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Polypeptides. V. The Infrared Spectra of Polypeptides Derived from γ-Benzyl-L-glutamate

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It has been shown that the polymerization of γ-benzyl-N-carboxy-L-glutamate anhydride with *n*-hexylamine initiation using anhydride–initiator mole ratios less than 20 yields mixtures of α- and β-forms of polypeptides. The amount of β-polypeptide increases with decreasing anhydride–initiator ratio. The β-polypeptides may be extracted from the mixtures by virtue of their solubility in 98% formic acid. The β-polypeptides show a C=O (amide I) infrared absorption band at 1630 cm.$^{-1}$ in the solid state, but this is shifted to frequencies as high as 1678 cm.$^{-1}$ in dilute solution or by heating more concentrated solutions. This spectral behavior parallels that of low molecular weight secondary amides, and it is on this basis as well as on the elementary analyses and low solution viscosities of the β-polypeptides that it is concluded that such materials are of very low molecular weight. The formic acid insoluble fractions, and those polypeptides made from anhydride–initiator ratios over 100, exist in the α-form, have higher solution viscosities, and higher molecular weights. The α-form is characterized by a C=O amide I infrared absorption band at 1655 cm.$^{-1}$ in the solid. The position of this band in α-polypeptides does not change in most solvents or upon heating. No evidence has been found with poly-γ-benzyl-L-glutamate to indicate an $\alpha \rightarrow \beta$ transformation upon treatment with formic acid. Evidence is presented which indicates that preparations which are mixtures of α- and β-polypeptides lose their β-component upon heating to 280° *in vacuo*. Since there is a marked loss of weight and no increase in the intensity of the characteristic "α" infrared absorption bands, it is concluded that no $\beta \rightarrow \alpha$ transformation occurs.

Infrared spectral studies of synthetic polypeptides have been important in the development of the current ideas of the molecular configuration of such materials and their relationship to the structure of certain proteins.[2] Previous work by Elliott,[3,4] Bamford[5,6] and their colleagues have shown the existence of α- and β-forms of synthetic polypeptides. In particular, Elliott has shown that there is a correlation between the α-form and a C=O stretching mode (amide I) at about 1660 cm.$^{-1}$ having parallel dichroism, whereas the β-form has a C=O stretching mode around 1630 cm.$^{-1}$ with perpendicular dichroism. Bamford has described X-ray investigations and in addition $\alpha \rightleftarrows \beta$ transformations in synthetic polypeptides. Often these studies have been made on polypeptides whose method of preparation has not been stated, and little regard has been paid to determining other physical and chemical properties—especially the molecular weight. In this communication, we describe the infrared spectra of polypeptides prepared from γ-benzyl-N-carboxy-L-glutamate anhydride and relate the spectra and hence the molecular configuration to both the mode of preparation and to the molecular weight of the polypeptides. Our interest has been particularly directed toward the preparation of the β-form of poly-γ-benzyl-L-glutamate, the separation of the α- and β-forms from each other, and an investigation of $\alpha \rightleftarrows \beta$ conversions.

Poly-γ-benzyl-L-glutamate (PBLG) was selected for this work because it is readily soluble in a variety of solvents and because previous studies[7,8] have shown that polypeptides of very high molecular weight (mol. wt. >500,000) could be prepared from the corresponding anhydride. The polymerizations of the N-carboxyanhydride were carried out in dioxane solution using either *n*-hexylamine or sodium hydroxide in methanol[8] as initiators over the range of anhydride–initiator mole ratios (A/I) from 4 to 1100.[9]

Results

The polymers obtained from A/I of 100 or more using either *n*-hexylamine or sodium hydroxide as initiator showed identical infrared spectra when measured as films or in solution. A typical spectrum is shown in Fig. 1 curve D and note should be taken of the position of the amide I (due to C=O stretching motions) and amide II (associated with C=N stretching and N–H deformation[10]) frequencies which lie at 1655 and 1550 cm.$^{-1}$, respectively. We have determined the infrared spectra of many preparations of poly-γ-benzyl-L-glutamate and in all those where the molecular weight was greater than 30,000 the spectra were substantially identical and the position of the amide I and amide II bands were consistent with those reported for "α"-polypeptides by Ambrose and Elliott.[3,4,11]

However, on examination of the infrared spectra of the products obtained from A/I's less than 100, a different situation is found to exist. In Fig. 1 there are shown the spectra of films cast from chloroform solution of materials initiated by *n*-hexylamine where the A/I ranged from 4 to 50. It is apparent that *the spectra changed markedly with decreasing A/I*, especially in the location of the characteristic amide bands between 1500 and 1700 cm.$^{-1}$. With the A/I 4 polymer the amide I absorption lies at 1630 cm.$^{-1}$, which position has been described[3] as

(1) Chemical Research Laboratory, Polaroid Corporation, Cambridge 39, Mass.

(2) L. Pauling, R. B. Corey and H. R. Branson, *Proc. Nat. Acad. Sci.*, **37**, 205 (1951); L. Pauling and R. B. Corey, *ibid.*, **37**, 235 (1951).

(3) E. J. Ambrose and A. Elliott, *Proc. Roy. Soc.* (*London*), **A205**, 47 (1951).

(4) E. J. Ambrose and A. Elliott, *ibid.*, **A208**, 75 (1951).

(5) C. H. Bamford, W. E. Hanby and F. Happey, *ibid.*, **205**, 30 (1951).

(6) C. H. Bamford, W. E. Hanby and F. Happey, *ibid.*, **206**, 407 (1951).

(7) E. R. Blout, R. H. Karlson, P. Doty and B. Hargitay, THIS JOURNAL, **76**, 4492 (1954).

(8) E. R. Blout and R. H. Karlson, *ibid.*, **78**, 941 (1956).

(9) We use the terminology A/I to designate polypeptide preparations since this provides a simple description of the general preparative conditions.

(10) R. D. B. Fraser and W. C. Price, *Nature*, **170**, 490 (1952).

(11) To conform with previous nomenclature we designate a material as "α" if it has an amide I band around 1655 cm.$^{-1}$, as "β" if it has an amide I band around 1630 cm.$^{-1}$. By the use of this nomenclature we do not imply that the molecular structures associated with these absorption bands are those ascribed to them by previous workers.[3]

characteristic for the "β"-form of polypeptides. With the A/I 8, 13.4 and 20 products two bands appear in the amide I region—one at 1655 ± 3 cm.$^{-1}$ and one at 1630 ± 2 cm.$^{-1}$.

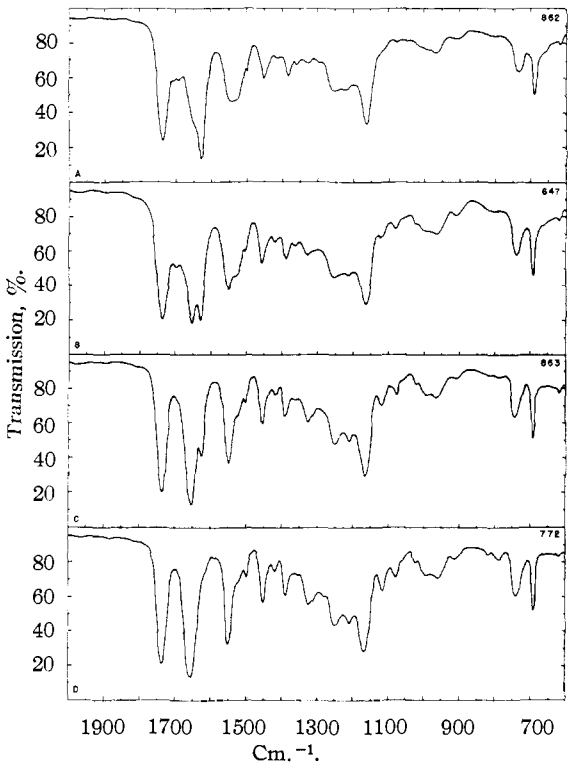

Fig. 1.—Infrared spectra of poly-γ-benzyl-L-glutamates of different molecular weights. All spectra were determined on films cast from chloroform. Anhydride-initiator mole ratios as follows: A, 4; B, 8; C, 20; D, 50; see Table II.

Previous workers, using similar polypeptides have pointed out that different preparations contain varying amounts of α- and β-forms (as indicated by the location of the amide I frequency in the infrared spectra and by X-ray evidence) and that it is possible to effect an $\alpha \rightarrow \beta$ transformation depending on the solvent from which the polypeptide was cast.[5] In particular formic acid has been described as the preferred solvent for the production of β-forms.[6] On attempting to dissolve the various PBLG polymers in formic acid we found that the solubility was inversely proportional to the A/I (Table II).[12] With the A/I (*n*-hexylamine) 4, 8, 13.4 and 20 materials the formic acid soluble fractions gave spectra of cast films which had an amide I absorption around 1630 cm.$^{-1}$ (β). The A/I 50 and A/I 100 materials were soluble to a much lesser extent in formic acid, and gave spectra of cast films from formic acid solution with amide I absorption maxima about 1655 cm.$^{-1}$ (α). The formic acid insoluble fraction in all cases gave spectra of films cast from chloroform which showed amide I absorption around 1655 cm.$^{-1}$ (α) only (see Fig. 2).

In view of these results it appeared likely that the low A/I polymers were a mixture of two or more

(12) We use the designation FS following the preparation number to indicate the formic acid soluble fraction, and FI for the formic acid insoluble fraction.

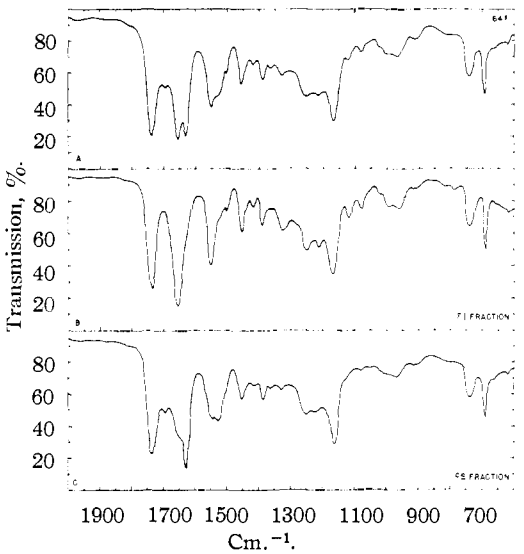

Fig. 2.—A, Infrared spectrum of poly-γ-benzyl-L-glutamate preparation from A/I 8 initiation with *n*-hexylamine (647). Note presence of approximately equal intensities in "α"- and "β"-amide I bands at 1655 and 1630 cm.$^{-1}$, respectively. B, Infrared spectrum of formic acid insoluble fraction of preparation 647 (647FI). C, Infrared spectrum of formic acid soluble fraction of preparation 647 (647FS). All spectra on films cast from chloroform.

chemical species—which had different solubilities and different spectral properties. Therefore we used the solubility difference in formic acid to separate the species and indeed in each case we were able to separate a formic acid soluble fraction whose percentage of the total polymer decreased with increasing A/I. The formic acid insoluble fractions analyzed correctly for high polymers of PBLG and had intrinsic viscosities greater than 0.20 in dichloroacetic acid (DCA). On the other hand, the formic acid soluble fractions from A/I < 20 materials all had significantly lower intrinsic viscosities than 0.20 (DCA) and gave lower C and higher N analyses than can be accommodated by a high molecular weight polymer of PBLG.

Spectral studies were made on the formic acid soluble "β"-polymers, that is, those which showed a "β"-amide I band at 1630 cm.$^{-1}$ in cast films (Fig. 3A). In such materials the band is often somewhat asymmetric and a small amount of α-fraction is probably present. The spectra of the formic acid soluble fraction of such material in chloroform solution is shown in Fig. 3 curves B, C and D. In the 10% solution 2 bands are noted, the 1630 cm.$^{-1}$ "β"-band and a weaker band at 1660 cm.$^{-1}$ which we designate a σ-band, since we ascribe it to solvated amide C=O absorption (*vide infra*). As the solution is diluted—and the path length increased correspondingly—the band at 1630 cm.$^{-1}$ becomes weaker (and at 0.5% solute disappears) while the 1660 cm.$^{-1}$ band increases in intensity. Analogous behavior is noted in dioxane solutions, although it is necessary to go to lower concentrations for the 1630 cm.$^{-1}$ band to completely disappear.

The disappearance of the 1630 cm.$^{-1}$ band with a concomitant increase in intensity of the 1660 cm.$^{-1}$

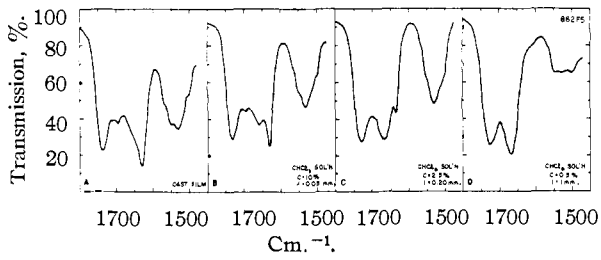

Fig. 3.—Infrared spectrum of formic acid soluble PBLG fraction from A/I 4 *n*-hexylamine initiated polymerization (862FS): A, film cast from chloroform; B, C, D, chloroform solutions at indicated concentrations of solute (*c*) and cell thicknesses (*t*).

band also has been caused to occur by heating a dioxane solution to 88°. On cooling to room temperature the 1630 cm.$^{-1}$ band reappears. These spectra are shown in Fig. 4.

We have noted further that the exact position of the 1660 cm.$^{-1}$ σ-band depends on the concentration of the solute and the nature of the solvent. It varies from 1660 to 1678 cm.$^{-1}$ in chloroform and dioxane solutions.

The same experiments as those just cited, namely, dilution to low concentration, heating and solution in various non-ionic solvents, have been performed with several samples of high molecular weight "α"-poly-γ-benzyl-L-glutamate. In contrast to the results with "β"-polypeptides the *"α"-amide I band at 1655 cm.$^{-1}$ remains constant* and invariant in position within the limits of measurement. A summary of the infrared data on the amide I band for these various fractions is shown in Table I.

TABLE I

LOCATION OF AMIDE I BAND

Polypeptide	Designation of form	Band location in cm.$^{-1}$ Film	Chloroform solutions 10%	Chloroform solutions 0.5%	Dioxane solutions 10%	Dioxane solutions 0.5%
High A/I and low A/I formic acid insol.	α	1656	1655	1655	1655 (at 5%)	1651
Low A/I formic acid soluble	β	1630	1632 and		1631 and	
	σ		1660	1668	1669	1678

Several other significant infrared spectral differences may be noted in the α- and β-polypeptides. First, in cast films the N–H deformation frequency lies at 3290 cm.$^{-1}$ in the "β"-material and about 10 cm.$^{-1}$ higher in the "α"-material. Second, the amide II band lies at 1525 cm.$^{-1}$ in the "β"-compounds and at 1550 cm.$^{-1}$ in the α-compounds (*cf.* ref. 4). Thirdly, almost all other bands in the infrared spectra as low as 650 cm.$^{-1}$ are identical except that the β-material has a weak band at 1690 cm.$^{-1}$ and the α-compounds show 1080 and 1117 cm.$^{-1}$ bands that are not seen in the spectra of β-material (compare Fig. 1, curves A and D). These bands may prove to be useful, along with the position of the amide I band, in determining α-material in the presence of β.

In the light of the foregoing spectral data on separated "α"- and "β"-polypeptides it is now easier to consider the spectral data obtained from a polypeptide preparation which contains both "α"- and "β"-material. Preparation #647, which shows approximately equal intensity of the α- and β-amide I bands (Fig. 2, curve A) was made by initiation with *n*-hexylamine to A/I 8 in dioxane solution. It has been noted already that formic acid dissolves only part of the product of low A/I polypeptides. Treatment with this solvent dissolves approximately 67% of the 647 material (Table I) and films may be cast from chloroform solution of both the formic acid insoluble (Fig. 3, curve B) and formic acid soluble portions (Fig. 2, curve C). It appears that a separation into α- and β-material was effected through the difference in their solubilities in formic acid. This conclusion is based on the position of the amide I band in the two fractions and the presence of the bands at 1117 and 1080 cm.$^{-1}$ in the formic acid insoluble α-fraction.

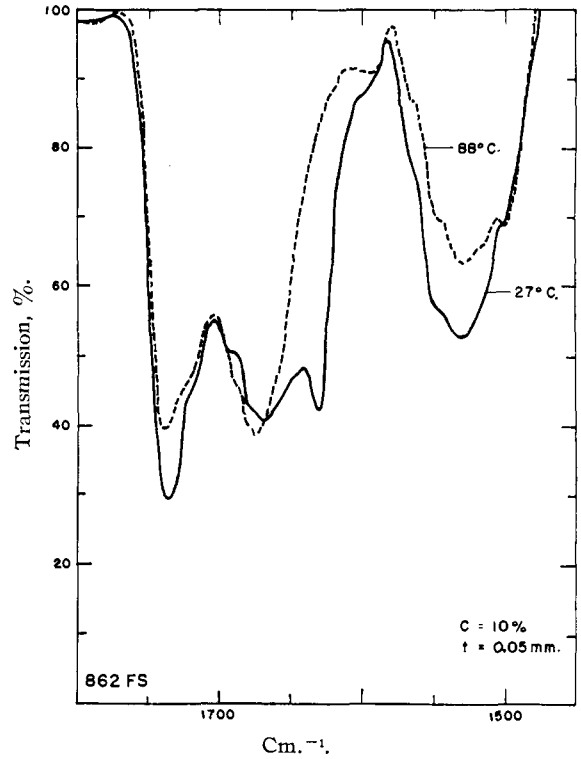

Fig. 4.—Change in infrared spectrum of formic acid soluble PBLG fraction (862FS) upon heating to 88°. Spectra were determined in dioxane solution.

We have prepared cast films of this preparation from a variety of solvents including benzene, chloroform, dimethylformamide, dichloroacetic acid and trifluoroacetic acid. The spectral data (Fig. 5) indicate that such solvents have essentially no effect on the proportion of α- and β-forms, although there is a broadening of some bands, possibly because of retained solvent. Spectral data on solutions of this preparation are consistent with a mixture of α- and β-polypeptides. These data are shown in Figs. 6 and 7 for solutions in chloroform and dioxane, respectively. Although the β-amide I band at 1630 cm.$^{-1}$ disappears upon dilution, the 1660 cm.$^{-1}$ σ-amide I band and the 1655 cm.$^{-1}$ α-amide I band (from the α-material present) lie at frequencies so close to each other that they are

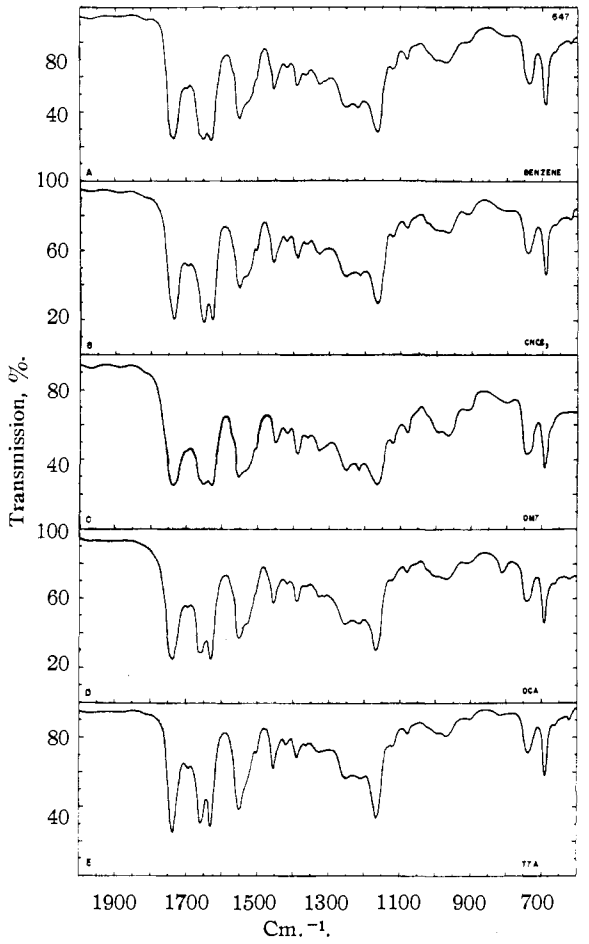

Fig. 5.—Infrared spectra of preparation 647. Films cast from: A, benzene; B, chloroform; C, dimethylformamide; D, dichloroacetic acid; E, trifluoroacetic acid.

not resolved, but a broadening of this absorption may be observed in the dioxane solutions.

The spectral behavior of the 647 preparation upon heating in dioxane solution is analogous to that obtained with β-polypeptides. The results are shown in Fig. 8 where one sees the shift of the 1630 cm.$^{-1}$ band to 1670 cm.$^{-1}$ upon increasing the temperature. Because α-form is present in this preparation an approximately corresponding amount of an α-polypeptide was placed in the reference cell, thus effectively cancelling the α-band at 1655 cm.$^{-1}$.

Experimental

Preparation of Polymers.—γ-Benzyl-N-carboxy-L-glutamate anhydride, m.p. 93°, prepared as previously described,[8] was added to make 5% solutions in purified dioxane containing the initiator. The polymerizations were run at room temperature in flasks protected from moisture. Polymerization was completed within 24 hours in all cases as evidenced by titration of the residual anhydride with sodium methoxide.[13] No polymers were isolated until the titration indicated less than 5% residual anhydride. Titration in this manner also reveals any large amounts of acidic by-products such as substituted hydantoin acetic acids. The titration results indicated that less than 5% of acid and/or anhydride was present in the reaction mixtures when the polymers were isolated.

(13) A. Berger, M. Sela and E. Katchalski, *Anal. Chem.*, **25**, 1554 (1953).

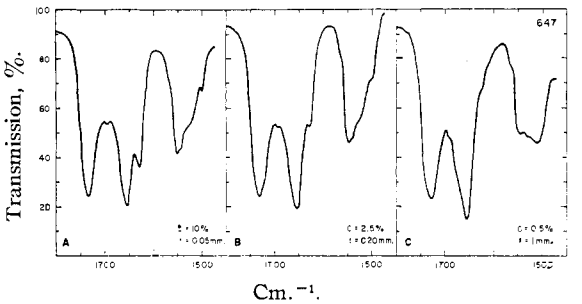

Fig. 6.—Spectra of chloroform solutions of PBLG (preparation 647) at indicated concentration (c) of solute and cell thickness (t).

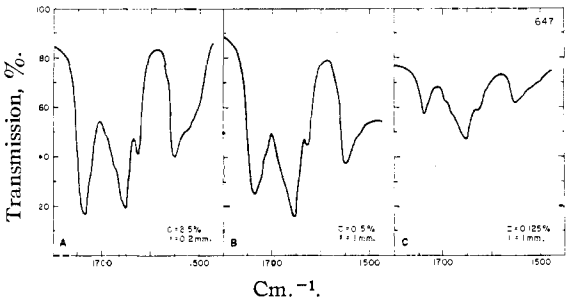

Fig. 7.—Spectra of dioxane solutions of PBLG (preparation 647) at indicated concentrations of solute (c) and cell thickness (t).

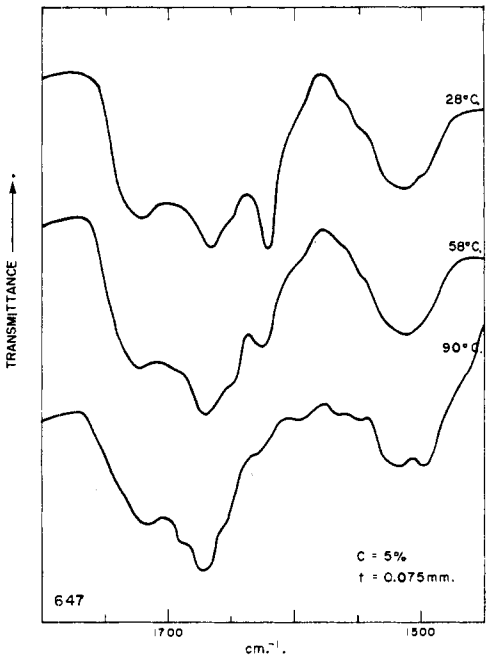

Fig. 8.—Infrared spectra of 5% dioxane solution of preparation 647 of PBLG at various temperatures. Note disappearance of 1630 cm.$^{-1}$ "β"-amide I band with increasing temperature. Reference cell contained 2% pure "α"-polymer to balance "α"-polymer in preparation 647.

When the titration indicated that anhydride had been consumed, the reaction mixture was poured, with stirring, into a 20-fold excess of diisopropyl ether, except preparations 450 and 396B where ethyl alcohol was used as a precipitant. The precipitates were filtered and dried at 50° *in vacuo*. Some details of the various preparations and properties of the isolated products are shown in Table II.

TABLE II

Prepn. no.	Mole ratio (A/I)	Initiator	[η] in DCA	Weight average[a] Degree of polymerization	Weight average[a] Molecular weight	Weight % insoluble in formic acid	Form and approximate %[b] (from amide I infrared band)
862	4	*n*-Hexylamine	0.08	44	9,700	9	>90 β
862FS[c]			.065[h]				>95 β
862FI[d]			.234	155	34,000		100 α
647	8	*n*-Hexylamine	.11	64	14,000	33	50 β + 50 α
647FS[e]			.06[h]				>90 β
647FI[f]			.20	128	28,000		100 α
720	13.4	*n*-Hexylamine	.18	112	25,000	55	70 α + 30 β
720FS			.075[h]				>90 β
720FI			.30	200	44,000		100 α
863	20	*n*-Hexylamine	.21	137	30,000	64	80 α + 20 β
863FS			.10[h]				85 β
863FI			.24	150	33,000		100 α
772	50	*n*-Hexylamine	.27	178	39,000	78	>98 α
772FS							90 α
772FI							100 α
773	100	*n*-Hexylamine	.34	228	50,000	95	>99 α
773FS							>98 α
773FI							100 α
980R	4	NaOH	.204	132	29,000	85	>99 α
980RFS							>98 α
980RFI							100 α
450[g]	60	NaOH	.48	350	76,000	98	100 α
450FS							100 α
450FI							100 α
396B	1100	NaOH	1.40	1160	255,000	>99.8	100 α

[a] From intrinsic viscosity correlated with light scattering see refs. 17 and 18. We are much indebted to Dr. Paul Doty for the measurements of intrinsic viscosity reported herein. [b] Assuming equal molecular extinction coefficients in the α-amide I band (1655 cm.$^{-1}$) and the β-amide I band (1630 cm.$^{-1}$). [c] Calculated for the hexylamine initiated benzylglutamate trimer–formic acid salt $C_{43}H_{56}N_4O_{11}$: C, 64.3; H, 7.0; N, 7.0. Found: C, 64.5; H, 7.1; N, 7.8. [d] Calculated for a high polymer of benzylglutamate $(C_{12}H_{13}NO_3)_x$: C, 65.8; H, 6.0; N, 6.4. Found: C, 66.3; H, 6.3; N, 6.2. [e] Calculated as in (*c*) $C_{43}H_{56}N_4O_{11}$: C, 64.3; H, 7.0; N, 7.0. Found: C, 64.0; H, 6.5; N, 7.4. [f] Calculated for polybenzylglutamate $(C_{12}H_{13}NO_3)_x$: C, 65.8; H, 6.0; N, 6.4. Found: C, 65.6; H, 6.3; N, 6.6. [g] Calculated for polybenzylglutamate $(C_{12}H_{13}NO_3)_x$: C, 65.8; H, 6.0; N, 6.4. Found: C, 66.3; H, 6.0; N, 6.2. [h] We do not assign molecular weights corresponding to viscosities below 0.10 since the viscosity–molecular weight curve has not been calibrated in this region as yet; see ref. 18.

Fractionation of Polymers.—Separations of the products into two fractions were achieved by treatment with 98% formic acid with occasional shaking at room temperature for four hours using 50 times the sample weight of solvent. After filtering and washing the residual insoluble material twice with formic acid, the soluble fraction was dried by lyophilization. With the low molecular weight polypeptides subsequent treatment of the formic acid insoluble fractions with additional formic acid yielded a second formic acid soluble fraction which showed a strong α-amide I band and a weak β-amide I band. A third extraction of the formic acid insoluble fraction yielded only a small amount (less than 10%) of polypeptide—all of which showed only the α-amide I absorption. In the polypeptides made with A/I 20 and 50 the first treatment with formic acid extracted all the β-form. It is evident that formic acid treatment of the reaction products dissolves all the material showing the β-amide I absorption band, but in addition a small amount of α-material (probably of low molecular weight) is also soluble in formic acid.

High molecular weight α-PBLG polypeptide is insoluble in formic acid under the above conditions. It is interesting to note that prolonged treatment of relatively high molecular weight α-PBLG (sample 450) with 98% formic acid or with 9:1 trifluoroacetic acid:water results in production of formic acid soluble material, but such material shows only α-amide I absorption.

High Temperature Experiments.—Films were cast from chloroform solutions of preparations 647 and 720 on silver chloride plates (*cf.* Table II and Fig. 1). The infrared spectra were determined and the plates containing the cast films were then heated *in vacuo* at 270–280° for 30 minutes. On cooling to room temperature the spectra were measured again (see Fig. 9) and loss in weight determined. Preparation 647 lost 30% of its weight; preparation 720 lost 10% of its weight.

Spectral Determinations.—All infrared spectra were run in a model 21 Perkin–Elmer double beam spectrometer using a sodium chloride prism. Slit widths were approximately 0.040 mm. at 1800 cm.$^{-1}$, 0.050 mm. at 1600 cm.$^{-1}$ and 0.100 mm. at 1000 cm.$^{-1}$. Films for these determinations, when cast on silver chloride plates from low boiling solvents, were dried at room temperature. The films cast from high boiling solvents were dried *in vacuo* for 24 hours.

The spectral data reproduced in the figures are direct tracings of the curves obtained from the infrared spectrometer. The data were obtained using a noise level of about 1%. The 100% transmission curve was straight to within 1.5% in the region 650 to 2000 cm.$^{-1}$. The frequency calibration was accurate to ±2 cm.$^{-1}$ in the region 1500 to 1800 cm.$^{-1}$.

Discussion

The work of Ambrose and Elliott[3,4] has elegantly shown the correlation of the α- and β-forms of synthetic polypeptides with C=O stretching modes at 1660 and 1630 cm.$^{-1}$, respectively. However, the interconversion of these forms is a matter of some interest. Bamford, *et al.*,[5] state that formic acid is unique in producing the β-modification in their polypeptide preparations and that by treatment with formic acid an α → β transformation may be effected as evidenced by changes in the X-ray diffraction patterns. Furthermore, Bamford[6] indicates that the β-forms of two polypeptides revert to the α upon treatment at 280° *in vacuo*. In general our work confirms the existence of two forms, α and β, in low molecular weight PBLG preparations, but

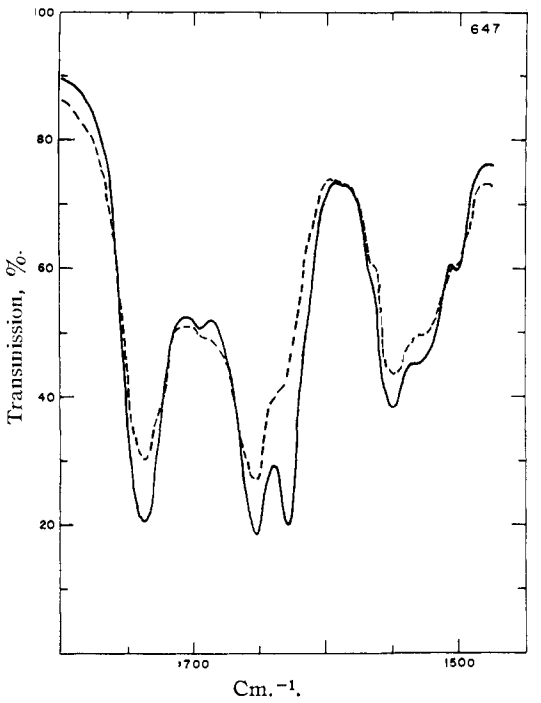

Fig. 9.—Infrared spectra showing effect of heating PBLG (preparation 647) as a film to 280° *in vacuo* for 30 minutes: ——, original film; - - - - -, same film after heating. Both spectra were determined at room temperature.

our results with PBLG indicate that with these polypeptide preparations no $\alpha \rightleftarrows \beta$ transformations occur. Furthermore, *we find a direct correlation between the α- and β-forms and the molecular weight of the PBLG polypeptides investigated.*

The β-Form.—From our experimental work it is quite apparent that PBLG polypeptides may be prepared which show amide I absorption bands at 1655 and 1630 cm.$^{-1}$ in the infrared (and thus are presumably α- and β-forms). Such polypeptides are prepared from A/I's <20 using *n*-hexylamine initiation. These bands have approximately the same relative intensities (in any one preparation) irrespective of the solvent from which the material is cast, *provided* it is a solvent for both forms (see Fig. 5). Initiation with sodium hydroxide with comparable A/I's produces polypeptides which show only α-amide I absorption.

From the materials which show both α- and β-bands *it is possible to extract all the β-material as a formic acid soluble fraction.* Once the β-material has been extracted, further treatment of the remaining formic acid insoluble material yields no additional β-material. Thus it must be concluded that for γ-benzyl-N-carboxy-L-glutamate anhydride, *initiation with primary amines to low A/I yields a mixture of α- and β-polypeptides and these can be separated* by virtue of their different solubilities. The β-fraction is formed during the polymerization process and is not produced during treatment with formic acid.

The separated β-polypeptides behave, insofar as their infrared spectra are concerned, like low molecular weight secondary amides.[14,15] That is, both the β-polypeptides and secondary amides have a C=O stretching frequency in the solid state around 1630 cm.$^{-1}$. In dilute $CHCl_3$ solution this frequency lies around 1660 cm.$^{-1}$ and in dilute dioxane solution around 1680 cm.$^{-1}$.[14] Furthermore the absorption maxima in solvents shift with concentration of the solute. In addition, in both β-polypeptides and in model secondary amides heating a concentrated solution shifts the amide I absorption to higher frequencies.[16] These facts clearly indicate that β-polypeptides are in a non-helical configuration which is strongly associated at high concentrations but is dissociated upon dilution or heating. In addition the low viscosities and the elementary analyses reported above lead us to the conclusion that the PBLG *β-polypeptides are very low molecular weight materials.*

The α-Form.—The spectral data and the molecular weight determinations[17,18] summarized in Table II indicate that PBLG polypeptides having molecular weights greater than about 28,000 (degree of polymerization (D.P.) ~128) exist in the α-form. We do not mean to imply that this is the minimum D.P. at which the α-form exists, but rather that our evidence indicates that at this D.P. and higher it is the preferred form in solid films and many solvents.[17,18]

The α-form of PBLG is produced, to the essential exclusion of β-material, by initiation with sodium hydroxide with A/I > 4. With *n*-hexylamine initiation A/I up to 20 produces substantial amounts of β-form, but above this value of A/I the α-form is by far the major product. The percentage of α-polypeptide resulting from the *n*-hexylamine initiated polymerizations appears to increase in relation to A/I over the range 4 to 50.

The α-polypeptides have characteristic infrared spectra which are remarkably insensitive to changes in environment and temperature. It may be concluded from the spectral data that the α-form is exceedingly stable to changes in solution environment, which fact is further evidence for helical structures previously suggested.[2,17,18] In fact, the only solvents in which changes in the spectra were noted were dichloroacetic acid and trifluoroacetic acid. Since these solvents can only be used over a limited spectral range, no extensive studies were made with them, but it was observed that the amide I and amide II frequencies of α-polypeptides were shifted between 10 and 15 wave numbers toward lower frequencies in these solvents.

The α → β Transformation.—Previous workers[5] reported that poly-D,L-phenylalanine could be transformed from α → β, according to X-ray data, by a few minutes immersion in formic acid. As indicated above, treatment of PBLG polymers with formic acid results in the extraction of the β-material which is soluble in formic acid. Treatment of a pure α-PBLG polymer with formic acid for periods up to 48 hours (*e.g.*, 450) results in no observable production of β-material according to the infrared spectral data.

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From our results with PBLG preparations it would appear that formic acid possesses no unique properties with respect to $\alpha \rightarrow \beta$ transformations, but rather that it is a good solvent for low molecular weight (β)-polypeptides and a poor solvent for high molecular weight (α)-polypeptides. It may be possible, of course, that the differences between our results with formic acid treatment of PBLG, and those of the previous workers who used D,L-polypeptides for the most part, are due to the greater stability of the α-helix when only amino acid residues of one optical configuration are involved. This matter is under investigation and will be reported shortly.

The $\beta \rightarrow \alpha$ Transformation.—It has been reported[6] that certain polypeptides may be made to undergo a $\beta \rightarrow \alpha$ transformation by heating to 280° *in vacuo*. The conversion from $\beta \rightarrow \alpha$ was determined by both X-ray and infrared determinations. On heating cast films of our PBLG polypeptides in a similar manner to that described, we observe that typical spectral changes indicated in Fig. 9—namely, that the original material, which was a mixture of α and β, *on heating to 280°* and cooling to room temperature, appears to be predominantly α. However, from the intensities of the infrared bands and the weights of the samples before and after heating, it is apparent that there is *a loss of β-material* from the sample (by sublimation) *and no increase in the amount of the α-material.* Therefore no actual $\beta \rightarrow \alpha$ change has occurred and it is suggested that the previously reported $\beta \rightarrow \alpha$ transformation may have the same explanation.

In view of these studies it may be concluded that the *configuration of PBLG in the solid state is dependent on the molecular weight* and not on the prior solvent treatment or thermal history of the polypeptide. The infrared spectral results reported above with preparations of poly-γ-benzyl-L-glutamate are representative of those obtained with several other polypeptide polymers and copolymers which results will be published in due course. These data, from synthetic polypeptides of known composition and molecular weight, will be related to infrared data from proteins.

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"Enzymoid" Properties of Lysozyme Methyl Ester

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Conditions are defined under which an "enzymoid," an inactive enzyme derivative which retains affinity for substrate, can be expected to inhibit the activity of the native enzyme. Lysozyme methyl ester has been found to inhibit the lytic activity of lysozyme, and the data are consistent with the assumption that competition between enzyme and enzymoid for substrate occurs. Approximate K_m values for lysozyme–substrate and methyllysozyme–substrate interaction are 1.1 and 0.015, respectively. The implication of these findings for the problem of the relationship of structure to biological activity of proteins is discussed.

A large body of information is available concerning the effects of chemical alteration upon enzymes and other biologically active proteins. Usually, the effects of such alterations have been followed by measurement of changes in the activity of the protein being studied, sometimes correlated with quantitative estimates of the extent of chemical change. These studies have established the relationship between activity and specific structural features for a variety of enzymes and protein hormones.[2] However, the question of just what part a group which has been found to be essential plays in enzyme or hormone function has generally gone unanswered.

In this paper there will be outlined a method by means of which the function of essential groups of some enzymes can be defined, and its application to lysozyme will be described.

According to current concepts, an enzymatically catalyzed reaction proceeds through the initial formation of an enzyme–substrate complex, which then decomposes into reaction product(s) and free enzyme. If it is assumed that the affinity of enzyme for substrate and its catalytic property are functions of different parts, or at least different structural features, of the molecule, it follows that inactivation of an enzyme can occur in either of two possible ways: (1) modification or destruction of groups which are necessary for combination of enzyme and substrate, or (2) alteration of a structural element, the integrity of which is necessary for catalytic activity, *i.e.*, the decomposition of the enzyme–substrate complex into enzyme and reaction products. It is further readily apparent that specific modifications corresponding to type (2) would give rise to a derivative which would retain the characteristic affinity of the native enzyme for its substrate, but which would be devoid of catalytic activity. Under appropriate conditions, such a derivative might be expected to inhibit the apparent activity of the native enzyme by competing with the latter for available substrate. It should

(1) Guggenheim Foundation Fellow, University of California at Los Angeles, 1953–1954. Presented in part at the April, 1955, Meeting of the Federation of American Societies for Experimental Biology, San Francisco, California.

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