1965) illustrates that this effect is cumulative.

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References

Finamore, F. J., and Warner, A. H. (1963), *J. Biol. Chem. 238*, 344.

King, E. J. (1932), *Biochem. J. 26*, 292.

Mejbaum, W. Z. (1939), *Z. Physiol. Chem. 258*, 117.

Razzell, W. E. (1963), *Methods Enzymol. 6*, 236.

Reiss, J. R., and Moffatt, J. G. (1965), *J. Org. Chem. 30*, 3381.

Smith, M., and Khorana, H. G. (1958), *J. Am. Chem. Soc. 80*, 1141.

Staehelin, M. (1961), *Biochim. Biophys. Acta 49*, 11.

Verheyden, D. L. M., Wehrli, W. E., and Moffatt, J. G. (1965), *J. Am. Chem. Soc. 87*, 2257.

Warner, A. H., and Finamore, F. J. (1965a), *Biochim. Biophys. Acta 108*, 525.

Warner, A. H., and Finamore, F. J. (1965b), *Biochemistry 4*, 1568.

Wehrli, W. E., and Moffatt, J. G. (1965), *J. Am. Chem. Soc. 87*, 3760.

Wylie, V., and Smith, M. (1964), *Can. J. Biochem. 42*, 1347.

Synthetic Polypeptides Containing Side-Chain Amide Groups. Water-Soluble Polymers*

Larry H. Krull and Joseph S. Wall

ABSTRACT: Synthetic high-molecular weight polypeptides containing glutamine and glutamic acid residues were employed as model systems to investigate the influence of side-chain amides on protein conformation and aggregation in aqueous solutions. The polypeptides were prepared by converting ester groups of mixed polymers of glutamine and glutamyl esters to free carboxyls.

In some polymers, ester groups were retained to permit evaluation of hydrophobic forces. Solubilities and optical rotatory dispersion measurements at various pH values established that the presence of amide groups increased the minimum pH for solubility and decreased the helical content of the polymers. The minimum pH for solubility of the polymers was lowered with urea. The presence of ester residues also decreased polypeptide solubility but stabilized helical structure. These findings suggest that in high-molecular weight polypeptides or proteins, containing high levels of asparagine or glutamine, conformation and aggregation in aqueous solution may result from hydrogen bonding between amide groups.

In many proteins, glutamine, or asparagine residues occur in high concentration, especially in such prolamines as corn zein or wheat gliadin. The numerous side-chain amide groups may be responsible in part for the tendency toward aggregation and insolubility of such proteins. To investigate this possibility, high-molecular weight synthetic polypeptides, consisting of glutamine residues alone or together with γ-glutamyl ester residues, were synthesized and their properties investigated by Krull and associates (1965). These polymers did not dissolve in water, but when they were dissolved in organic solvents or were in a solid state, pronounced interactions between side-chain amide groups could be demonstrated. This effect was indicated by a decrease in polymer solubility and helical content in organic solvents as the ratio of glutamine–γ-glutamyl ester was increased. X-Ray and infrared studies established that the conformation and crystalline structure of polyglutamine were markedly different from those of poly-γ-ethyl-L-glutamate.

Since the normal environment of proteins is water, it was essential to evaluate the behavior of glutamine-containing synthetic polypeptides in aqueous solution.

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By incorporating glutamic acid residues into the polypeptides containing glutamine, we could then dissolve the copolymers in neutral water solutions. The behavior of these water-soluble polymers supports the thesis that hydrogen bonds between amide groups of proteins or other polymers of high-molecular weight can exist in aqueous solutions under certain conditions. Such bonds between amide groups can be disrupted by urea solutions. The properties of the polymers containing hydrophobic ester side-chain residues, as well as glutamine and glutamic acid, indicate that hydrophobic interactions stabilize polypeptide secondary structure but render the polymer less soluble in aqueous solvents.

Methods

Preparation of Copolymers. PBG,[1] PEG, and PMG were prepared by polymerization of the appropriate γ-ester of L-glutamyl-*N*-carboxyanhydride or were from a commercial source as described previously (Krull *et al.*, 1965). Conversions of ester to amide groups were carried out on the polyglutamyl esters by an adaptation (Krull *et al.*, 1965) of the procedure used by Bruckner *et al.* (1953).

The ethyl and methyl ester groups of some partially amidated samples were removed by a modification of the procedure of Green and Stahmann (1955). The polymer containing ester residues was stirred under nitrogen with 0.1 N NaOH for *ca.* 48 hr in the cold. The residual insoluble material was removed by centrifugation and the supernatant solution neutralized, dialyzed, and finally lyophilized. The GAM content was determined by amide nitrogen analysis and the residual ester groups by alkoxyl analysis. The liberated GA was determined by titration and by difference between total residues and the sum of esterified and amidated residues.

Hydrolysis by base was suitable for producing mixed polymers containing acid, amide, and ester groups but unsatisfactory for preparing polymers completely devoid of ester groups. Because a small fraction of ester groups resisted base hydrolysis, much longer reaction times were required to remove all of them completely. Treatment with alkali for the required prolonged periods was avoided since cleavage of peptide groups may occur and also racemization of amino acid residues (Bohak and Katchalski, 1963).

Alternative methods were, therefore, developed to prepare GA- and GAM-containing copolymers. Acid hydrolysis of side-chain amide groups from PGAM was carried out in 2 N H_2SO_4 at 80° for *ca.* 1 hr. Increasing the reaction time gave polymers with a greater proportion of glutamic acid residues. The reaction mixture was cooled to 0° and neutralized. After insoluble material was removed by centrifugation, the clear solution was dialyzed and lyophilized. This process yielded polymers completely free of ester groups, but the preparation of PGAM required considerable time, and the acid hydrolysis resulted in some peptide cleavage.

Another procedure employed for preparing mixed GA–GAM copolymer was *via* partial amidation of PBG and debenzylation of the residual benzyl ester groups with anhydrous hydrogen bromide in trifluoroacetic acid. The latter operation was carried out according to the method of Idelson and Blout (1958). This procedure was the most satisfactory as it gave minimal amide or peptide cleavage.

Physical Measurements. Viscosity determinations were made with a Cannon-Ubbelohde dilution viscometer,[2] size 50, at 25.05° on solutions containing copolymer concentrations 0.50–0.10% in 0.2 M NaCl at pH 7.3 or dichloroacetic acid as indicated.

Optical rotatory dispersion measurements of polymer solutions were obtained with a Model 200 Rudolph photoelectric polarimeter equipped with a mercury light source. The emission lines between 313 and 579 mμ were used for rotation measurements. Solutions containing 0.2–0.5% of polymer were analyzed in 1.0-dm water-jacketed polarimeter tubes with fused-quartz end plates.

Solutions for optical rotatory dispersion measurements were prepared from concentrated, neutral solutions, which were diluted with water or aqueous urea solution and titrated to the proper pH with 0.1 N HCl. Additional water was added to adjust the solution to volume to obtain a 0.2% solution. A Beckman Model H2 pH meter and a Beckman 39183 combination electrode were used for pH measurements. Immediately after preparation of the solutions, they were transferred to the polarimeter tube and allowed to equilibrate for 20 min; then rotation measurements were made.

Titration curves of the polymers were obtained automatically by means of a Radiometer Model TTT-1 titrator and a Model SBR/2 titragraph with single-probe combination electrodes GK-2026. Each polymer (10 mg) was titrated with 0.0107 N KOH in a volume of 5 ml of water. The samples for titration were prepared by dialyzing them *vs.* dilute acid, then *vs.* water, and finally by lyophilizing them. When added to water, these samples did not dissolve. They were titrated with base either in fine suspension or after being solubilized in minimal base and then brought to incipient turbidity with acid. No additional salt was added.

The solubility of the copolymers was determined at 0.2% concentration. The criterion for complete solubility at this level was the optical transparency of the solutions when viewed through a 1.0-dm polarimeter tube, a condition necessary for the optical rotatory dispersion measurements.

[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; PGA, poly-L-glutamic acid; 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; GAM, L-glutamine.

[2] Mention of suppliers of chemicals or equipment does not constitute preferential endorsement of their products by the U. S. Department of Agriculture.

TABLE I: Composition of Copolymers.

Copolymer (%)	Mole Ratio of Glutamine–Glutamic-γ-Glutamyl Ester	[η]	Amide Content	
			mg of N/g	Glutamine (%)
GAM–GA[a] (10:90)	1.0:9.0:0.0	0.525[b]	11.2	10.3
GAM–GA[a] (25:75)	2.5:7.5:0.0	0.415[b]	26.6	25.4
GAM–GA[c] (30:70)	3.0:7.0:0.0	0.365[b]	33.7	30.9
GAM–GA[a] (35:65)	3.5:6.5:0.0	0.378[b]	38.7	35.5
GAM–GA[c] (40:60)	4.0:6.0:0.0	0.410[b]	43.9	40.3
GAM–GA[c] (47:53)	4.7:5.3:0.0	0.445[b]	51.4	47.1
GAM–EG[d] (21:6)	2.1:7.3:0.6	0.290[e]	22.4	20.6
GAM–BG[a] (21:2)	2.1:7.7:0.2	...	22.9	21.0
GAM–MG[f] (34:2)	3.4:6.4:0.2	0.360[e]	36.8	33.8
GAM–MG[f] (20:6)	2.0:7.4:0.6	0.280[e]	22.1	20.3
GAM–EG[d] (21:12)	2.1:6.7:1.2	...	22.6	20.8

[a] Prepared from partially amidated poly-γ-benzyl-L-glutamate. [b] Determined in 0.2 M NaCl at pH 7.3. [c] Prepared from polyglutamine. [d] Prepared from partially amidated poly-γ-ethyl-L-glutamate. [e] Determined in dichloroacetic acid. [f] Prepared from partially amidated poly-γ-methyl-L-glutamate.

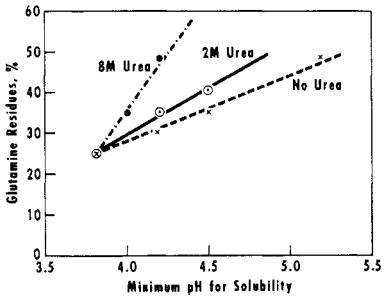

FIGURE 1: The effect of urea and pH on the solubility of glutamine–glutamic acid copolymers.

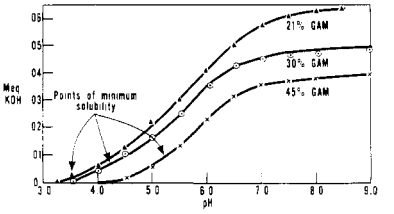

FIGURE 2: Titration curves of glutamine–glutamic acid copolymers (GAM, L-glutamine).

Results

Composition of Copolymers. The composition of the GAM–GA copolymers is shown in Table I. Intrinsic viscosities of the copolymers in the unfolded state, measured at pH 7.3 in 0.2 M NaCl, are also listed in Table I. These viscosities were used to obtain estimates of the polymer molecular weights by relationships previously used by Doty *et al.* (1957), Lenormant *et al.* (1958), Fasman *et al.* (1964), and Nagasawa and Holtzer (1964).

Also shown in Table I is the compositional data for several GAM–GA copolymers that also contain nonpolar ester chains. Molecular weight estimates of these copolymers were determined from the intrinsic viscosity in dichloroacetic acid, by means of the calibration curve of Doty *et al.* (1956). These copolymers were produced by stopping the deesterification reactions before complete removal of ester groups. In neutral solutions, these mixed polymers contained ionized carboxyl groups, hydrophilic amide groups, and hydrophobic ester groups on the ends of the side chains.

Despite wide variation of GAM content, the molecular weight estimates for the different copolymers were in the range 20,000–50,000. All the copolymers were of sufficient size to assume a helical conformation (Blout, 1962; Goodman and Schmitt, 1960) at acid pH, since steric factors were favorable (Bloom *et al.*, 1962), providing that side-chain interactions were not prevalent.

Solubility of the Copolymers. As the ratio of GAM–GA residues in the copolymers increased, the minimum pH at which the copolymers were soluble in water solution increased (Figure 1). The titration curves (Figure 2) establish that more carboxyl groups had to be ionized on polypeptides with greater side-chain amide content to dissolve them than on polymers with less GAM residues. Consequently, greater electrostatic force is required in aqueous media to separate associations involving amide groups than those due to carboxyl groups. When the copolymers of GAM and GA exceeded 60% GAM content, they could not be dissolved in aqueous systems even at neutral pH.

TABLE II: Optical Rotatory Parameters of Glutamine–Glutamic Acid Copolymers at Various pH's and Urea Concentrations.

		Urea Concn (mole/l.)					
		0		2		8	
Copolymers (%)	pH	b_0	a_0	b_0	a_0	b_0	a_0
All copolymers	7	0		0		0	
GAM (10)	5.5	−137	−498	...	...	...	...
	4.5	−596	−133	...	...	...	...
	4.0	−641	−120	...	...	...	...
	3.8	−630	−27	−650	−29	...	...
GAM–MG (20:6)	5.2	Insol	...	−411 (0.1 M urea)		...	...
	4.2	Insol	...	−627 (1.0 M urea)		...	...
GAM–EG (20:6)	5.9	−2	−553	...	...	...	...
	5.2	Insol	...	−371	−118	...	...
	4.2	Insol	...	−426	−89	...	...
GAM (25)	4.5	−292	−196	...	...	...	...
	4.2	−393	−136	...	...	...	...
	3.8	−490	−102	−328	−193	−296	...
GAM (35)	4.5	−313	−87	...	...	...	...
	4.2	Insol	...	−300	−120	...	...
	4.0	Insol	...	...	...	−256	−244
GAM (40)	5.2	−90	−209	−38	−253	...	...
	4.5	Insol	...	Insol	...	−16	−111
	4.2	Insol	...	Insol	...	0	−271
GAM (46)	5.2	−40	−5	−20	−9	...	...
	4.5	Insol	...	Insol	...	−13	...
	4.2	Insol	...	Insol	...	0	...
PGA	5.2	−423	−24	−426	−62	−342	−147
	4.5	−625	+69	−448	+18	−450	+9

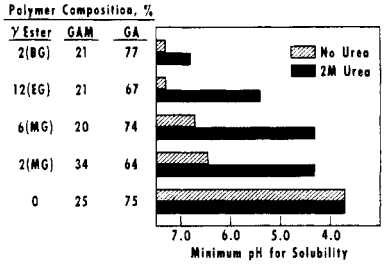

FIGURE 3: Influence of pH and urea on the solubility of copolymers of glutamine–glutamic acid and γ-glutamyl esters.

The addition of urea to the aqueous solvent diminished association and permitted solubilization of the GAM-containing polymers at lower pH values. The reduction of the minimum pH for solubility of several GAM–GA polymers by the addition of 2 M urea is illustrated in Figure 1. An 8 M urea solution was considerably better than 2 M in effecting polymer solvation at lower pH; *e.g.*, the 35% GAM copolymer was soluble at pH 4.5 with no urea, pH 4.2 with 2 M urea, and pH 4.0 with 8 M urea.

Ester groups in GAM–GA copolymers markedly decreased the polypeptide solubility at low pH (Figure 3). A polymer containing 34% GAM, 64% GA, and 2% EG dissolved in solutions only above pH 6.6, whereas a GAM–GA (35:65%) copolymer dissolved at pH 4.5. The effect of slightly larger quantities of hydrophobic ester side chains, 6 and 12%, is greater insolubility of the polypeptides near neutrality.

Urea does increase the solubility of mixed polymers containing ester as well as amide and acid functions in the side chains, although solubility in urea solutions is not so great as when hydrophobic groups are absent. As shown in Figure 3, the 12% EG, 21% GAM, and 67% GA polymer was not dissolved below pH 7.3 without urea, but was soluble in 2 M urea as low as pH 5.4. Comparison of the polymers containing similar levels of GA, but differing in that one contains glutamyl ester residues in place of some GAM, established that the effectiveness of solvation with urea is diminished by the replacement of side-chain amides by ester groups. Even 8 M urea was only slightly effective in improving solubility of a preparation containing 2% BG, 21% GAM, and 77% GA. Evidently, the hydrophobic interaction of the benzyl groups was not readily disrupted by urea.

Titration Curves. The titration curves of Figure 2

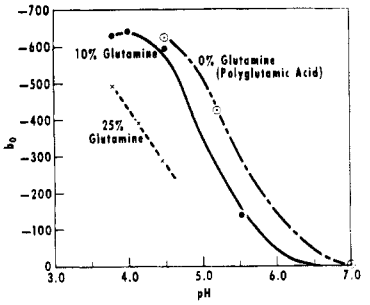

FIGURE 4: Effect of pH and glutamine content on rotatory dispersion of glutamine–glutamic acid copolymers.

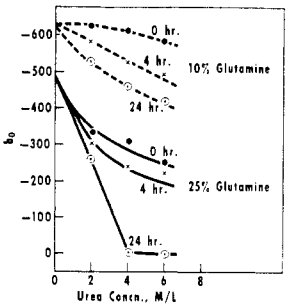

FIGURE 5: Effect of urea as a function of time on optical rotatory dispersion of glutamine–glutamic acid copolymers at pH 3.8.

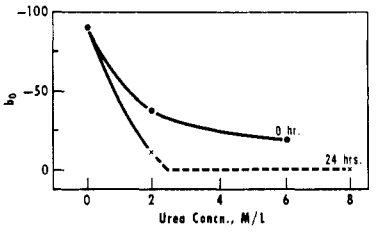

FIGURE 6: Effect of urea as a function of time on optical rotatory dispersion of 40% glutamine–glutamic acid copolymer at pH 5.2.

compare the amount of ionized residues on equal amounts of polymer at various pH values. The introduction of additional glutamine residues into the polymers reduced the concentration of titratable carboxyls and caused changes in apparent acidity as indicated by differences in the titration curves. The slightly higher acidity at higher levels of ionization of polymers richer in GAM is probably due to charge separation. The loss in apparent acidity of carboxyls at low levels of ionization has been attributed to hydrogen bonding or inaccessibility. Nagasawa and Holtzer (1964) found that helical or aggregated polyglutamic acid exhibited different titration parameters than the random-coil polymers. The variations in the titration curves of GAM–GA copolymers are consistent with the premise that amide groups reduce helical content but promote molecular aggregation. The addition of urea to the polymer solutions permitted titrations to be initiated at lower pH, reduced the apparent pK of the carboxyl, and gave titration curves generally associated with random-coil polymers.

Optical Rotatory Dispersion. The influence of side-chain amide content, pH, and urea concentration upon conformation of the GAM–GA copolymers was determined in aqueous solution. Rotatory dispersion measurements were made on solutions of the various polymers having different GAM content at different pH and urea concentrations. The b_0 and a_0 values were calculated from the rotatory dispersion data by means of the equation of Moffitt and Yang (1956), assuming 212 mμ for λ_0. The helical content of the copolymers is proportional to the $-b_0$ value. However, b_0 values of -625, -700, and -520 have been assigned to the completely helical conformation and 0 to $+100$ for the random coil (Blout and Idelson, 1958; Shechter *et al.*, 1964; Nagasawa and Holtzer, 1964). Because of uncertainty of the direct correlation of b_0 with helical content, the relative values of b_0 are stressed.

In the data reported in Table II, no additional salt was added to the polymer solutions beyond that produced during pH adjustment in order to observe maximum helix formation under conditions permitting solubility. Fasman *et al.* (1964) demonstrated that increased ionic strength shifts the helix–coil transition of GA-containing copolymers to lower pH. In limited studies in this laboratory, GA–GAM polymers in solutions containing 0.2 M NaCl exhibited less negative values of b_0 as compared to those in a water solution containing only minimal salt. Small variations in reproducibility and departures from trends may be due to slight differences in ionic strength of the solutions.

Both increase in pH and increase in GAM content of the copolymers diminished the tendency of the polymers to assume a helical conformation, as shown in Figure 4. The electrostatic repulsion, due to increased charge at higher pH, not only increased solubility by diminishing intermolecular association, but also caused the helical structure to unfold. Also illustrated in Figure 4 is the helix–random-coil transition with pH for PGA, first demonstrated by Idelson and Blout (1958). The data of Figure 4 illustrates that the introduction of uncharged glutamine groups reduces the relative concentration of charged residues per polymer molecule at any given pH (Figure 2). However, the polymers having larger contents of glutamine residues exhibited less tendency to form helices as indicated by optical rotatory dispersion measurements made at identical pH (Figure 4).

The effect of addition of urea to the polymer solution at various pH values upon polymer conformation was also investigated. At levels where urea was effective in dissolving the polymer, it only slowly unfolded its helical structure. As shown in Figure 5, urea was most active in disrupting the secondary structure of polymers

richest in GAM residues. At pH 3.8, a polymer containing only 10% GAM had a high helical content as evidenced by b_0. Initially, the addition of urea in concentrations up to 6 M did not decrease the helical content of the polymer; however, on standing for 4 and 24 hr, the helical content decreased. The extent of this time-dependent unfolding was greater with higher urea concentrations, but even after 24 hr in 6 M urea the polymer was not completely unfolded. The 25% GAM polymer in pH 3.8 aqueous solution had a high helical content, but upon addition of only 2 M urea immediate unfolding occurred (Figure 5). Upon standing for 24 hr in 4 M urea, the secondary structure in the polymer appeared to be highly disrupted. The b_0 of the 40% GAM copolymer was determined at pH 5.2 since it was not possible to dissolve this polymer in aqueous solution at lower pH. In 2 M urea the b_0 immediately became more positive (Figure 6), and upon standing in 8 M urea, b_0 approached 0. Data in Table II contain additional examples which demonstrate that susceptibility to disruption of polymer secondary structure by urea is increased by higher content of amide side-chain groups.

Even low amounts of γ-glutamyl ester residues in the polymer, together with GA and GAM moieties, promoted increased helical content of the polymers; *e.g.*, a polymer containing 20% GAM, 6% MG, and 74% GA when solubilized in 0.1 M urea at pH 5.2 and at 1 M urea at pH 4.2 had b_0 values more negative than that of copolymers of GAM and GA, having slightly larger (25%) or smaller (10%) GAM content in the same solvent (Table II).

The a_0 values of the various polymer solutions, calculated from rotatory dispersion data by the Moffitt and Yang (1956) equation, are tabulated in Table II. The a_0 values appear to become less negative with increases in amide content of polymers maintained at the same pH. When the pH is reduced and carboxyl ionization is suppressed, a_0 increases as does helical content and polymer insolubility.

Discussion

The introduction of a high level of GA residues into polymers containing varying amounts of GAM and γ-glutamyl esters produced a series of polypeptides soluble in neutral aqueous solutions and exhibiting a wide range of properties. Polymers similar in chemical composition and physical properties were prepared by different chemical pathways, establishing that the properties were due to residue composition rather than to any extraneous side reaction such as racemization. Solution of the polymers was primarily due to the electrostatic repulsion of the polymer segments by negative charges upon ionization. The repulsion force not only overcame noncovalent intermolecular bonds favoring aggregation, but also tended to disrupt the helical conformation of the polymers.

The incorporation of GAM residues into the predominantly GA polymers decreased the water solubility of the polymers. This decrease was true even when the solubilities of the different polymers were compared at pH values at which the same fraction of residues in each polymer was ionized. Since the chain length of the polymers was similar, molecular size was not a factor in solubility differences. All the amino acids were derived from γ-glutamyl esters so that the side-chain aliphatic moieties were identical and their hydrophobic interactions similar. Thus, solubility differences between polymers must be due primarily to the variations in the side-chain terminal functional groups.

It was observed previously (Krull *et al.*, 1965) that copolymers of GAM and γ-glutamyl esters exhibited decreased helical content in nonaqueous solutions as their GAM content increased. Incompatibility of the hydrophilic amide groups for the nonaqueous solvent was one suggested factor for the decline in helical content. However, a similar change in the secondary structure is observed with the GAM–GA copolymers in aqueous systems. The molecular size of the polymers and the steric structure of the GAM side chains were probably conducive to helical conformation (Bloom *et al.*, 1962). The reduction of helical content of the polypeptides, in aqueous solution, as the GAM content increases may be due to the association of the side-chain amide residues with other amide groups both intra- and intermolecularly.

The changes in a_0 that occur when more GAM residues are introduced into the copolymers, or when the pH of polymer solution is changed, are consistent with the observations of Tanford *et al.* (1960) and Harrap and Stapleton (1963). They found that the a_0 of the polymer solution decreased with an increasing polarity of the environment of the peptide group which accompanies molecular unfolding. Increasing values of a_0 also have been shown to occur with molecular aggregation (Herskovits *et al.*, 1964).

Retention of even small amounts of γ-glutamyl ester groups on the polymers produced considerable changes in solubility and conformation of the polymer in aqueous solution, all of which may be attributed to hydrophobic influences. The presence of ester groups reduced the solubility of the polypeptides in water at a given pH to a far greater extent than a comparable number of GAM residues. Nonpolar side-chain groups in the polymers also stabilized the helical structure even in the presence of urea.

Even in low concentrations, urea was effective in solubilizing GAM-containing polymers at low pH in aqueous solutions, possibly by disrupting intermolecular amide associations which cause aggregation of the molecules. But the action of urea at low concentrations in disrupting helical content was less pronounced and much slower. Evidently, the hydrogen bonds between amides of the peptide backbone, which forms the helical conformation, are more numerous and less accessible; perhaps these bonds are more resistant to disruption by urea because they are protected by the hydrophobic sheath of the side-chain methylenes.

In the presence of urea, the pH at which polymers containing hydrophobic ester groups go into solution was higher than the pH which solubilized copolymers

consisting solely of GAM–GA. The helix in polymers with ester groups was also more stable to low levels of urea than that of ester-free polymers. The hydrophobic groups strengthened the aggregation and secondary structure of the polypeptides against the disassociating action of urea.

These results indicate that the hydrogen bonds and hydrophobic bonds act synergistically to maintain molecular structure and to induce aggregation at the isoelectric point. Disturbing either type of bond may render the molecular associations sufficiently unstable so that the solvating action of water may overcome the remaining interactions. This phenomenon is consistent with observations by Beckwith *et al.* (1963) on changes in wheat gliadin protein solubility when some of the numerous glutamine residues of gliadin were converted to γ-methyl glutamyl esters. It is concluded that side-chain amide groups, when sufficiently numerous in polypeptides or proteins, may associate even in aqueous systems and contribute to protein insolubility and influence conformation.

References

Beckwith, A. C., Wall, J. S., and Dimler, R. J. (1963), *Arch. Biochem. Biophys. 103*, 319.

Bloom, S. M., Fasman, G. D., de Lozé, C., and Blout, E. R. (1962), *J. Am. Chem. Soc. 84*, 458.

Blout, E. R. (1962), *in* Polyamino Acids, Polypeptides, and Proteins, Stahmann, M. A., Ed., Madison, University of Wisconsin Press, p 275.

Blout, E. R., and Idelson, M. (1958), *J. Am. Chem. Soc. 80*, 4909.

Bohak, Z., and Katchalski, E. (1963), *Biochemistry 2*, 228.

Bruckner, V., Kovács, J., and Kovács, K. (1953), *J. Chem. Soc.*, 1512.

Doty, P., Bradbury, J. H., and Holtzer, A. M. (1956), *J. Am. Chem. Soc. 78*, 947.

Doty, P., Wada, A., Yang, J. T., and Blout, E. R. (1957), *J. Polymer Sci. 23*, 851.

Fasman, G. D., Lindblow, C., and Bodenheimer, E. (1964), *Biochemistry 3*, 155.

Goodman, M., and Schmitt, E. E. (1960), *Ann. N. Y. Acad. Sci. 88*, 669.

Green, M., and Stahmann, M. A. (1955), *J. Biol. Chem. 213*, 259.

Harrap, B. S., and Stapleton, I. W. (1963), *Biochim. Biophys. Acta 75*, 31.

Herskovits, T. T., Townend, R., and Timasheff, S. N. (1964), *J. Am. Chem. Soc. 86*, 4445.

Idelson, M., and Blout, E. R. (1958), *J. Am. Chem. Soc. 80*, 4631.

Krull, L. H., Wall, J. S., Zobel, H., and Dimler, R. J. (1965), *Biochemistry 4*, 626.

Lenormant, H., Boudros, A., and Blout, E. R. (1958), *J. Am. Chem. Soc. 80*, 6191.

Moffitt, W., and Yang, J. T. (1956), *Proc. Natl. Acad. Sci. U. S. 42*, 596.

Nagasawa, M., and Holtzer, A. (1964), *J. Am. Chem. Soc. 86*, 538.

Shechter, E., Carver, J. P., and Blout, E. R. (1964), *Proc. Nat. Acad. Sci. U. S. 51*, 1029.

Tanford, C., De, P. K., and Taggart, V. G. (1960), *J. Am. Chem. Soc. 82*, 6028.