Synthetic Polypeptides Containing Side-Chain Amide Groups: Water-insoluble Polymers*

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ABSTRACT: Synthetic high-molecular-weight polymers containing glutamine residues were employed as model systems for studies of the contribution of side-chain amides to protein properties. Prolamine and glutelin proteins have unique properties that have been related to their high content of glutamine residues. The amide-rich polypeptides were prepared by reaction of poly-γ-methyl-, γ-ethyl-, or γ-benzyl-L-glutamate with liquid ammonia. Changes occurred in solubility and optical rotatory dispersion in organic solvents and in X-ray diffraction patterns and infrared spectra of the solid polypeptides as amides replaced ester groups. These observations indicate that side-chain amide groups may associate with peptide amides or side-chain amides in the same or other molecules through hydrogen bonding to effect solubility and conformation of the protein.

Synthetic high-molecular-weight polypeptides have found use as model systems for the study of the contribution of specific functional groups to protein behavior (Bamford *et al.*, 1956). The use of polypeptides eliminates the consideration of properties contributed by other residues in the protein or complications arising from the more complex protein conformation. To evaluate the contribution of numerous side-chain amide groups to protein properties, synthetic polypeptides were prepared that contained L-glutamine alone or L-glutamine and γ-L-glutamyl esters. The solubilities and rotatory dispersion curves of these polymers were determined in various solvents; the conformation and crystal structure of the solids were examined by infrared spectroscopy and X-ray diffraction.

The glutamine-containing polypeptides may be regarded as models for prolamine proteins, like zein and gliadin, and also for glutelin proteins, like wheat glutenin. Such proteins contain as much as 35% glutamine, a high content of nonpolar amino acids, and a sparsity of charged residues. The prolamines and glutelins are insoluble in neutral water but dissolve in solutions of urea or guanidine and in solutions of high or low *p*H at low ionic strength. The prolamines are also soluble in 70% ethanol. When side-chain amides of wheat gluten were converted to ester groups by Beckwith *et al.* (1963), evidence was obtained that the solution characteristics of the protein are influenced by hydrogen bonding between side-chain amides and by hydrophobic association of nonpolar residues. Now synthetic polypeptides give further support to the function of side-chain amides in protein interaction.

Methods

Preparation and Chemical Modification of Polypeptides. PBG,[1] PEG, and PMG were prepared by polymerization of the appropriate γ-ester of L-glutamyl *N*-carboxyanhydride. The anhydrides were obtained by reacting the corresponding γ-L-glutamyl ester with phosgene in anhydrous dioxane (Hanby *et al.*, 1950; Blout *et al.*, 1954). The *N*-carboxyanhydrides were polymerized in 5% solutions in anhydrous dioxane with sodium methoxide as the initiator. An anhydride-to-initiator ratio of 200 was used to obtain high-molecular-weight polymer (Blout and Karlson, 1956). Polymerization was allowed to proceed until more than 95% of the anhydride reacted (Berger *et al.*, 1953). The polymer was precipitated by pouring the dioxane solution into a large volume of vigorously stirred 95% ethanol.

In some of the later experiments PBG samples used as starting materials were obtained from Pilot Chemicals, Inc.[2] (lot G-12, mw 255,000 and lot G-19, mw 350,000).

The polyglutamyl esters were amidated by a modification of the procedure of Bruckner *et al.* (1953). Samples of the polymers (1–2 g) were sealed in a glass tube with 50 ml of anhydrous liquid ammonia and maintained at

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[1] The following abbreviations are used in the text: PBG, poly-γ-benzyl-L-glutamate; PEG, poly-γ-ethyl-L-glutamate; PMG, poly-γ-methyl-L-glutamate; and PGAM, poly-L-glutamine. Also the following abbreviations designate residues: BG, γ-benzyl-L-glutamate; EG, γ-ethyl-L-glutamate; MG, γ-methyl-L-glutamate; GA, L-glutamic acid; and GAM, L-glutamine.

[2] Pilot Chemicals, Inc., Watertown, Mass. Mention of suppliers of chemicals or equipment does not constitute preferential endorsement of their products by the U.S. Department of Agriculture.

TABLE I: Copolymers of γ-L-Glutamyl Esters and L-Glutamine.

Sample	Mole Ratio GAM:γ-Glutamyl Ester	[η]	mw_w[a]	DP	Amide Nitrogen (mg/g)	GAM Residues (%)
PBG	0:10	1.40	250,000	1140		
PMG	0:10	0.77	130,000	900		
PEG	0:10	1.07	190,000	1210		
10% GAM[b]	1:9	0.62	100,000	650	11.7	10.7
25% GAM[b]	2.5:7.5	0.50	78,000	520	27.5	25.1
36% GAM[b]	3.6:6.4	0.37	55,000	370	38.9	35.6
60% GAM[c]	6.0:4.0	0.37	36,000	410	66.1	60.4
68% GAM[c]	6.8:3.2	0.34	50,000	370	73.8	67.5
93% GAM[c]	9.3:0.7				101.9	93.2
PGAM[d]	10:0	0.24	34,000	270	109.4	100.0

[a] Estimated from viscosity, using molecular-weight calibration for PBG (Doty *et al.*, 1956). [b] Prepared from PEG. [c] Prepared from PMG. [d] Prepared from PBG.

room temperature with occasional shaking for as long as 14 days. The liquid ammonia was then allowed to distill from the opened tube, and the remaining product was washed with hot water. Its amide content was determined by digesting the polymer with 2 N H_2SO_4 for 1 hour in an autoclave at 120° and titrating the released ammonia in a Kjeldahl distillate of the digest.

Measurement of Physical Properties of Polypeptides. Infrared spectra were obtained with a Perkin-Elmer Model 21 double-beam spectrophotometer equipped with a sodium chloride prism. For determination of infrared spectra in solutions, closely matched BaF_2 cells with a path length of 50 μ were used to contain the dissolved sample and solvent. For measurements of dichroism in the infrared spectra of oriented films, polarized radiation obtained with a silver chloride polarizer (Makas and Shurcliff, 1955) was used. The films were cast on silver chloride slides and oriented by stroking in the direction of orientation or by rolling the slides in one direction until they were stretched 600%. In addition, some infrared spectra were determined on material incorporated into KBr disks.

For optical rotatory dispersion measurements a Rudolph Model 200 photoelectric polarimeter was employed with a mercury light source. The optical rotations were determined at wavelengths corresponding to emission maxima of the source from 313 to 579 mμ. The solutions, containing 0.2–0.5% polypeptide, were analyzed in 1-dm water-jacketed polarimeter tubes with fused quartz end-plates.

X-Ray patterns were determined on a Norelco diffraction unit by using nickel-filtered Cu-K radiation for 30 minutes at 35 kv and 15 ma. Kodak No-Screen film was exposed to the patterns at a distance of 5 cm. Polymer samples for X-ray analysis were either finely ground powders or films cast from various solvents.

Viscosity measurements were carried out with a Cannon-Ubbelohde dilution viscometer, size 100, at 24.8° on solutions containing polymer concentrations of 0.25–0.50% in anhydrous dichloroacetic acid.

Amino nitrogen determinations (Van Slyke, 1929) were conducted in an apparatus having a special chamber to allow the introduction of a solid sample (Williams and Lang, 1956).

Solubilities of the copolymers were determined at 0.2% concentration. The criterion for complete solubility was optical transparency of the solutions when viewed through a 1.0-dm polarimeter tube.

Results

Molecular Weights of Polypeptides. The weight-average molecular weights of polyglutamyl esters and the derived polymers are tabulated in Table I. They were estimated from the reduced specific viscosity in dichloroacetic acid by means of the viscosity–molecular weight correlation for PBG determined by Doty *et al.* (1956). To minimize peptide and side-chain amide hydrolysis in the acid solvent, all viscosity determinations were conducted within a few hours after solution of the polypeptide in dichloroacetic acid was complete. The stability of the polymers in dichloroacetic acid during the periods required for solution was established by the close agreement of amide content on the original polymers and those isolated from that solvent. However, a gradual decline in viscosity of a PGAM preparation was noted if the polymer was allowed to remain in solution in dichloroacetic acid for periods in excess of 1 week.

Number-average molecular weights of some polymer samples were obtained by the Van Slyke (1929) amino nitrogen analysis. A value of 35,000 was obtained for the molecular weight of a PGAM preparation by the Van Slyke method, whereas viscosity gave 34,000. A molecular weight of 190,000 was obtained by both procedures for a PEG sample. The close agreement be-

tween these molecular weights suggests that the samples are not highly heterogeneous in molecular weight since samples having a wide weight distribution should have significantly different weight-average and number-average molecular weights.

The reaction of polyglutamyl esters with ammonia, to yield polymers containing varying amounts of side-chain amide groups, resulted in a small amount of peptide cleavage. Thus the products have a lower molecular weight than the starting material.

Amidation Reaction. The conversion of ester residues in polyglutamyl esters to GAM residues was carried out in a two-phase system with liquid ammonia. The PEG reacted with the ammonia at a much slower rate than either PMG or PBG. Thus the rate of reaction was not entirely determined by the size or polarity of the leaving alcohol. The degree of crystallinity of the solid polymer and the type of structure may have some influence on the rate of reaction since, for example, some β structure was evident from the X-ray patterns of the PEG. The initial rate of amidation of PMG was rapid in contrast to the other polymers (55% in 6 days), whereas PEG and PBG were amidated 14 and 30%, respectively, in that time.

Exposure to ammonia longer than 10 days led to a diminished rate of amidation of the polymer with little further increase in GAM residues. Therefore, after 10–12 days of reaction, the ammonia and free alcohol were removed from the polymer and fresh liquid ammonia was added. Periods of amidation greater than 10 days indicated in Table II are for the two-stage process. A sample of PBG amidated in two stages of 12 days each was converted to 100% GAM residues, but another sample amidated continuously for 25 days contained only 78% GAM.

Attempts were made to increase the rate of amidation by modifying the reaction conditions. The reaction was run in a single-phase system obtained by dissolving the PBG in dioxane and adding liquid ammonia, but amidation was slow in this system (2% in 7 days). Catalysis of amidation by methoxide ion as suggested by Bunnett and Davis (1960) was also investigated. The product obtained was completely soluble in hot water, and although amide determination revealed it to be completely amidated, amino nitrogen analysis established it to be of low molecular weight, averaging only 10 residues in length. Amidations run at elevated temperature in an autoclave at 100° and 900 psi for 24 hours also yielded low-molecular-weight product. In the standard amidation reaction with liquid ammonia, small amounts of the hot water–soluble material are produced, and the quantity is proportional to the reaction time; but the high-molecular-weight, water-insoluble polymer is the main product.

Solubility of Copolymers of GAM and γ-L-Glutamyl Esters and of PGAM. Marked changes were noted in the solubilities of the polypeptides after introduction of GAM residues as shown in Table II, which summarizes the solubility of the various polymers in a number of organic solvents. The polyglutamyl esters were soluble in organic solvents like chloroform and *m*-cresol. PMG and PEG dissolved in the more polar solvent, formic acid, with extensive mixing, whereas PBG did not. Dichloroacetic acid dissolved all three polymers.

After 10% GAM residues were introduced into the polymer, it no longer dissolved in chloroform. The more polar solvent, *m*-cresol, dissolved the 10, 25, and 35% GAM–containing polymers but not those of higher GAM content. Polymers consisting of 60 and 68% GAM residues dissolved in formic acid. PGAM dissolved only in solutions containing strong hydrogen bond–breaking agents, such as dichloroacetic acid. Thus, as the quantity of GAM residues increased in the polymer, a greater hydrogen bond–disrupting capacity was required of the solvent. However aqueous solutions of urea at concentrations as great as 8 M were not adequate to dissolve PGAM.

Differences in rate of solution were noted for the various polymers in dichloroacetic acid. PEG and PMG were dissolved rapidly by dichloroacetic acid. As the GAM content became greater the time for complete solution also increased. The polymer containing 10% GAM required 1 hour to be dissolved; 60%, 8 hours; and 100%, 70 hours. Thus, the larger amounts of GAM residues resulted in changes in crystal structure and greater cohesive forces which maintain the particle structure.

High-molecular-weight PGAM (DP 300) was not soluble in water, even at elevated temperatures. This insolubility permitted the use of hot water as a means of extracting the low-molecular-weight polypeptides (DP

TABLE II: Solubility of Glutamine-containing Polymers in Organic Solvents.

Polymer	Solvent			
	Chloroform	*m*-Cresol	90% Formic Acid	Dichloroacetic Acid
PBG	+[a]	+	−	+
PEG	+	+	+	+
PMG	+	+	+	+
10% GAM–90% EG	−	+	+	+
25% GAM–75% EG	−	+	+	+
36% GAM–67% EG	−	+	+	+
60% GAM–40% MG	−	−	+	+
68% GAM–32% MG	−	−	+	+
PGAM	−	−	−	+

[a] (+) indicates polymer soluble at 0.2% concentration.

~10), which were soluble, from the high-molecular-weight PGAM. Although insoluble in water, the high-molecular-weight PGAM exhibited its hydrophilic character by hydrating and swelling when exposed to water.

Infrared Spectra of Solutions of Polymers. The infrared spectra of polymers containing different amounts of glutamine residues were examined in solutions of 25% dichloroacetic acid–75% ethylene dichloride. Polymer concentrations were varied from 0.2 to 2.0%. The absorbance at 1650 cm^{-1} was associated with random or helical conformation, while that at 1610 cm^{-1} was considered a measure of β structure (Blout and Asadourian, 1956).

In all samples, ranging from PEG to PGAM, the peak at 1650 cm^{-1} was highly predominant and that at 1610 cm^{-1} appeared only as a small shoulder. It must be concluded that in this solvent, irrespective of polymer composition, the β form of the polymers is not stable and that most of the molecules occur as random coils or α-helical structures. This conclusion is consistent with the demonstration by Wada *et al.* (1961) that low levels of dichloroacetic acid added to solutions of low-molecular-weight PBG in nonpolar solvents disrupted the β structure. Furthermore, no increase in the relative magnitude of the 1610 cm^{-1} peak occurred in the solutions of higher polymer concentration, which suggests that the 1610 cm^{-1} absorption may not be due to intermolecular β structure.

Since the samples of polymer containing high levels of glutamine residues were soluble only in solvents containing dichloroacetic acid or related substances, only limited studies of infrared spectra in solution were undertaken because dichloroacetic acid absorbs strongly in many regions of the infrared, including the amide I region. Problems encountered in canceling all traces of solvent absorption rendered data interpretation difficult at low polymer concentrations.

Optical Rotatory Dispersion of GAM-Containing Copolymers. The influence of side-chain amide groups on conformation of the polymer in various solutions was also investigated by means of optical rotatory dispersion measurements. Yang and Doty (1957) demonstrated that addition of ethylene dichloride to solutions of PBG in dichloroacetic acid will induce α-helical conformation. The rotatory dispersion characteristics of polymers containing GAM residue in solution in 25% dichloroacetic acid–75% ethylene dichloride are summarized in Table III.

Values of b_0 were calculated from the optical rotatory dispersion data by means of the relationship developed by Moffitt and Yang (1956) using 212 mμ as the λ_0. The approximate relative helical content is calculated from b_0 assuming a b_0 value of 0 for the completely random conformation and −640 for the completely helical structure for the polyglutamyl esters. The value of b_0 obtained for PMG was −630. As amidation increased helical content of the polymers in this solvent decreased. In 1:1 dichloroacetic acid–ethylene dichloride, b_0 of each polymer became more positive. In pure dichloroacetic acid b_0 values of 0 for each polymer in the series confirmed random-coil structure in that solvent.

The values of the a_0 parameter calculated from rotatory dispersion data by the Moffitt and Yang (1956) equation for the polypeptides dissolved in 25% dichloroacetic acid–75% ethylene dichloride are also tabulated in Table III. The a_0 values become less positive with increasing amide content and are negative for PGAM.

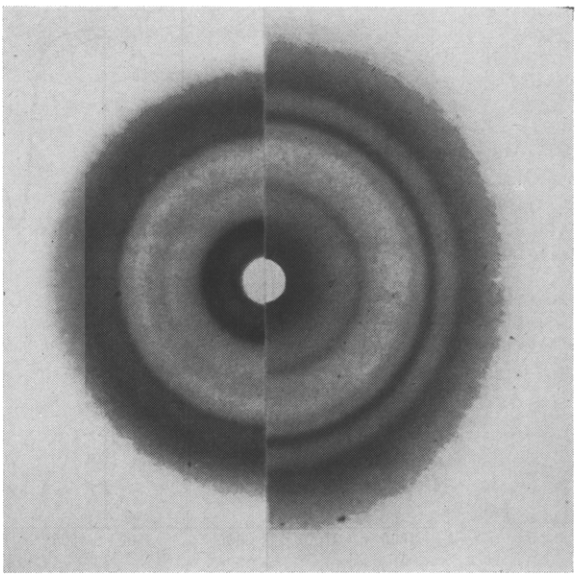

FIGURE 1: X-Ray powder patterns of synthetic polypeptides, PBG on left and PGAM on right. Interplanar spacings in angstroms for PBG 14.2 (S), 7.6 (W), 6.7 (VW), 5.26 (S), 4.46 (M); for PGAM 8.40 (M), 5.34 (W), 4.78 (S), 4.10 (M), 2.74 (W). Line intensities are indicated as strong (S), medium (M), weak (W), and very weak (VW).

TABLE III: Optical Rotatory Dispersion of Glutamine-containing Polypeptides in 75% Ethylene Dichloride–25% Dichloroacetic Acid.

Polymer	a_0	b_0	Helix[a] (%)
PMG	+182	−630	98
10% GAM–90% EG	+120	−552	86
25% GAM–75% EG	+76	−539	84
36% GAM–64% EG	+64	−445	68
60% GAM–40% MG	+49	−484	76
68% GAM–32% MG	+44	−449	70
PGAM	−27	−261	41

[a] Based on the assumption that $b_0 = 0$ in completely random conformation and −640 in the completely helical form of PBG, and that the amount of polymers having β structure in this solvent is small.

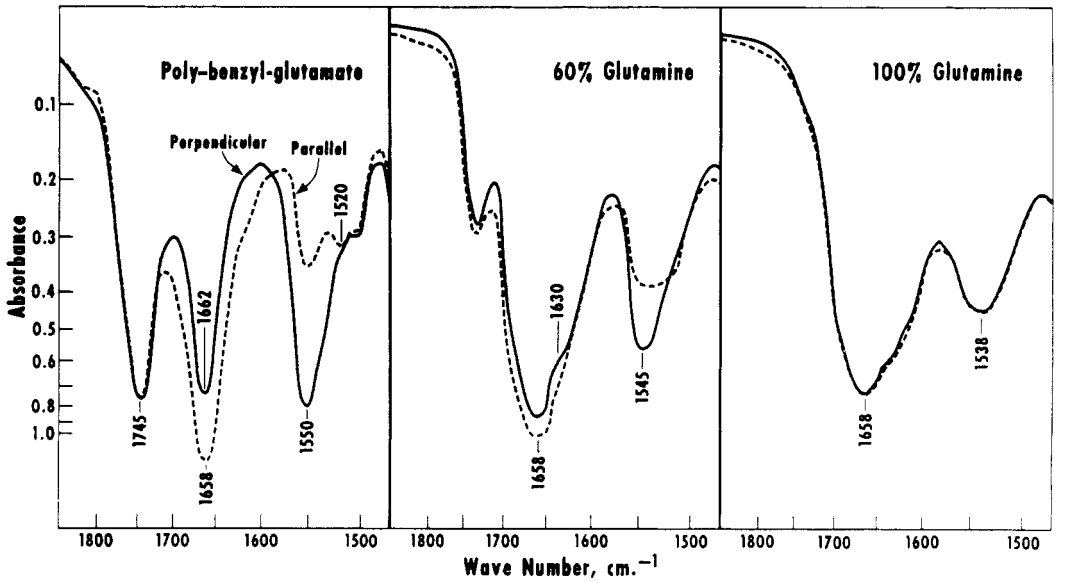

FIGURE 2: Infrared spectra of oriented polypeptide films obtained with polarized light.

Imahori (1960) indicates that β structure is associated with a positive value of a_0. The decline in a_0 further establishes that in this solvent increasing the GAM content of the polymers results in converting the α-helical conformation of the polymer to random-coil rather than β structure.

X-Ray Diffraction Studies. X-Ray powder patterns (Figure 1) demonstrate that a striking transformation occurs in the conformation of the polypeptide upon complete substitution of amide groups for the benzyl ester groups on the γ-carboxyl of the glutamyl residues. PBG shows an α pattern and PGAM exhibits what is probably a β pattern of a highly crystalline fraction that is in the solid granular polymer. In the absence of oriented materials, the judgment about β is based on the powder patterns that closely resemble those shown by known β structures. The transition shown in Figure 1 holds for the PEG and PMG also, although initially each of these esters appeared to have minor β-crystalline components.

Infrared Studies on Solid Polymers. Oriented films of PBG, PEG, and PMG were readily obtained by stroking highly concentrated solutions of the sample in chloroform on a silver chloride plate as described by Ambrose and Elliott (1951). However, the copolymers containing GAM residues, cast from slowly evaporating dichloroacetic acid or formic acid solutions, were poorly oriented by stroking, if at all. For these samples the polymers were oriented by stretching the dried films on a slide.

The infrared spectra of three polymers, PBG, 60% GAM–40% EG, and PGAM are compared in Figure 2. The progressive chemical transformations are evident in the spectra. The absorbance at 1745 cm^{-1} due to the ester group diminishes as amide groups are introduced into the polymer. Concomitantly there is an increase in size of the amide I band at 1658 cm^{-1}, which is due to the primary amide groups of the GAM residues formed by the amidation reaction.

The PBG molecules in the film were well oriented and existed predominantly in the α-helical conformation as evidenced by the marked dichroism of the amide I peak at 1658 cm^{-1} and of the amide II peak at 1550 cm^{-1} in polarized light (Figure 2) (Miyazawa and Blout, 1961). The amide I peak was most pronounced when the polymer orientation was parallel to the electrical vector of the radiation, but the amide II was greatest when polymer orientation was perpendicular to the electrical vector.

The infrared patterns confirmed the fact that as the GAM content of the polymers increased the polymers became less subject to molecular orientation. The 60% GAM copolymer showed diminished dichroism in patterns of stretched films and no dichroism was apparent in the PGAM film. Increased interactions between polymer chains due to the added amide groups on the side chains evidently were responsible for our inability to orient the amidated films.

However, the infrared spectra do indicate a shift from α to β conformation of the peptide backbone upon conversion of glutamic ester residues to GAM. In both 60 and 100% GAM polymers, a shoulder is evident at 1630 cm^{-1} in the amide I peak. The shoulder is most pronounced in the 60% GAM film when the polymer orientation is perpendicular to the electrical vector of radiation of the polarized light, which is characteristic of the parallel β structure (Ambrose and Elliott, 1951). In addition, there is an increase in the magnitude of the amide II peak in the parallel orientation of the 60% GAM polymer. A shift of this band to lower wave numbers occurs with increased GAM content as shown in the 60 and 100% GAM infrared patterns (Figure 2).

The broad absorption peak that is given by the amidated polymers at 1658 cm^{-1} shows little dichroism. The

magnitude of this band is probably due to the additional absorbance of hydrogen-bonded side-chain amide groups that characteristically absorb in the region of 1650 cm^{-1} (Bellamy, 1956). The position of this band gives support to the occurrence of side-chain amide associations in the solid amidated polymers.

A highly crystalline preparation of PGAM identical to that employed for the X-ray pattern of Figure 1 when incorporated into KBr pellets gave better-defined patterns than material cast from dichloroacetic acid. Well-defined maxima at 1630 and 1538 cm^{-1} were detected. This evidence gave further support to the existence of the peptide backbone of PGAM in a β conformation in regions of crystallinity. However, considerable random-coil structure is apparent in these preparations and even more in material deposited from dichloroacetic acid.

Discussion

The chemical modifications of polyglutamyl esters yielded mixed polymers, which possess certain advantages for use as model systems. The different residues are most probably randomly distributed, whereas mixed polymers formed by condensation of different monomers may not have a random distribution. The side chains are of fairly uniform structure, differing only at their termini. Thus any change in the properties are reflections of the side-chain terminal groups. The terminal ester groups, selected to impart hydrophobic character, were ethyl or methyl so as to minimize the variation in chain size. The structure of the side chains in each case should not interfere sterically with helical conformation, as established by Bloom *et al.* (1962). The glutamyl polypeptides are more suitable model systems for the study of solvent effects on solubility and conformation of protein than many substances used for this purpose since the aliphatic side chains exhibit actions analogous to those of side-chain groups in proteins.

During preparation of the polymers the DP of the original polyglutamyl esters was reduced. There is a direct correlation between amount of amide substitution and decrease in chain length. However, the alteration of properties, such as helical content or solubility, is probably not greatly influenced by the molecular weight change, since the DP values of the materials studied are well above the limiting DP values which can form α-helical conformations for many polypeptides. Blout (1962) indicates that the α-helix is the conformation of solid poly-L-leucine, PBG, and poly-L-methionine at DP values of 35, 100, and 135, respectively, and that DP values over 35 gave helical conformation in polymers when the residue content favors that structure.

The GAM-containing polypeptides are also well above the minimum DP for α-helix conformation in solution, as shown by Blout and Asadourian (1956) for PBG, and Goodman and Schmitt (1960) for PMG. However in certain other polymers, even if the chain length exceeds this minimum value, the polymer will not form a helical conformation because of side-chain interactions (Blout, 1962). These interactions, causing a nonhelical structure, are presumably the factors opposing helix formation in the GAM-containing polypeptides. The viscosity measurements yield information on weight-average molecular weight. If the weight distribution is broad, then there is still a possibility that substantial amounts of lower-molecular-weight material may be present that could not form α-helices and would favor β conformation. Limited comparison of number-average with weight-average molecular weights indicates that, despite the randomness of the peptide cleavage resulting from the polypeptide modification, a suitably narrow weight distribution was probably obtained after the preparations were washed with hot water to remove very low-molecular-weight polypeptides.

Visual appearance, infrared spectra, and X-ray diffraction patterns established that definite changes in the crystal structure of the solid polymer occurred as the γ-ester groups were replaced by amide groups. The polyglutamyl esters were fibrous materials readily cast from solution in organic solvents into water-resistant plastic films. In contrast, PGAM was a granular solid that could not be successfully cast into orientable films, and although it was not dissolved by water, it hydrated rapidly. Intermediates in the conversion of the polyglutamyl esters to PGAM exhibited transitional properties. The change in polymer conformation from α to β form with increase in amide content probably accounts for much of the change in the properties of the solid. In the α-helical conformation of the poly-γ-glutamyl esters association between side chains can occur only through hydrophobic bonding, whose weaker nature allows a considerable degree of plasticity in the films. In the parallel β structure extended chains are joined in sheets by hydrogen bonds involving peptide amides. Thus the β structure generally seems to impart strength and rigidity to a solid. In the PGAM, additional hydrogen bonding through the numerous side-chain amide groups may result in strongly associated sheets in a crystalline β structure. It is also possible that a multiplicity of hydrogen bonds in an amorphous PGAM could lead to hard, granular, and difficultly soluble materials that also resist orientation.

These observations are pertinent to the production of useful films from prolamines. Plasticity and tensile strength are retained only as long as moisture or polyol plasticizer is used to disrupt or weaken some intermolecular hydrogen bonds. Thus unplasticized prolamine films become brittle and disrupt readily upon drying.

Molecular models of polyglutamine and other polyglutamic acid derivatives, constructed with space-filling atomic units prepared in this laboratory and those of Dr. Elkan Blout (personal communication), establish that optimum association between side-chain amides is facilitated by β conformation. In contrast, in the α structure the side-chain amides are distributed helically about the molecule so as to offer less opportunity for molecular interactions. In solid PGAM, the greater degree

of intermolecular hydrogen bonding possible in the β form may account for the greater stability over that of the α form.

The dependence of conformation in solution and solubility of synthetic polypeptides upon the relative interaction of side chains with the solvents has been pointed out by Blout (1962). The tendency of strongly hydrophobic side chains to orient toward an organic solvent, while the peptide amide groups associate, favors α-helix formation and solubility in organic solvents. Introducing hydrophilic groups such as the amide group onto the side chains of the polypeptides not only reduces polypeptide solubility in organic solvents but also results in the hydrophilic group's orienting away from the nonpolar solvent so as to interact with the peptide backbone and contribute to helix disruption. Side-chain amides may also associate with those of adjacent molecules so as to cause insolubility. The need for increasing polarity of solvents, to dissolve and dissociate the polypeptides as their amide content is increased, probably arises from the hydrogen bond–breaking ability of these solvents. With the completely amidated polymer the only effective solvents are strong hydrogen bond–breaking agents, which also tend to disrupt any helical conformation. Since a high level of helical content may be exhibited in solutions of the polymer in ethylene dichloride–dichloroacetic acid, which contain little β structure, the dichloroacetic acid may preferentially associate or exchange protons with the side-chain amide groups.[3]

The decreasing values of a_0 observed for solutions of the polymers in 25% dichloroacetic acid–75% ethylene dichloride with increase in amide content are consistent with the observations of Tanford *et al.* (1960) and Harrap and Stapleton (1963). They found that decreases in a_0 may be related to an increase in the polarity of the environment of the peptide group. Not only are the side-chain amide groups in the glutamine copolymers more polar than the ester groups they displace, but they disrupt the helix so as to permit solvation of the peptide group by the highly polar dichloroacetic acid in the solvent.

The effectiveness of hydrogen bonding in stabilizing conformations or aggregations of polymers in aqueous media evidently depends on the chain length of the polymers. Thus, PGAM of high molecular weight did not dissolve in water, whereas low-molecular-weight GAM-containing peptides did. Similarly, the solubility in water of other linear polymers that contain hydrogen-bonding groups, such as amylose or polyacrylamide, is known to depend on polymer size, the larger polymers being insoluble.

Steric factors also have an influence on the solubility characteristics. Bohak and Katchalski (1963) found that high-molecular-weight, pure poly-L-serine, which exists in the β conformation, was not soluble in water or numerous other solvents in contrast to partially racemized polymers of serine, which were water soluble. It is possible that steric regularity increases the effectiveness of hydrogen bonding between the serine side chains and may enhance intermolecular association of glutamine residues in polyglutamine.

Klotz and Franzen (1962) concluded from studies with low-molecular-weight model compounds that the equilibrium constant for association of amide groups in dilute solution in water is below 0.01. This value indicated that the thermodynamics of interamide hydrogen-bond formation is not favorable in aqueous media. In the case of high-molecular-weight, sterically regular polypeptides, a multiplicity of interacting groups are in close proximity. The high local concentration in PGAM of amide groups may be sufficient to shift the equilibrium between interamide and amide-water hydrogen bonds to favor the interamide. A hydrophobic local environment, due to the numerous aliphatic side-chain methylenes, may further stabilize the hydrogen bonds between side-chain amide groups.

The finding that side-chain amide groups contained in the synthetic polypeptides can associate by means of hydrogen bonds may be evidence for similar behavior of these groups in the prolamine proteins where they are also present in large quantity. An analogy may also be drawn between the solution characteristics of the mixed polymers containing both hydrogen-bonding groups and hydrophobic terminal side-chain groups and that of prolamines. Their characteristic behaviors probably result from the interplay of both of these factors. Hydrophobic groups, by rendering hydrogen bonds less accessible to water, strengthen the hydrogen bonds, which in turn give strength and specificity to molecular conformation and intermolecular aggregation.

An extension of these studies to the behavior of glutamine and glutamic acid–containing polypeptides dissolved in aqueous systems at different *p*H values and in the presence of varying amounts of urea will be presented in another communication.

[3] The solubility and random conformation of synthetic polymers in dichloroacetic acid have been ascribed to the protonation of the amide groups rather than hydrogen-bond formation (Hanlon *et al.*, 1963).

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