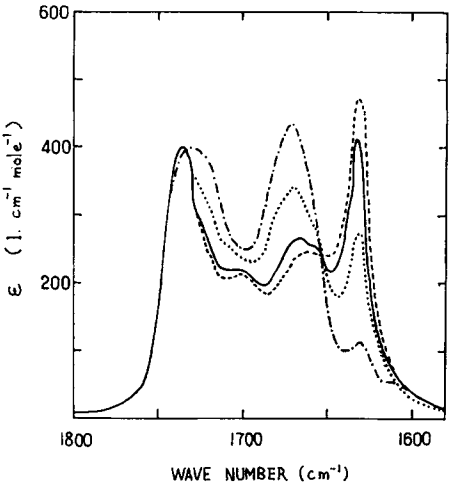

Fig. 4. Infrared spectra of poly-γ-benzyl-L-glutamate A/I 4 in ethylene dichloride. -·-·-, 0.19 g/dl; ·····, 0.61 g/dl; ——, 2.00 g/dl; - - -, 5.77 g/dl.

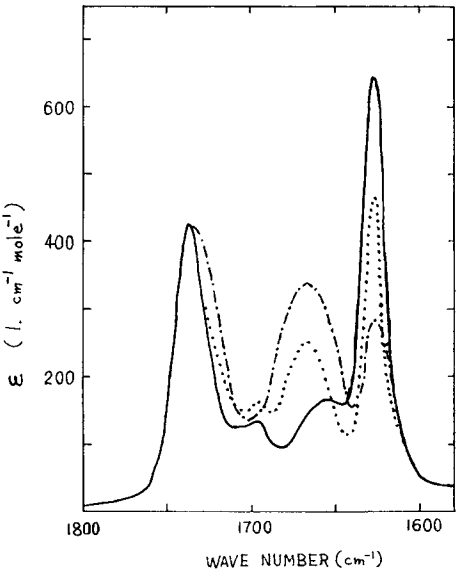

Fig. 5. Infrared spectra of poly-γ-benzyl-L-glutamate A/I 8 in ethylene dichloride. -·-·-, 0.01 g/dl; ·····, 0.10 g/dl; ——, 3.78 g/dl.

TABLE II
Wave Number of the Amide I Band

[A]/[I]	Wave number (cm^{-1})			
	In ethylene dichloride		In dioxane	
	$\bar{\nu}_\sigma$	$\bar{\nu}_\beta$	$\bar{\nu}_\sigma$	$\bar{\nu}_\beta$
3	1675	1639	1674	1639
4	1669	1632	1674	1631
8	1667	1627	1672	1627

Vapor-Pressure Osmometry

Figure 6 represents the results of vapor-pressure osmometry for the polypeptides in both solvents and shows the plots of reciprocal apparent number-average molecular weight against concentration. The number-average molecular weight obtained by extrapolation to infinite dilution is approximately equal to the formula molecular weight of each polypeptide, irrespective of solvent species and temperature. These values are tabulated in Table III. Thus molecular association is absent from the solu-

TABLE III
Molecular Weight of the Polypeptides

[A]/[I]	Formula molecular weight	$\bar{M}_n$[a]	$\bar{M}_w$[b]	$[\eta]$, dl/g[c]
3	758.9	760	930	0.061
4	1008.2	910	1080	0.062
8	1901.1	2000	2000	0.069

[a] Number-average molecular weight determined by vapor-pressure osmometry at infinite dilution in ethylene dichloride and dioxane.

[b] Weight-average molecular weight estimated from $[\eta]$ according to Mitchell, Woodward, and Doty.[8]

[c] Intrinsic viscosity measured in dichloroacetic acid at 25°C.

tions at infinite dilution. Table III also includes values of weight-average molecular weight derived from the intrinsic viscosity in dichloroacetic acid.[8] The number-average molecular weight is approximately equal to the weight-average molecular weight. Thus the polypeptides are fairly homogeneous in molecular weight, as far as the observed ratios of the weight-average to number-average molecular weights are concerned.

The homogeneity of the polypeptide samples will deserve some comments. It has been shown that low-molecular-weight poly-γ-benzyl-L-glutamates prepared by the *N*-carboxyanhydride method have very broad molecular-weight distributions.[8] However, it was also demonstrated that they can be fractionated by treatment with formic acid into α- and β-polypeptides,[2,6,8] each having high and low molecular weights and giving a single spot on the chromatogram, respectively. It is not yet certain whether each fraction is homogeneous or not, but various evidence

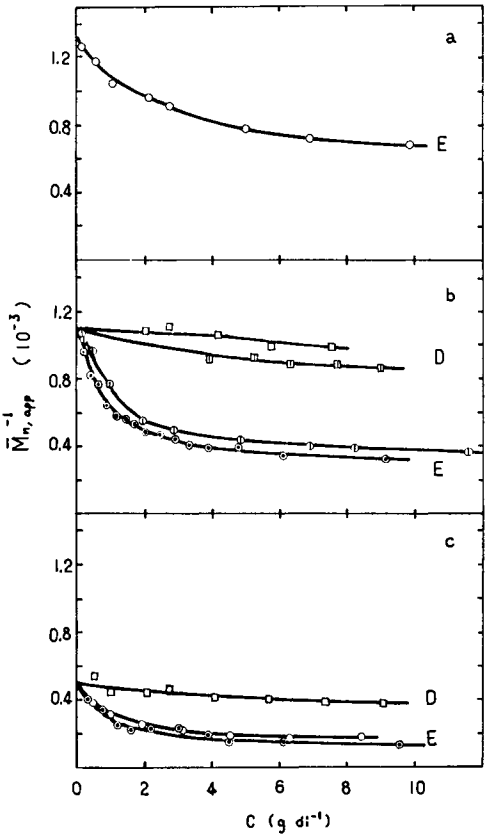

Fig. 6. Relations of $\bar{M}_{n,app}^{-1}$ with c for poly-γ-benzyl-L-glutamate in ethylene dichloride (E, circles) and in dioxane (D, squares). (*a*) A/I 3, O 50°C; (*b*) A/I 4, ⊙ 25°C, ◑◨ 37°C, □ 50°C; (*c*) A/I 8, ⊙ 25°C, O□ 50°C.

seems to suggest a low degree of polydispersity, especially, for the formic acid-soluble fraction. Thus it must be concluded that the fractionation of the polypeptide samples with formic acid was effectively performed to obtain the low-molecular-weight polypeptides, whose molecular-weight distributions were relatively sharp.

With increasing concentration, however, the polypeptide molecules associate intermolecularly, and the association proceeds more sharply in ethylene dichloride than in dioxane. The effect of temperature on the association is rather small in both solvents. It can also be seen that the average number of association is not very high, even at the highest concentration measured. This usually suggests that only small aggregates are formed even at very high concentrations, although reservations must be made that the number-average molecular weight is relatively insensitive to the presence of large aggregates and the effect of second virial coefficient acts to reduce the number of association.

DISCUSSION

Content of β-Structure in Solutions

Low-molecular-weight poly-γ-benzyl-L-glutamate can take three conformations in ethylene dichloride and dioxane that are distinguishable by infrared spectral measurements. These are the σ-form characterized by the 1670 cm^{-1} amide I band, the β-form with the 1630 and the weak 1696 cm^{-1} bands, and a small amount of the α-form having the 1653 cm^{-1} band.

The content of β-structure, f_β, is defined by the weight (mole) fraction of residues in the β-structure. These residues would be hydrogen bonded with other residues through both imino and carbonyl groups and contribute to the intensity of the 1630 cm^{-1} band. The content of β-structure is determined from the observed extinction coefficients of amide I bands under the following assumptions. (*1*) The content of α-form is put equal to zero. An analysis of the spectra for the A/I 8 polymer at high concentrations, in which the 1653 cm^{-1} band appears as a weak peak, has shown that the content of α-form is less than 10%. At lower concentrations the content would not exceed this. For the lower A/I polymers the content would be much less and can be safely neglected. (*2*) The amide I band characteristic of each structure has an identical spectral shape with identical residue extinction coefficient at the peak, irrespective of different A/I values, although its peak position depends on the A/I value. This permits us to treat all the data on different A/I polymers in the same solvent as a single set of different contents of β-structure. (*3*) Concentration dependence of the residue extinction coefficient characteristic of each structure is not considered. Actually it could occur in such a wide concentration range as covered in the present experiment.

The residue extinction coefficient, $\epsilon_{\tilde{\nu}}$, at a wave number, $\tilde{\nu}$, is given by

$$\epsilon_{\tilde{\nu}} = (1 - f_\beta)\, \epsilon^{\sigma}_{\tilde{\nu}} + f_\beta \epsilon^{\beta}_{\tilde{\nu}} \tag{3}$$

where $\epsilon^{\sigma}_{\tilde{\nu}}$ and $\epsilon^{\beta}_{\tilde{\nu}}$ are residue extinction coefficients for the pure σ- and β-forms, respectively. Choosing the two wave numbers of the peak position for the 1670 and 1630 cm^{-1} bands as $\tilde{\nu}$, equation (3) can be solved and a linear relation between ϵ_{1670} and ϵ_{1630} is derived.

$$\frac{\epsilon_{1670} - \epsilon^{\beta}_{1670}}{\epsilon^{\sigma}_{1670} - \epsilon^{\beta}_{1670}} + \frac{\epsilon_{1630} - \epsilon^{\sigma}_{1630}}{\epsilon^{\beta}_{1630} - \epsilon^{\sigma}_{1630}} = 1 \tag{4}$$

The wave numbers of the two peaks change with the A/I value and the solvent species, as given in Table II, and in the application of equation (4) values of the extinction coefficients at those wave numbers were taken as ϵ^{σ}_{1670} and ϵ^{β}_{1630}. The effects of tail of the other band, ϵ^{β}_{1670} and ϵ^{σ}_{1630}, which were needed for the evaluation of numerators in the left side of equation (4), were estimated as follows. A spectrum of the A/I 8 polymer at a high concentration was assumed to approximate to that for $f_\beta = 1$ and graphically resolved into components, i.e., 1670 and 1630 cm^{-1} bands. The

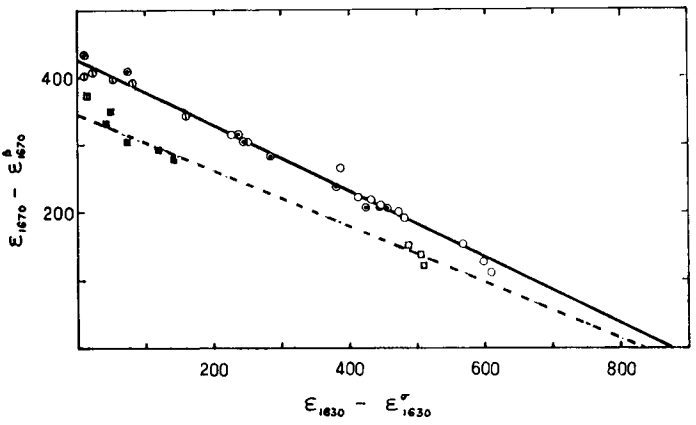

Fig. 7. Relations between $\epsilon_{1670} - \epsilon^{\beta}_{1670}$ and $\epsilon_{1630} - \epsilon^{\sigma}_{1630}$ for poly-γ-benzyl-L-glutamate in ethylene dichloride (——, circles) and in dioxane (- - -, squares). ⊕□, A/I 3; ⊙⊡, A/I 4; ○□, A/I 8.

ϵ^{β}_{1670} value was read at each wave number associated with the A/I value and used for estimate of $\epsilon_{1670} - \epsilon^{\beta}_{1670}$. Similarly, a spectrum of the A/I 3 polymer at a low concentration was approximated to that for $f_\beta = 0$ and resolved into components, and the ϵ^{σ}_{1630} value was read at the characteristic wave number. These procedures were performed for both solvents, and the obtained values ranged from 10 to 30 for ϵ^{β}_{1670} and 50 to 30 for ϵ^{σ}_{1630}.

Figure 7 shows the linear relations between $\epsilon_{1670} - \epsilon^{\beta}_{1670}$ and $\epsilon_{1630} - \epsilon^{\sigma}_{1630}$ in ethylene dichloride and dioxane, obtained in this way. The linear relation permits to evaluate the residue extinction coefficient, $\epsilon^{\beta}_{1630} - \epsilon^{\sigma}_{1630}$, from the intercept with the abscissa. Then the content of β-structure is readily obtained by

$$f_\beta = \frac{\epsilon_{1630} - \epsilon^{\sigma}_{1630}}{\epsilon^{\beta}_{1630} - \epsilon^{\sigma}_{1630}} \tag{5}$$

and is given in Figure 8 as a function of concentration.

Association of the Polypeptides in Solutions

From the observed dependence of apparent number-average molecular weight on concentration, the mode of association of the polypeptides in solutions can be derived by means of the Steiner's analysis.[9,10] For such an analysis the following assumptions are made: (*1*) Monomer molecules are monodisperse in molecular weight. This is supported by the fact that the number-average and the weight-average molecular weights are approximately equal to each other. (*2*) The polypeptide solutions are ideal except for the association–dissociation equilibria involved, that is, the second virial coefficient is omitted: $B = 0$ for both monomers and aggregates. Then the apparent molecular weight is equal to the number-average molecular weight, i.e., $\bar{M}_{n,\mathrm{app}} = \bar{M}_n$, at any finite concentration. This is

partly supported by the trend of curves, which do not exhibit any upward rise of $\bar{M}_{n,\mathrm{app}}^{-1}$ with concentration.

If the monomer molecule, whose molecular weight is M_1, is represented by A and the m-mer molecule is by A_m, the concentration-dependent association of the polypeptide in solution can be completely described by a series of association equilibria

$$mA \rightleftarrows A_m \quad m = 2,3, \ldots . \tag{6}$$

for which the equilibrium constants on the weight concentration basis are given by

$$K_m' = \frac{c_m}{c_1^m} \quad m = 2,3, \ldots \tag{7}$$

where c_1 and c_m are the weight concentrations (g/ml) of monomer and m-mer, respectively.

For total concentration, c, the weight fraction of monomer, w_1, is given by c_1/c, and it can be shown that

$$\ln w_1 = \int_0^c \left(\frac{M_1}{\bar{M}_n} - 1\right)\frac{dc}{c} + \left(\frac{M_1}{\bar{M}_n} - 1\right) \tag{8}$$

The integration of the first term in the right side can be performed graphically. Since the weight fraction of monomer is just equal to the weight

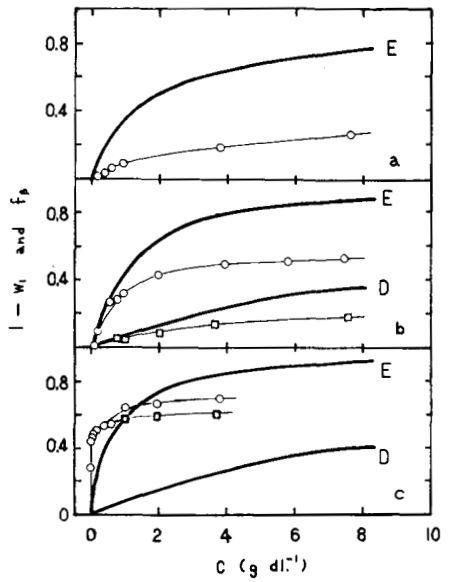

Fig. 8. Variations of fraction of association, $1 - w_1$, and content of β-structure, f_β, with concentration. E and circles, ethylene dichloride; D and squares, dioxane. ——, $1 - w_1$; ——, f_β. (*a*) A/I 3 (50°C); (*b*) A/I 4 (37°C); (*c*) A/I 8 (50°C). Temperatures indicated are for $1 - w_1$ only.

(mole) fraction of residues in unassociated monomers, it is then ready to find the fraction of association, $1 - w_1$, at a given concentration, where the fraction of association is defined as the weight (mole) fraction of residues in associated molecules (dimers and higher aggregates). The results are shown in Figure 8 as a function of concentration.

Inter- and Intra-molecularly Hydrogen Bonded β-Conformations

Figure 8 shows both the content of β-structure and the fraction of association as functions of concentration. At infinite dilution the polypeptide molecules are free from association, but the fraction of association increases with concentration, rather sharply in ethylene dichloride and gradually in dioxane. It is clear that ethylene dichloride promotes the association of polypeptide more than dioxane. The fraction of association is not very different for different A/I polymers in each solvent.

On the other hand, all the polypeptide molecules or residues are in the σ-form at infinite dilution. Thus, if all the residues in a molecule belong to the σ-form, the molecule should be isolated from other molecules in solution. With increasing concentration, some of these residues in the σ-form are transformed into those in the β-form, by making linkages of hydrogen bonds either between molecules or within a molecule, and the content of β-structure increases.

For the A/I 3 and 4 polymers, the fraction of association is larger than the content of β-structure at all concentrations. This means that all the residues in associated molecules are not in the β-form, but some of them are in the σ-form. Molecules constituting an aggregate are linked together through a relatively small number of residues, which are assigned to being in the β-form. Thus an intermolecularly hydrogen-bonded conformation is formed with these polypeptides, but its β-structure is incomplete in those solvents. Formation of the intramolecularly hydrogen-bonded β-structure would be excluded from those short-chain polypeptides. It can be seen that intermolecular hydrogen bonding or association occurs more favorably in ethylene dichloride than in dioxane. However, it seems unlikely that hydrogen bonding would prevail over all residues in the aggregates in those solvents.

On the other hand, the hydrogen bonding in the A/I 8 polymer proceeds quite differently from that in the A/I 3 and 4 polymers, while the association behavior is similar. At very low concentrations, the content of β-structure rapidly increases with concentration and exceeds the fraction of association considerably. The latter implies that the total number of hydrogen bonds is much larger than the number of intermolecular hydrogen bonds as expected from the amount of associated molecules. Then some of the polypeptide molecules must be in the intramolecularly hydrogen-bonded β-structure, most of which will be isolated from one another. In this structure, an extended polypeptide chain is folded back on itself at its middle portion, and peptide bonds are hydrogen-bonded intramolecularly. The A/I 8 polymer has a polypeptide chain long enough to

accommodate with this folded structure. In the concentration range, where association is not very appreciable, the content of β-form is close to a half. This is also consistent with the formation of the intramolecular β-structure, since a half number of residues can form hydrogen bonds in it.

Upon further increase in concentration, the association proceeds continuously, depending on the solvent species, while the contents of β-structure in those two solvents are almost equal and their rates of increase are considerably depressed. Thus the fraction of association higher than the content of β-structure is observed at high concentrations in ethylene dichloride, while the reverse relation remains to hold for all concentrations in dioxane. These observations can be attributed to the difference in number of hydrogen bonds that are participating in the intermolecular linkages in the two solvents. In ethylene dichloride the intermolecular hydrogen bonding is predominant, while in dioxane the intramolecular linkages prevail and the association is much suppressed. Since the total number of hydrogen bonds does not increase appreciably with concentration after its initial sudden increase, the intermolecularly hydrogen-bonded aggregates would be formed from the molecules of the intramolecularly folded β-structure by rearrangement of hydrogen bonds from intramolecular to intermolecular. In such a process polypeptide chains must undergo unfolding or refolding, and then they would have more extended or less folded configurations in the aggregates.

Finally it is concluded that, at a given concentration, the number of hydrogen bonds participating in the intermolecular association is mainly determined by specific interaction of polypeptide residue with solvent, and thus the mode of association in the same solvent is almost indifferent to the polypeptide chain length.

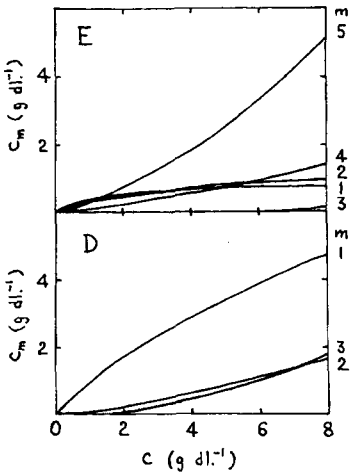

Fig. 9. Weight concentrations of m-mers, c_m, as functions of total concentration, c, for poly-γ-benzyl-L-glutamate A/I 8. E, ethylene dichloride; D, dioxane. Temperature 50°C.

Mode of Association

In order to see the difference in modes of association of the polymer in ethylene dichloride and in dioxane, fractions of aggregates of different sizes were estimated at given concentrations, by applying the Steiner's successive analysis.[9,10] This analysis would reveal, at least, a qualitative aspect of association, although it accumulates errors successively and the results become less reliable for higher aggregates.

As the fraction of monomer, w_1, has been determined by means of equation (8), the dimer association constant, K_2', can be assessed by

$$c - cw_1 = \sum_{m \geq 2} K_m'(cw_1)^m \tag{9}$$

from the intercept of the plot of $(c - cw_1)/(cw_1)^2$ against cw_1. The trimer association constant, K_3', can be evaluated in a similar way by plotting $\{(c - cw_1)/(cw_1)^2 - K_2'\}/cw_1$ against cw_1. The higher association constants can be obtained successively. Then it is ready to find the weight concentrations of m-mers, c_m, by means of equations (7).

Figure 9 illustrates the change of weight concentrations of aggregates or m-mers as functions of total concentration. In ethylene dichloride, aggregates as large as tetramer and pentamer are readily formed even at very low concentrations, and the concentrations of larger aggregates increase more rapidly with increasing total concentration. In dioxane, however, most molecules are free from association, and only small amounts of small aggregates, which are, at most, dimeric or trimeric, are formed. Quite similar features are seen for all the polymers and at all temperatures examined, as can be also expected from Figure 6. Thus it can also be concluded that size of aggregates and its distribution are determined by the interaction of polypeptide residue with solvent at a given concentration. However, it is to be noted that the results have been derived from the data of number-average molecular weight, which is less sensitive to the presence of large aggregates, and are based on the treatment for ideal systems as well as on the assumption of monodisperse monomers.

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