Studies on Long Chain Alkyl Amides of Water-soluble Poly-α-amino Acids. II. Infrared Spectra of Poly-DL-alanine Dodecylamide

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Infrared spectral studies of synthetic polypeptides have been an important method for elucidation of the molecular conformation. Experimental investigations have been made by Elliott[1-4], Bamford[1,5] and Blout[6-8] to correlate the conformation of various polypeptide specimens with the frequency of characteristic band in infrared spectra, and recently a semiempirical interpretation of the spectra has been developed by Miyazawa[9,10].

Blout and Asadourian[6] found that an L-polypeptide, poly-γ-benzyl-L-glutamate, consists of different amounts of the α- and β-forms, depending on the mode of preparation and the degree of polymerization. The α-form was characterized by an absorption around 1655 cm^{-1} in the amide I band, and the β-form by an absorption around 1630 cm^{-1}. Bamford, Happey and Hanby[5] reported the dependence of the infrared spectra and the conformation of copoly-1 : 1-(DL-phenylalanine, DL-leucine) on the degree of polymerization, and Robinson and Bott[11] correlated the conformation of copoly-1 : 1(γ-methyl-L-glutamate, DL-phenylalanine) with the degree of polymerization.

On the other hand, the mechanism of the polymerization reaction of *N*-carboxy-α-amino acid anhydride with primary amine initiation[12] leads us to expect that the polypeptide produced by this reaction should link with the amine residue on its terminal carboxyl group by a peptide bond. No direct evidence for such a linkage has yet been found, however. Infrared spectral study of this kind of polypeptides would also provide information to demonstrate such a terminal structure, if the

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[1] C. H. Bamford, A. Elliott and W. E. Hanby, "Synthetic Polypeptides", Academic Press, New York (1956).

[2] E. J. Ambrose and A. Elliott, *Proc. Roy. Soc.*, **A205**, 47 (1950).

[3] E. J. Ambrose and A. Elliott, ibid., **A208**, 75 (1951).

[4] A. Elliott, ibid., **A221**, 104 (1953).

[5] C. H. Bamford, W. E. Hanby and F. Happey, ibid., **A206**, 407 (1951).

[6] E. R. Blout and A. Asadourian, *J. Am. Chem. Soc.*, **78**, 955 (1956).

[7] E. R. Blout and M. Idelson, ibid., **80**, 4909 (1958).

[8] E. R. Blout, C. deLozé, S. M. Bloom and G. D. Fasman, ibid., **82**, 3787 (1960).

[9] T. Miyazawa, *J. Chem. Phys.*, **32**, 1647 (1960).

[10] T. Miyazawa and E. R. Blout, *J. Am. Chem. Soc.*, **83**, 712 (1961).

[11] C. Robinson and M. J. Bott. *Nature*, **168**, 325 (1951).

[12] S. G. Waley and J. Watson, *Proc. Roy. Soc.*, **A199**, 499 (1949).

primary amine for initiation was properly chosen.

We have studied a long-chain alkyl amide of water-soluble poly-α-amino acid, which we call poly-DL-alanine dodecylamide$^{13)}$. In this paper we will report the results of the infrared spectral study on poly-DL-alanine dodecylamide (PADA) with reference to poly-DL-alanine. Our original interests have been directed towards the confirmation of the presence of a dodecyl group in the molecule and the dependence of the molecular conformation on the mode of preparation or on the degree of polymerization. For these purposes, the polymers were investigated in the solid state. However, the spectra of the polymers in Nujol mull or in potassium bromide disk were found to exhibit some complications$^{13)}$. Thus the polymers were exclusively examined in the state of film which had been cast from solutions in various solvents.

The observed infrared spectra themselves reflected the difference in structure as well as in conformation, depending on the mode of preparation. Furthermore, they were found to be also strongly dependent on the state of solution from which the film had been cast. These were interpreted as a result of conformational change of the polymer in the dissolved state.

Experimental

Materials.—Poly-DL-alanine dodecylamide was prepared as previously described, and all the specimens except one were the same as in the previous investigation$^{13)}$. PADA obtained from the anhydride/initiator molar ratio, [A]/[I], of 5 to 30 was found to have a number average degree of polymerization approximately equal to [A]/[I]. Poly-DL-alanine was prepared by triethylamine initiation or by sodium methoxide initiation.

The number average degree of polymerization of the polymers was determined by the anhydrous titration of a terminal amino group.

PADA and poly-DL-alanine were soluble in formic acid, dichloroacetic acid, trifluoroacetic acid and *m*-cresol. When PADA of [A]/[I] higher than 5 and poly-DL-alanine were dissolved in formic acid to a concentration of about 10%, the solutions gelled after a few hours to a few days. The gelation occurred faster, as the [A]/[I] value was lower. The polymer solutions in the other solvents did not show any such tendency to gel.

PADA of [A]/[I] higher than 15 and poly-DL-alanine were soluble in distilled water*. All the polymers were soluble in aqueous hydrochloric acid solution, the pH value of which was lower than 5.5. When PADA of [A]/[I] higher than 5 or poly-DL-alanine was dissolved in aqueous hydrochloric acid or in distilled water to a concentration of about 10%, the solution gelled at room temperature after several hours to several days, and the gelation occurred faster as the pH of the solution was lower. The solution gelled also by heat.

Some properties of these polymers are listed in Table I.

Infrared Measurements.—Films for infrared measurements were cast on silver chloride plates from about 10% polymer solutions in various solvents, whether they were fluid or gelled. Films cast from formic acid solution or from aqueous solution were dried at room temperature for 4 hr. or more. Dichloroacetic acid or trifluoroacetic acid could be removed from cast films with great difficulty, merely by drying them by heat (ca. 50°C) in vacuo (4 mmHg) for several hr.; the remaining solvent greatly affected the spectra and the conformation of the polymer. It was more difficult to remove the solvent, as the [A]/[I] value of the polymer was higher.

All infrared spectral measurements were performed on a Perkin-Elmer 221 spectrophotometer using a sodium chloride prism. The temperature was about 20°C, and the relative humidity was about 40%.

Results

Poly-DL-alanine. — Poly-DL-alanine obtained from [A]/[I] of 50 or more with either sodium methoxide or triethylamine initiation showed identical spectra when measured in

TABLE I. PROPERTIES OF PADA AND POLY-DL-ALANINE

Sample No.	[A]/[I]	Degree of polymerization	Amount of HCl added to bring aqueous solution to pH 5.5 (mmol./g.)	Approximate % of each conformation	
				Random coil	β-Form
A431 (PADA)	5	5.4	1.13	5	95
A432 (PADA)	10	10.2	0.50	15	85
A433 (PADA)	15	14.6	0.24	45	55
A434 (PADA)	20	18.5	0.12	85	15
A605 (PADA)	30		—	98	2
A203 (Et_3N)	50		—	100	0
A808 (NaOMe)	150	49	—	100	0

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13) T. Isemura, S. Ikeda, F. Tokiwa and J. Noguchi, This Bulletin, **34**, 1236 (1961).

* Poly-DL-alanine contained a small amount of insoluble material, which was stated to have a cyclic structure at the terminal residue (Ref. 1, p. 298).

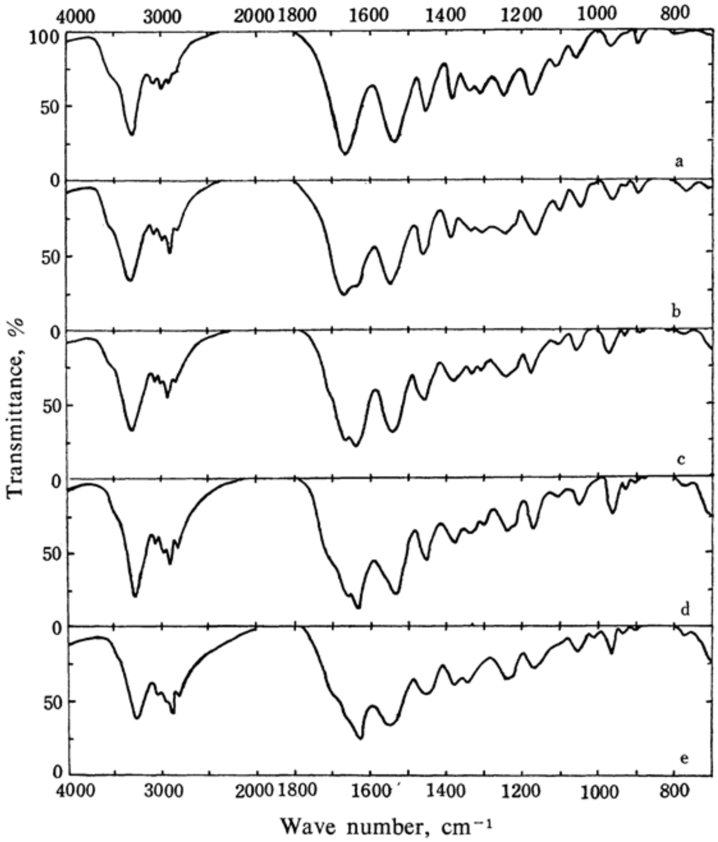

Fig. 1. Infrared spectra of poly-DL-alanine and poly-DL-alanine dodecylamides. Films cast from formic acid solutions. a. Poly-DL-alanine, A808; b. [A]/[I]=20; c. [A]/[I]=15; d. [A]/[I]=10; e. [A]/[I]=5

films cast from formic acid solution or from aqueous solution. A typical spectrum is shown in Fig. 1, curve a. This spectrum is identical with that reported by Elliott[1,4]. The characteristic absorptions at the amide I and II bands were relatively broad and were located at 1661 cm^{-1} and 1536 cm^{-1} respectively. Elliott[4,14] attributed the amide I band at 1661 cm^{-1} to the α-form. However, from the results referred to below, it is more likely that this absorption is due to the random coil form of poly-DL-alanine. Furthermore, it is now established that the amide I band of the random coil form lies at almost the same frequency as that of the α-helix[10,15]. Deuterium-hydrogen exchange experiments provide additional evidence for the random coil form of poly-DL-alanine, although it is in an aqueous solution[16].

The correlation of the amide II frequency with the polypeptide conformation is not clear. An absorption in the amide III band appeared at 1248 cm^{-1}, whatever the polypeptide conformation was, as will be described below. The N–H stretching mode was observed at a relatively high frequency, 3311 cm^{-1}. The relatively weak absorptions at 2985 and 2933 cm^{-1} may be assigned to the antisymmetric and the symmetric C–H stretching modes of the side chain methyl group respectively. The data concerning the main absorption bands are summarized in Table II.

Film cast from gel obtained by aging the solution either in formic acid or in distilled water gave an absorption spectrum as shown in Fig. 2, curve b. In comparison with curve a, obtained from a fresh, fluid solution, the amide I band had a weak shoulder at 1630 cm^{-1} in addition to the original peak at 1661 cm^{-1}. The shoulder may be assigned to the amide I band of the β-form, following Elliott[14] and after Miyazawa and Blout[10]. Thus, the gelation of the poly-DL-alanine solutions accompanies the partial conversion of the random coil form to the β-form.

Structure and Conformation of PADA.—The infrared spectra of PADA in films cast from a

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[14] A. Elliott, *Nature*, **170**, 1066 (1952).

[15] G. D. Fasman and E. R. Blout, *J. Am. Chem. Soc.*, **82**, 2262 (1960).

[16] W. P. Bryan and S. O. Nielsen, *Biochim. Biophys. Acta*, **42**, 552 (1960).

TABLE II. CHARACTERISTIC ABSORPTIONS OF PADA AND POLY-DL-ALANINE films cast from formic acid solutions

Characteristic band[a]		Wave number (cm^{-1}) and intensity[b]		
		PADA	[A]/[I]=5 → 30	Poly-DL-alanine
NH str.			3279(s) → 3300(s)	3311(s)
Antisym. CH_3 str.			2967(m) → 2985(m)	2985(m)
Sym. CH_3 str.			— —	2933(m)
Antisym. CH_2 str.			2907(s) → 2924(w)	
Amide I	↑↓ β	ca 1690	w → 0	
	r	1661	w → s	1661(s)
	β	1630	s → w	
Amide II		1530~1550	s s	1536(s)
Amide III		1244	m m	1248(m)
	β	1222	m → 0	

a) r=random coil conformation, β=β-conformation, ↑↓ β=antiparallel β-conformation[9]
b) s=strong, m=medium, w=weak

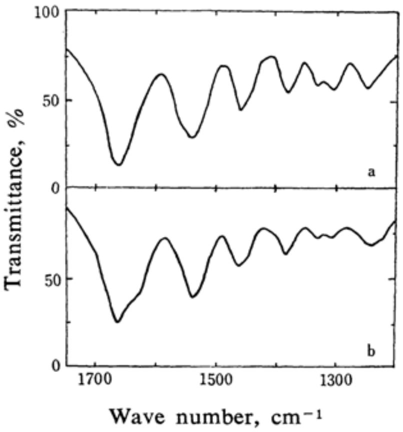

Fig. 2. Infrared spectrum of poly-DL-alanine. a. Film cast from formic acid solution; b. Film cast from gelled solution in formic acid

formic acid solution in a fluid state (immediately after dissolution) are shown in Fig. 1, curves b, c, d and e. It should be first noted that the intensity of the antisymmetric C–H stretching mode at 2920 cm^{-1} is varied from weak to strong with decreasing the [A]/[I] ratio or the degree of polymerization. No doubt this dependence of the spectra on the [A]/[I] ratio reflects the difference in the structure of the polymer dependent on the degree of polymerization. The band may be associated with the methylene groups of the terminal dodecyl group in the PADA molecule. Since the number of mole of dodecyl group per, say, peptide group increases with decreasing the [A]/[I] ratio, the change in intensity of the band at 2920 cm^{-1} indicates the formation of a linkage between the dodecylamino group and the poly-DL-alanine part.

While poly-DL-alanine of different modes of preparation was in the random coil conformation, as has been mentioned before, PADA obtained from [A]/[I] ratios of less than 30 exhibited a different situation. The amide I absorption of the [A]/[I] 20 preparation lay at 1661 cm^{-1} with a weak shoulder at 1630 cm^{-1}, suggesting that the polymer consisted mostly of the random coil form but partly of the β-form. With decreasing [A]/[I] ratio, the absorption at 1661 cm^{-1} became weaker and that at 1630 cm^{-1} stronger. For the [A]/[I] 5 preparation, the latter band was predominant and the former appeared as a weak shoulder. The dependence of the spectra on the [A]/[I] value manifests a conformational change of PADA with the degree of polymerization. That is, a greater amount of the β-form and a smaller amount of the random coil were included in PADA, as the [A]/[I] value or the degree of polymerization was lower. The lowest [A]/[I] preparation was mostly composed of the β-form. The relative amounts of the two conformations were estimated from the ratio of the intensities of the two amide I absorptions, assuming equal molar extinction coefficients. The results are given in Table I. In addition, a shoulder at 1690 cm^{-1} became apparent as the peak at 1630 cm^{-1} became higher. This indicates that the β-form consists of antiparallel chains, provided that Miyazawa's theory[9] can be extended to such low molecular weight polypeptides.

The amide II band showed no such striking correlation of spectra with the [A]/[I] value as the amide I band, but it appeared to be slightly broader as the [A]/[I] value was decreased.

A characteristic change of spectra was also observed in the amide III band. While this band was located at 1244 cm^{-1} for the [A]/[I] 20 and 30 preparations, corresponding to 1248 cm^{-1} for poly-DL-alanine, the lower [A]/[I] preparations absorbed two frequencies, 1244 and 1222 cm^{-1}, the latter becoming stronger with decreasing [A]/[I] value. Accordingly, the absorption at 1222 cm^{-1} should be associated with the β-form, while the one at 1244 cm^{-1} is independent of the conformation.

The N–H stretching mode at about 3300 cm^{-1} changed not in intensity but in frequency as the [A]/[I] value was varied. While poly-DL-alanine gave an absorption at 3311 cm^{-1}, the observed frequency of PADA of [A]/[I] 5 was 3279 cm^{-1} and the higher [A]/[I] preparations absorbed an intermediate frequency, 3289 to 3300 cm^{-1}. This variation also seems to accompany the conformational change with the degree of polymerization. PADA absorbed a higher frequency of the N–H stretching vibration in the random coil form, and, with an increasing amount of the β-form, it absorbed a lower frequency. This kind of behavior has already been observed for other polypeptides by Ambrose and Elliott[2].

All the above correlations of the infrared spectra with the mode of preparation are tabulated in Table II. The conformation of PADA and poly-DL-alanine can be most distinctly identified by observing the amide I absorptions at 1661 and 1630 cm^{-1}.

Film cast from dichloroacetic acid or trifluoroacetic acid solution in which the solvent was still retained always showed a stronger absorption at 1661 cm^{-1} than that from a formic acid solution. This observation suggests that PADA is strongly solvated by dichloroacetic acid or trifluoroacetic acid, which is known to be a "random coil solvent", and that PADA is more favorably stabilized in the random coil form in the presence of the solvent. The band at 1661 cm^{-1} may, therefore, be associated with the random coil form rather than with the α-helix.

Ionization of the Terminal Amino Group of PADA.—The pH value of an aqueous solution of PADA was dependent on the [A]/[I] value when dissolved or suspended in aqueous hydrochloric acid of the same concentration, since PADA combined with different numbers of hydrogen ions[13]. The pH value of the solution should be lower as the [A]/[I] value is higher. Thus the [A]/[I] 5 preparation was insoluble in 0.1 N hydrochloric acid solution, while the others were soluble. However, we may use the concentration of hydrochloric acid instead of the pH value as a measure of the ionization of the terminal group of dissolved polymer.

The infrared spectra of film cast from aqueous hydrochloric acid solution or suspension, the acidity of which was varied from 0.1 N to 0.4 N, were compared with that from formic acid solution. It was found that the spectra of PADA of [A]/[I] higher than 15 were not distinguished from those cast from the formic acid solution and were scarcely influenced by the acid strength. However, the lower [A]/[I] preparations revealed the effect of the ionization on the spectra. For the preparations of [A]/[I] 5 and 10, these changes are illustrated in Fig. 3, in which are shown the spectra of films cast from formic acid solution and from 0.1 N and 0.2 N hydrochloric acid solutions. The effect of the ionization was more enhanced as the [A]/[I] value was higher, and the absorption at 1661 cm^{-1} was stronger and the one at 1630 cm^{-1} was weaker as the degree of ionization was higher. Thus the random coil form is more stable in the ionized state than the β-form.

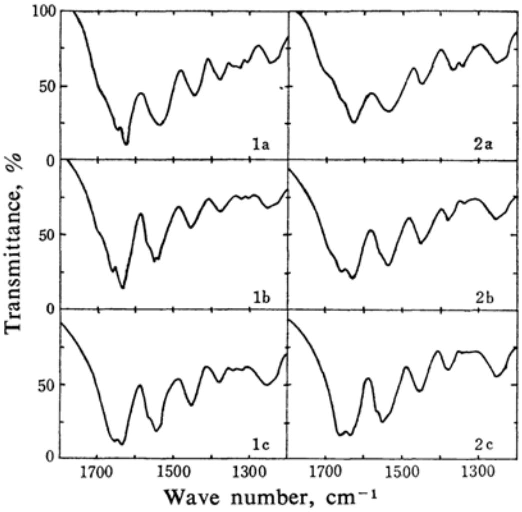

Fig. 3. Infrared spectra of poly-DL-alanine dodecylamide. Films cast from solutions in different solvents. 1. [A]/[I]=10; 2. [A]/[I]=5; a. Formic acid; b. 0.1 N Hydrochloric acid; c. 0.2 N Hydrochloric acid

Gelation of PADA Solution in Formic Acid or in Aqueous Hydrochloric Acid.—When PADA was dissolved in formic acid, the lower [A]/[I] (10 and 15) preparations gelled within several hours, while the higher [A]/[I] preparations gelled after a day or two. Film cast from the gel gave a spectrum different from that obtained from the original fluid solution. With the higher [A]/[I] preparations, the absorption at 1661 cm^{-1} became weaker and that at 1630 cm^{-1} stronger, as in the case of poly-DL-alanine. Correspondingly, the bands at 1690 and 1222 cm^{-1} appeared or became slightly stronger. These are shown in Fig. 4, curves 1. Thus, the gelation of the higher [A]/[I] preparations in formic acid accompanies a conformational change characterized by the transformation of a part of the random coil to the β-form.

With the lower [A]/[I] preparations the

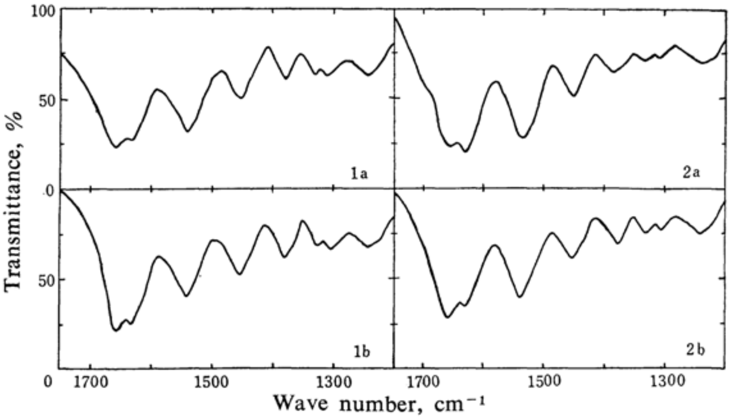

Fig. 4. Infrared spectra of poly-DL-alanine dodecylamide. 1. [A]/[I]=20; 2. [A]/[I]=15; a. Film cast from formic acid solution; b. Film cast from gelled solution in formic acid

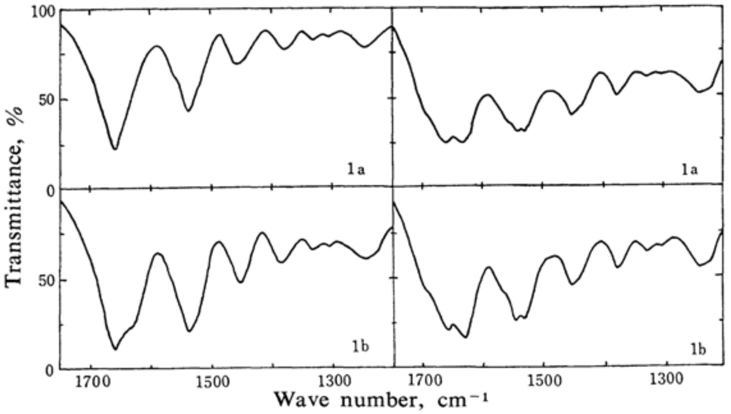

Fig. 5. Infrared spectra of poly-DL-alanine, A 808 (1) and poly-DL-alanine dodecylamide, [A]/[I] 15 (2). a. Film cast from 0.2 N hydrochloric acid solution; b. Film cast from gelled solution in 0.2 N hydrochloric acid

TABLE III. CONFORMATIONAL CHANGE DURING GELATION[a)]

PADA	Formic acid solution			0.2 N HCl Solution		
	Sol	Gel		Sol	Gel	
[A]/[I] 5	r, % 5 β, % 95	not gelled		r, % 45 β, % 55	not gelled	
[A]/[I] 10	r, % 15 β, % 85	30 70	β→ r	r, % 40 β, % 60	15 85	r →β
[A]/[I] 15	r, % 45 β, % 55	75 25		r, % 45 β, % 55	40 60	
[A]/[I] 20	r, % 85 β, % 15	75 25	r →β	—	—	
[A]/[I] 30	r, % 98 β, % 2	—		—	—	
Poly-DL-ala (A808)	r, % 100 β, % 0	90 10		r, % 100 β, % 0	90 10	

a) r=random coil conformation, β=β-conformation reverse situation was encountered during the gelation. The change of the spectra by gelation is illustrated in Fig. 4, curves 2. The absorption at 1661 cm^{-1} became stronger and that at 1630 cm^{-1} weaker. Simultaneously, the bands at 1690 and 1222 cm^{-1} vanished or weakened. In the case of the lower [A]/[I] preparations in formic acid, therefore, the β-form is partly transformed into the random coil during the gelation.

When PADA was dissolved in aqueous hydrochloric acid and kept at room temperature for several days or heated on boiling water for several minutes, the solution of PADA (except for the lowest [A]/[I] preparation) became gel. Film cast from the gel was always characterized by the increased intensity of the absorption at 1630 cm^{-1} and by the decreased intensity of 1661 cm^{-1}, as compared with that from the original fluid solution. Thus the gelation of PADA in aqueous hydrochloric acid is always associated with the change of a part of the random coil form to the β-form.

All these conformational changes followed by the gelation are summarized in Table III, in which the relative amounts of the random coil and the β-form estimated as before are given.

Discussion

According to Blout and Asadourian⁶⁾, low molecular weight poly-γ-benzyl-L-glutamate produced from the *N*-carboxyanhydride by *n*-hexylamine initiation could be separated into two components, the α- and the β-forms, by the difference in solubility in formic acid. The α-polypeptide had a higher molecular weight and absorbed a frequency at 1655 cm^{-1} in the amide I band when cast in film, but the β-polypeptide was of a lower molecular weight and showed an absorption at 1630 cm^{-1}. The portion of each form in poly-γ-benzyl-L-glutamate was independent of the solvent species from which film had been cast.

According to Bamford et al.[5,17), on the other hand, low molecular weight copoly-1:1-(DL-phenylalanine, DL-leucine) was less soluble in benzene as the degree of polymerization was lower. The copolymer was composed of the α- and β-forms, and the decreased solubility was attributed to the increased amount of the β-form. The α-form absorbed 1655 cm^{-1}* and the β-form absorbed 1636 cm^{-1} when the copolymer was examined in a film state. Unlike as in poly-γ-benzyl-L-glutamate, however, the portions of the two forms were dependent on the solvent species and the method for the regeneration into film. Formic acid was more favorable in producing the β-form, and dichloroacetic acid preferred the formation of the α-form. Similar results were obtained for poly-DL-phenylalanine¹⁷⁾.

Poly-DL-alanine obtained from dodecylamine initiation, PADA, was found to consist of the random coil form and the antiparallel β-form. The former form gave an absorption at 1661 cm^{-1} in the amide I band, while the latter absorbed at 1630 cm^{-1}. The portion of the β-form was greater, and the solubility in distilled water was lower, as the degree of polymerization was lower. However, the solubility of this polymer in water might not be attributable to the conformational difference alone but also to the hydrophobicity of the dodecyl group. As compared with that from formic acid solution, film cast from dichloroacetic acid solution contained a greater amount of the random coil form, but the difference would be caused by the presence of remaining solvent and will disappears after the complete removal of the solvent. Thus, the conformation of PADA in the solid state is defined mainly by the degree of polymerization.

There are two factors to stabilize a given conformation of a polymer chain, i. e., energy and entropy contributions. The former is determined by the potential of internal rotation around backbone linkages, the hydrogen bonding between peptide bonds, etc. The latter arises mainly from the Brownian motion of the constituent residues. In high molecular weight PADA and poly-DL-alanine, the entropy contribution is greater than the energy contribution; then the random coil form is made more stable. With lowering of the degree of polymerization, the conformational entropy decreases and the energy contribution becomes predominant. The energetically stable form would be the interchain hydrogen-bonded β-form, and then the β-form becomes abundant in low molecular weight PADA. In PADA, the van der Waals cohesion between dodecyl groups should play an additional role in stabilizing the β-form, the long chain alkyl groups tending to be held together. Further, the antiparallel-chain β-form would be more stable than the parallel one, as far as the potential of the internal rotation was concerned¹⁸⁾.

The effect of the dissolved state on the regenerated conformation of PADA may be interpreted as follows. In dichloroacetic acid or trifluoroacetic acid solution, the polymer is probably in a solvated random coil form,

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17) C. H. Bamford, W. E. Happy and F. Happey, *Proc. Roy. Soc.*, A205, 30 (1950).

* Elliott⁴⁾ recorded this frequency as 1661 cm^{-1}.

18) T. Miyazawa, *J. Polymer Sci.*, 55, 215 (1961).

irrespective of the degree of polymerization, owing to the strong ability of the solvent to break hydrogen bonds, and no further conformational change occurs in the course of time. The conformation of PADA in the dissolved state is considered to be reversible to the original one in the solid state when the polymer is made free from the solvent.

In formic acid solution, however, PADA will be in a randomly coiled form immediately after dissolution. This form may be less solvated and less randomly coiled than in dichloroacetic acid solution, since formic acid is a weaker hydrogen bond-breaking agent. However, this state of dissolution is considered to be reversible to the initial solid state of the polymer. The conformation of PADA in formic acid solution is no doubt much more abundant in the random coil form than in the initial solid state. However, this solution undergoes aging, probably owing to the weak polymer-solvent interaction and the high polymer concentrations such as 10%. In the course of aging, the dissolved random coils are in contact with one another and are then linked by interchain hydrogen bonds. As a result, the random coil is partly converted into the β-form, and the formation of cross-linkages makes the fluid solution into gel. PADA in the gelled state can no longer return to the initial solid state, even if the solvent is completely removed. When the regenerated solid state is compared with the original solid state, the aging or the gelation apparently accompanies the partial conversion of the β-form to the random coil for the [A]/[I] 10 and 15 preparations, but it produces the reverse conversion for the higher [A]/[I] preparations. The chain length of PADA of [A]/[I] 5 is too short to bring about gelation.

In aqueous hydrochloric acid solution, the terminal amino groups of PADA are, at least, partially ionized, as the pH titration data indicate. The electrostatic repulsion is then operative between polymers and the polymer chain takes a random coil. From the pH titration and the surface tension data, it is evident that most of the hydrochloric acid required to make the pH value of the solution lower than 5.5 is not bound to the polymer, but merely suppresses the electrostatic repulsion as neutral electrolyte does. When the solvent is removed, however, the free hydrochloric acid or the ionic atmosphere is reduced and the interchain distance is decreased. Thus, the electrostatic repulsion is very strong in the regenerated PADA, which is partially ionized. As compared with the unionized PADA regenerated from formic acid solution, the ionized PADA regenerated from aqueous hydrochloric acid solution has a higher portion of the random coil form, since the interchain hydrogen bonds maintaining the β-form are ruptured by the electric repulsion. The effect of the repulsive force is greater, as the degree of polymerization is lower and the degree of ionization is higher.

The aqueous solution of PADA or poly-DL-alanine forms a gel by aging. The random coil form of PADA in solution will be partly converted into the β-form during this process. The cross-linkages formed by the aging will be retained even after the removal of the solvent, and the increased amount of the β-form results in the ionized PADA regenerated from the gel. In solution, the contact or the aggregation of ionized PADA is disturbed by the interchain repulsion between ionized terminal groups. In concentrated hydrochloric acid solution, however, most of ions produced from the acid are free from PADA and only serve to reduce the electrostatic repulsion, Thus, the contact of ionized PADA with one another is made easier and the gelation can occur faster, as hydrochloric acid is more concentrated.

Summary

The infrared spectra of poly-DL-alanine and poly-DL-alanine dodecylamide were measured on film cast from various solutions, and the conformation of the polymers were elucidated on the basis of the location and intensity of absorptions, mainly in the amide I and III bands.

The polymer showed an absorption at 1661 cm^{-1} in the amide I band if it was in the random coil form, but it showed two absorptions at 1630 and 1690 cm^{-1}, if it was in the antiparallel β-form. While the amide III band was located at 1244 cm^{-1} irrespective of the polymer conformation, the band had an additional absorption at 1222 cm^{-1} for the β-conformation.

From the above correlation of the spectra with the polymer conformation, it was found that poly-DL-alanine and PADA prepared with high [A]/[I] ratios are in the random coil form but that PADA of [A]/[I] ratios lower than 30 is composed of both the random coil and the antiparallel β-forms when it is regenerated from formic acid solution. PADA contained a greater amount of the β-form as the [A]/[I] value or the degree of polymerization was lower. The [A]/[I] 5 preparation was mostly in the β-form.

In the ionized PADA obtained from aqueous hydrochloric acid solution, the portion of the random coil form increased relative to that in the unionized PADA derived from formic

acid solution, and the effect of the ionization was enhanced for the lower [A]/[I] preparations.

Poly-DL-alanine and PADA in formic acid or aqueous hydrochloric acid solution underwent a partial transformation from the random coil to the β-form during the aging of concentrated solution, which process accompanied gelation. The solution of the lowest [A]/[I] preparation did not gel. In the state regenerated from gel, poly-DL-alanine and PADA, except for the unionized lower [A]/[I] preparations, had an increased amount of the β-form as compared with that from an initial fluid solution. In the lower [A]/[I] preparations obtained from formic acid solution, the reverse transformation apparently occured, when the films cast before and after the gelation were compared.

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