THE OPTICAL ROTATORY AND VISCOMETRIC PROPERTIES OF γ-POLY-D-GLUTAMATE*

HAROLD EDELHOCH AND ROLAND E. LIPPOLDT

Clinical Endocrinology Branch, National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, Md. (U.S.A.)

(Received March 21st, 1960)

SUMMARY

The optical rotatory and viscometric properties of the γ-linked polypeptide, γ-poly-D-glutamate, are shown to be markedly dependent on the ionic state of the α-carboxyl side chain group. From the influence of various positively charged ions on the specific rotation of γ-poly-D-glutamate the relative order of their binding has been deduced: $H^+ > Pb^{++} > Cd^{++} > Zn^{++} > Ba^{++} \gg$ guanidinium $\sim Na^+$. The binding of the heavy metals follows quite closely their affinity constants to the carboxylate group in simple aliphatic acids. Optical rotational and viscometric evidence is reported which indicates that the un-ionized polypeptide exists in a hydrogen-bonded hyper-coiled form in water.

INTRODUCTION

In recent years considerable effort has been expended in trying to correlate the optical rotatory properties of α-polypeptides, native and denatured proteins with their molecular structure[1-3]. Since the asymmetric carbon atom of the peptide residue forms part of the backbone of the polypeptide chain, changes in optical rotatory behavior will reflect principally modifications in the helical configuration of the polypeptide chain[4,5]. For obvious reasons most interest has been focused on the rotatory properties of α-linked polypeptides. We have examined, however, the optical characteristics of a naturally occurring γ-linked polypeptide in order to compare the behavior of these two types of linkages in polyamino acids.

The γ-linked polypeptide of glutamic acid has been shown to exist in a randomly coiled structure when un-ionized[6,7]. Upon ionization the molecule undergoes considerable expansion as a result of intramolecular electrostatic repulsive forces. It has been demonstrated that α-polypeptides can exist in either a flexible chain or a helix, depending on environmental variables[1,8]. It was of interest, therefore, to determine the behavior of a γ-linked polypeptide in solvents which can lead to the formation of either type of structure.

Abbreviations: GP, γ-poly-D-glutamate; DCA, dichloroacetic acid; EDC, ethylene dichloride.

* A preliminary report of the optical rotatory aspects of this paper has been published by the present authors in this journal[41].

In addition we have explored the viscosity changes that occur in some of the solvents in order to ascertain whether any correlation exists between rotation and viscosity in a polypeptide which cannot form an α-helix. In certain proteins, *e.g.* serum albumin[9], ovalbumin[10–12], γ-globulin[13,14], and α-polypeptides[15], changes in rotation are paralleled by those in viscosity while in other proteins such as RNAse in H_2O–chloroethanol solutions[16] or gelatin in various neutral salt solutions[17] little or no relationship was found.

METHODS AND MATERIALS

We are greatly indebted to Dr. C. B. THORNE for all samples of γ-poly-D-glutamate. This material was obtained from the capsule of *B. anthracis* and consists of only D-glutamic acid[18] linked through the γ-carboxyl and α-amino group[19,20]. Unless otherwise stated all the data was obtained with a preparation No. 74. This material has been characterized by physical methods and has a molecular weight of 250,000 as determined by sedimentation and diffusion. In acid the sedimentation pattern shows a single peak with considerable spreading. The material is therefore heterodisperse with a mean sedimentation value in acid, at zero protein concentration, of 5.8 S.

Lithium bromide was decolorized with norite and then recrystallized several times. Guanidine hydrochloride was also recrystallized. The remaining salts were reagent grade and were used directly.

Viscosities were measured in an Ostwald viscometer held in a fixed position in a water bath regulated to ± 0.01°. Kinetic energy corrections were considered insignificant and were not applied. The specific viscosity, ηsp., was defined as $(t - t_0)/t_0$, where t and t_0 are the flow times in the solution and solvent respectively. The concentration, c, is in g/100 ml. All solutions were centrifuged at 20,000 × g for 30 min.

Optical rotation and rotatory dispersion measurements were performed with a Rudolph Model 80 photoelectric polarimeter. Measurements of the temperature coefficients were made initially with a Keston polarimeter attachment to the Model DU Beckman spectrophotometer. Several of the curves were verified subsequently in a thermostatted Rudolph polarimeter.

All the optical rotational data conformed to a single term Drude equation. The critical wavelength (λ_c) was determined from the slope of a plot of $[\alpha]\lambda^2 = [\alpha]\lambda^2_c + k$. Owing to the high molecular weight of preparation No. 74, solutions were fairly turbid and measurements could only be made with satisfactory precision between 700 and 400 mμ. A few experiments were made with another preparation (No. 75), of lower molecular weight ($s^0_{20,w} = 2.8$) where dispersion data could be obtained to wavelengths of ~ 350 mμ. The rotational constants of this preparation agreed with those of No. 74 in all respects. The observed rotational values have been corrected for the refraction of the solvent to that of water by the Lorentz factor $\frac{(n^2_w+2)}{(n^2_s+2)}$ and specific rotations are stated on the basis of the weight of the Na salt of GP. The temperature was between 23 and 25°.

At pH values below ~ 2.0 GP is completely un-ionized and this form will be described as HGP. At pH values above ~ 7 GP is 100% neutralized and will be referred to as NaGP. When evaluating properties of GP at intermediate levels of ionization, the degree of ionization will be explicitly stated. The degree of ionization was determined by a potentiometric titration curve of GP under conditions identical

with those cited for the particular property under investigation. Potentiometric titration curves of GP, at several ionic strengths, have been reported elsewhere[6].

The optical rotatory properties of GP

Effect of pH in salt-free solutions: The specific rotational values of GP from pH 2.9 to 7.2 in salt-free solution are illustrated in Fig. 1. When the specific rotation was plotted in accordance with the Drude equation ($[\alpha]\,\lambda^2$ *vs.* $[\alpha]$) linear relationships were always found. The curves in Fig. 1 are theoretical and are based on values of λ_c and k determined from Drude plots of the experimental data which are shown by the points. Values of λ_c less than ~ 200 mμ, especially when coupled with small values of $[\alpha]$, were particularly difficult to determine precisely. Two illustrative examples of the dependence of $[\alpha]$ on smaller values of λ_c, *i.e.*, $i100$ mμ and 0 are shown in Fig. 1. In the solid line at pH 3.60, λ_c was equal to $i140$ mμ. The k values have been adjusted so that all three curves intersect at 500 mμ*.

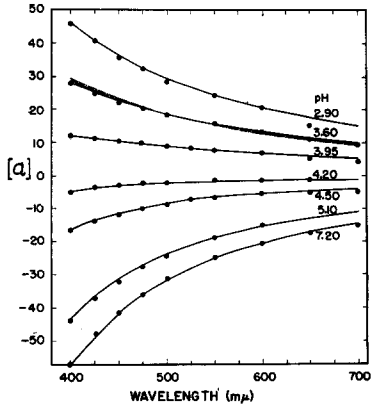

Fig. 1. The rotatory dispersion of GP as a function of pH. The curves are theoretical and based on the simple form of the Drude equation. The points are experimental. The dashed curve at pH 3.6 employed a value of $\lambda_c = i100$ instead of $i140$ as in the full curve. The dash-dot curve is for $\lambda_c = 0$. GP $\cong$ 1 %. Temperature, 23 to 25°.

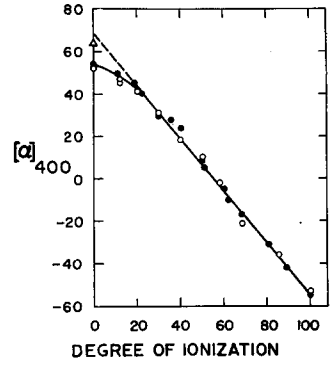

Fig. 2. Variation of specific rotation (at 400 mμ) with the degree of ionization of GP; ●, preparation No. 74; ⊙, No. 75. △, represents the optical rotation of HGP in 6.0 *M* guanidine from pH 2.2 to 1.5.

When the values of $[\alpha]$ at 400 mμ were plotted against the degree of carboxyl ionization a linear dependence was observed from 100 to 20 % ionization. At smaller values of ionization $[\alpha]$ increased somewhat less rapidly, as seen in Fig. 2.

Though $[\alpha]$ varied smoothly with ionization, values of the Drude constant, λ_c, showed an abrupt transition between ~ 50 and 60 % ionization. The values of λ_c became imaginary when $[\alpha]$ changed sign. The variations in λ_c with degree of ionization are shown in Fig. 3. Above 60 % ionization λ_c values are in the range of those

* The significance of the imaginary values of the Drude Dispersion constant, λ_c, is not apparent. It is perhaps more realistic to consider the Drude equation as an empirical formula where imaginary values of λ_c are observed. It is possible that some small systematic error (perhaps due to an optically active absorbing impurity) has distorted the data to produce the imaginary value of λ_c. On the other hand a slightly modified Drude equation could readily eliminate the imaginary value of λ_c.

reported for native and denatured proteins. Below 30 % ionization the Drude constant was indistinguishable from zero. Between 30 and 60 % it was imaginary. The abrupt change observed in λ_c between 50 and 60 % ionization has not been seen in any other property of GP evaluated to date.

Effect of ionic strength (NaCl): The rotatory constants for NaGP in NaCl solutions between 0 and 5.0 *M* are compiled in Table I. With the exception of the data for 5 *M* NaCl the values are similar to those reported for denatured proteins. The increase in rotation that occurs at high concentrations of NaCl is in the same direction as observed by reducing the degree of ionization of NaGP with acid. Therefore the influence of NaCl on the rotation of GP can be explained by the binding of Na^+ ions. The binding of Na^+ by synthetic polyelectrolytes is a well known phenomenon[21-23]. In contrast to the influence of NaCl on NaGP, the rotatory dispersion curve of HGP was unaffected by 1.0 *M* NaCl.

Urea, guanidine and LiBr: Both urea and guanidine are well known denaturing reagents[5,10-12]. As seen in Table I, 8.0 *M* urea produced very little alteration whereas

TABLE I

OPTICAL ROTATORY PARAMETERS OF NaGP IN VARIOUS SOLVENTS

Solvent	*Molarity*	$[\alpha]_{400}$	λ_c *(mμ)*
NaCl	0	—56°	220
	0.01	—55	211
	0.10	—48	208
	1.00	—21	204
	5.00	+14	~150
Urea	8.0	—35	208
Guanidine	3.0	+21	~0
	6.0	+36	170
LiBr	8.0	—15	280
		$[\alpha]_{425}$	
DCA		—30	270
DCA–EDC (53:47)		—19	270

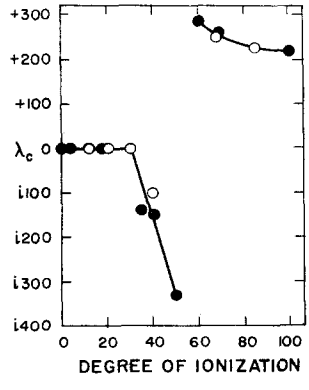

Fig. 3. Variation of the Drude dispersion constant (λ_c) with the degree of ionization of GP; ●, preparation No. 74; ⊙, No. 75.

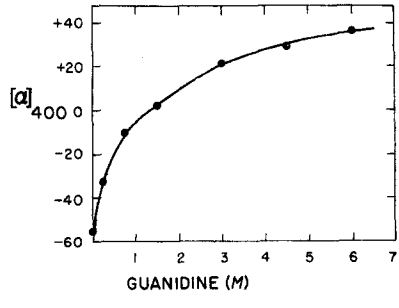

Fig. 4. Effect of guanidine on the specific rotation (at 400 mμ) of NaGP. NaGP concentration was 1%.

3.0 and 6.0 *M* guanidine had marked effects on the rotatory constants of NaGP. Indeed as shown in Fig. 4 an increase of 92° was observed in $[\alpha]_{400}$ in 6.0 *M* guanidine. The specific rotations were found to be independent of NaGP concentration (from 1.00 to 0.25 % NaGP) in 0.25 *M* and 4.5 *M* guanidine*.

When GP was uncharged, 6.0 *M* guanidine increased its specific rotation by 10°. A value of 68 ± 2° was obtained from pH 2.2 to 1.5 in 6.0 *M* guanidine solutions. This value of $[\alpha]$ is close to that which would be observed if the linear portion of the curve shown in Fig. 2 were extrapolated to zero degree of ionization. In this pH range HGP tends to become turbid in water, indicating a limited solubility. In 6.0 *M* guanidine HGP solutions were quite clear. The specific rotation of HGP was independent of concentration, from 0.25 to 1.0 %, in water or in 5.0 *M* guanidine at pH 1.75.

It has been shown by HARRINGTON AND SCHELLMAN[25] that moderate concentrations of LiBr, by reducing the activity of water, tend to enhance the enthalpy of a hydrogen bond and thereby promote hydrogen bonding in proteins. Although a γ-linked polypeptide, such as GP, cannot form a Pauling-Corey α-helix, it can engage in extensive intramolecular hydrogen bonding either between the backbone CO and NH groups, side chain carboxyl groups, or backbone and side chain groups.

HGP was soluble (1 % solutions were slightly opalescent) in 4.8 *M* LiBr and quite insoluble in 8.0 *M* LiBr. However, NaGP was readily soluble in 8.0 *M* LiBr and insoluble in 9.6 *M* LiBr. In 8 *M* LiBr the specific rotation of NaGP was −15° and λ_c was 280 mμ. The directional change of both these values, when compared to their values in water (pH ~7), resemble those found in α-polypeptides, native and denatured proteins in non-interacting solvents[1,25]. When NaGP in 8 *M* LiBr was acidified to a point just prior to precipitation (about 25 % of the acid needed to neutralize all the charged groups was added) $[\alpha]$ increased to + 8° and λ_c was *i*150 mμ. The rotational properties of HGP in 4.8 *M* LiBr were similar to those observed in water.

Non-aqueous solvents: YANG AND DOTY[1] have shown that certain organic solvents are good hydrogen-bond-breaking reagents while others, by providing a non-interacting milieu, tend to strengthen hydrogen bonds. Thus, by working with mixtures of DCA and EDC the α-helical content of a protein or polypeptide, as reflected by changes in both [α[and λ_c, may be diminished or augmented by varying the proportions of the two solvents. NaGP was quite soluble in DCA. Since DCA is a much stronger acid than glutamic, GP should be un-ionized in this solvent. The rotatory constants reported for DCA in Table I, however, are quite different from those found for HGP in water or even in 5 *M* guanidine solutions. When EDC was added to a DCA solution to a point just short of precipitating the GP (DCA–EDC 53:47), not much change was observed in either $[\alpha]$ or λ_c (see Table I).

Since alcohol (ethyl) has a considerable influence on the viscosity of HGP[26], its effect on the rotatory properties was also investigated. In both 25 and 50 % mixtures of methyl alcohol and water (v/v) the dispersion curves were unchanged from that observed in water.

* If we assume that the change in specific rotation of NaGP is proportional to the amount of guanidine bound, then at about 1 *M* guanidine half the sites are occupied. As a crude approximation this results in $K_A \cong 1$. TANFORD[24] found that the affinity constant (K_A) of acetate for guanidine is < 0.5. Electrostatic effects could readily account for the difference in constants.

The viscometric properties of GP

Effect of ionization: The dependence of the viscosity of GP on its charge, at several ionic strengths is shown in Fig. 5. The viscosity increased monotonically with the degree of ionization. At all three ionic strengths an initial lag occurred in the early stages of ionization. Subsequently the viscosity increased almost linearly until ionization was complete.

The concentration and ionic strength dependence of the viscosity of NaGP: The effect of NaGP and NaCl concentration on the specific viscosities of NaGP is illustrated in Fig. 6. Similar data for HGP appears in Fig. 7. It is evident that even 5 *M* NaCl fails to reduce the viscosity and hence the dimensions of NaGP to that of HGP. With other polyelectrolytes 1 *M* salt usually suffices to bring the viscosity of the ionized form close to that of the un-ionized molecule[27,28].

In Fig. 8 both the intrinsic viscosity and specific rotation of NaGP are plotted as a function of NaCl concentration. It is evident that the intrinsic viscosity and optical rotation depend on NaCl concentration in quite different ways. In low salt concentrations the viscosity falls rapidly as ionic strength increases whereas the optical rotation shows but little variation. In high salt concentrations the rotation changes considerably while the viscosity is much less affected.

Urea, guanidine and LiBr: It might be postulated that the very large values found

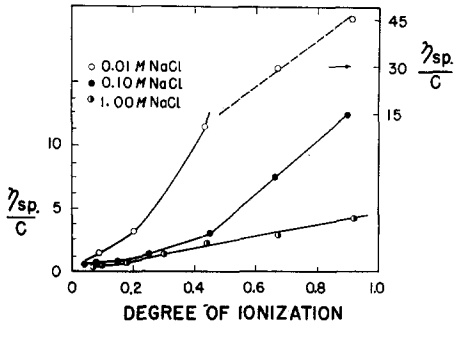

Fig. 5. Influence of the degree of ionization on the specific viscosity of GP at several ionic strengths. GP ≅ 0.125%. Temperature, 28.0°.

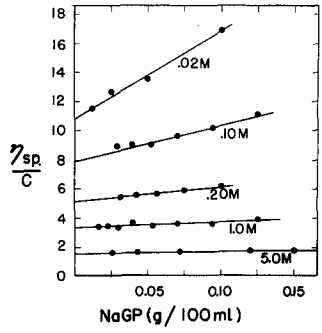

Fig. 6. Concentration dependence of specific viscosity of NaGP at several ionic strengths. Temperature, 28.0°.

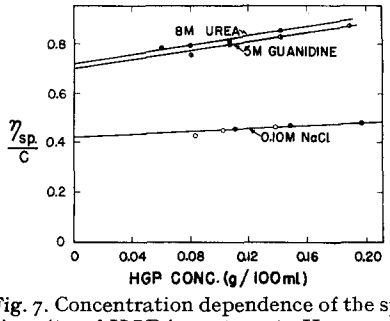

Fig. 7. Concentration dependence of the specific viscosity of HGP in water (at pH 1.9 and 1.55) and in 8 *M* urea and 5 *M* guanidine, at pH 1.55.

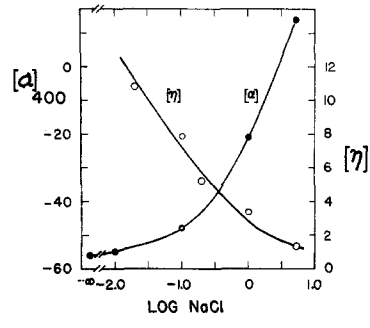

Fig. 8. Effect of NaCl on the specific rotation and intrinsic viscosity of NaGP.

for the intrinsic viscosity of NaGP (in 0.1 *M* NaCl) might be due to intramolecular hydrogen bonding between charged carboxylate and neighboring NH groups. Ring formation of this type would result in a reduction in rotational freedom with con-

TABLE II

INTRINSIC VISCOSITY OF GP IN VARIOUS SOLVENTS

Form of GP	*Molarity Solvent*	*pH*	*[η]*
HGP	0.10 NaCl	1.90	0.41
	0.10 NaCl	1.55	0.41
	5.0 Guanidine	1.55	0.70
	8.0 Urea–0.10 NaCl	1.55	0.72
NaGP	0.10 NaCl	7.0	7.9
	8.0 Urea–0.10 NaCl	7.0	10.0
	8.0 LiBr		0.66

siderable stiffening of the polypeptide chain. In order to check on this possibility the viscosity of NaGP (in 0.10 *M* NaCl) was determined in 8 *M* urea. In this solvent the intrinsic viscosity was close to 10 and thus somewhat greater than the value of 7.9 found in the absence of urea. Therefore intramolecular hydrogen bonding would not appear to be of significance to the structure of NaGP. Guanidine is usually more effective, at comparable concentrations, than urea in rupturing hydrogen bonds. Since guanidine at neutral pH values is a cation it would contribute to the ionic strength of the solution and therefore would not be likely to provide as unambiguous a result as urea.

It is possible that the disparity in viscosity between NaGP in strong salt and HGP results from hypercoiling in HGP due to random intramolecular hydrogen bonding. This possibility was tested therefore by measuring the viscosity of HGP in urea and guanidine. As seen in Fig. 7 there was about a 50 % increase in the viscosity of HGP in either 8.0 *M* urea or 5.0 *M* guanidine. Since both reagents produced comparable effects it would appear that the increase in viscosity resulted from the rupture of intramolecular hydrogen bonds which cross-link the linear polypeptide.

The viscosity of NaGP in 8 *M* LiBr was only somewhat greater than that observed for HGP in dilute acid. Since GP is still partially ionized in this solvent the low value of its intrinsic viscosity is particularly noteworthy.

The effect of temperature on optical rotation

In a random coil polymer an increase in temperature will enhance the rotational freedom of the molecule which should result in a decrease in the numerical value of the specific rotation[29]. The influence of temperature on the rotatory behavior of GP in several salt solutions is shown in Fig. 9. The decrease in rotation that occurs with HGP (in water or 0.10 *M* NaCl) at elevated temperatures is consistent with a structure which is not constrained to a specific configuration. An increase in rotation would be expected if intramolecular hydrogen bonds were broken at higher temperatures. However, this effect could be eclipsed by the negative temperature coefficient of rotation found for coiled polymers.

The increase in levorotation observed with NaGP in either 0.01 *M* or 5.0 *M* NaCl was unforeseen. A similar curve displaced by about 20° above the 0.01 *M* NaCl, was obtained with 90 % ionized GP in 0.10 *M* NaCl. The increase in 5.0 *M* NaCl,

however, may be a reflection of an increase in charge resulting from a reduction of Na^+ binding by GP at the higher temperatures.

In comparison with the other curves shown in Fig. 9 that of NaGP in 8 *M* LiBr was quite distinctive in that it showed only very minor changes in rotation with temperature. In this respect NaGP behaved in much the same way as α-linked polypeptides and proteins in this salt. According to HARRINGTON AND SCHELLMAN[25] this lack of a temperature coefficient is due to the greatly enhanced strength of hydrogen bonded groups which tends to lock the molecule into a specific configuration which is resistant to thermal agitation at the temperatures normally studied.

The interaction of heavy metals with NaGP

Though very high concentrations of NaCl were required to reduce significantly the viscosity of NaGP, certain heavy metallic ions were very effective at rather low concentrations. The influence of several of these cations on the viscosity of NaGP is illustrated in Fig. 10. In order to keep the ionic strength approximately constant, all solutions contained either 0.10 *M* NaCl or KNO_3. The data for all four curves extend close to the region where GP becomes insoluble. It is evident that all of these salts are vastly more efficient than NaCl in reducing the viscosity and hence the effective volume of GP. In fact the relative effectiveness of the four cations shown in Fig. 10 parallels their relative binding affinities for the carboxylate anion in simple carboxylic acids[30].

Since the optical rotation of GP has been found to vary regularly with the binding of protons it was not surprising to find that it also increased uniformly with

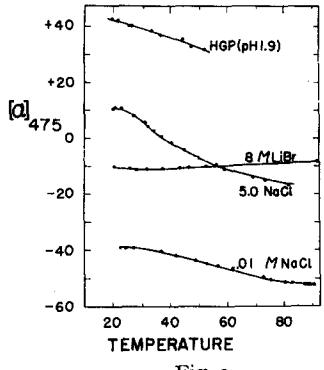

Fig. 9

Fig. 9. Temperature dependence of the specific rotation of GP at 475 mμ. GP concentration ≅ 1.0%.

Fig. 10. Effect of heavy metal salts on the specific viscosity of NaGP. All solutions contained 0.10 *M* KNO_3. Reagents were $Pb(NO_3)_2$, $Cd(NO_3)_2$, $ZnSO_4$, $BaCl_2$. NaGP ≅ 0.098%, Temperature, 28.1°.

Fig. 11. Effect of heavy metal salts on the specific rotation of NaGP. Reagents were the same as used in Fig. 10. The lead and zinc solutions contained 0.10 *M* KNO_3. The cadmium and barium solutions contained 0.10 *M* NaCl. NaGP ≅ 1.0%. Temperature, 23–25°.

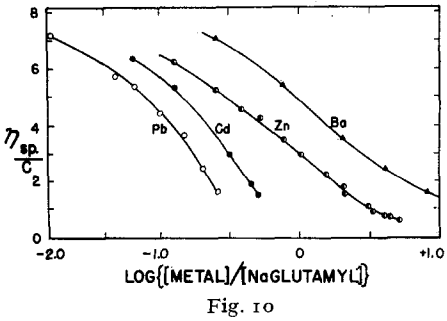

Fig. 10

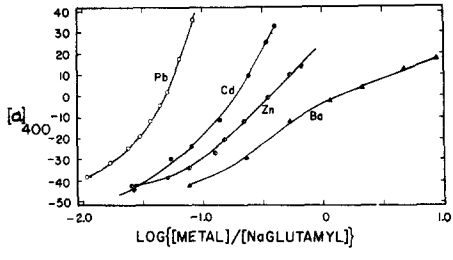

Fig. 11

the binding of heavy cations. The influence of Cd^{++}, Zn^{++} and Ba^{++} on the specific rotation paralleled their effect on the viscosity of NaGP. However, Pb^{++} appears to be more efficacious in modifying the rotatory than the viscosity properties. As a measure of the relative effectiveness of the metallic ions we have listed in Table III the amount of metal needed to increase the specific rotation from —48° to 0°. The affinity constants for the carboxylate anion are also shown. It is apparent that the relative influence of the metal corresponds quite well with its affinity for the simple carboxylate anion. This correspondence would tend to preclude any large effect of the peptide backbone on the interaction of GP with the investigated cations.

The binding of these ions to NaGP has been demonstrated also by potentiometric and electrophoretic methods[26].

TABLE III

CORRELATION BETWEEN THE AFFINITY OF CERTAIN METAL SALTS TO ACETATE WITH THAT TO NaGP

Salt	*log (M⁺⁺/GP)**	*Δ*	*log K*	*Δ¹*
$Pb(NO_3)_2$	—1.32		2.0	
		0.59		0.7
$Cd(NO_3)_2$	—0.73		1.3	
		0.30		0.27
$ZnSO_4$	—0.43		1.03	
		0.58		0.64
$BaCl_2$	+0.15		0.39	

* This expression indicates the amount of heavy metal ion (M^{++}) required to increase the specific rotation of NaGP from —48 to 0 at 400 mμ. GP refers to the glutamyl residue of molecular weight 129. The concentration of NaGP was close to 1%. Δ shows the numerical difference in the logarithmic function between neighboring salts. Δ^1 is the difference in the logarithm of the affinity constants between neighboring salts. K is the affinity constant of the metal ion for the carboxylate group and is taken from Table VII of GURD AND WILCOX[30].

DISCUSSION

It is clear from the continuous change in specific rotation with degree of carboxyl ionization that GP shows rotatory properties at variance with that of α-polypeptides and proteins. In the latter cases optical rotatory values are usually fairly independent of moderate changes in ionization[31] unless structural changes occur simultaneously[9–14]. It is well known that the specific rotation of amino acids and small polypeptides is markedly influenced by the charged state of the ionogenic groups[32]. However, in high polymeric α-polypeptides, composed of single amino acids, the influence of terminal groups rapidly vanishes as the number of residues becomes large and consequently the mean residue rotation becomes constant[3,33]. In GP the situation is peculiar since the ionizable side chain carboxyl groups are located α to the asymmetric carbon atom and consequently are the predominant influence in determining the rotatory behavior of GP in aqueous solvents. In proteins the charged groups, other than terminal, and with the exception of aspartic acid which is β, are never closer than the γ position to the asymmetric center. Hence their influence on the asymmetric carbon atom will be diminished materially.

The influence of metal ions, which are known to bind strongly to carboxylate anions, has demonstrated that the relative effectiveness of these ions in increasing the

specific rotation of NaGP is indeed related to their binding constants. The binding of cations can therefore influence the rotational properties of GP in much the same way as the binding of protons. The rather large amounts of Na^+ required to increase the rotation (to some arbitrary level), when compared with the relatively small amounts of strongly bound ions, attests to the weakness of its binding to GP. That guanidine is also bound to the carboxylate group is evident from the fact that urea, which shows comparable denaturing ability, has only a small effect on the dispersion characteristics of NaGP. Though the changes in $[\alpha]$ observed in NaCl and guanidine solutions are comparable to those produced by changes in ionization, the values of λ_c vary for identical values of $[\alpha]$. This is not surprising when one considers the chemical differences between the bound ions. Appreciable differences in the effects of salts on the optical rotatory properties of amino acids have been noted[34].

The binding of protons and cations to NaGP therefore is measurable by changes in optical rotation. It is apparent that the charge on the anionic GP molecule will be reduced as a consequence. Since the dimensional properties of polyelectrolytes are a function of net charge as well as ionic strength[35-37] the binding of oppositely charged ions should be reflected by modifications in the viscometric and other colloidal solution properties[21-23].

In dilute salt solutions strongly bound ions will attenuate the viscosity of NaGP by their influence on the net polymeric charge whereas weakly bound ions will depress the viscosity principally by contributing to the ion atmosphere. Consequently viscosity and rotatory changes will approximately parallel each other when they result from a change in charge as with the heavy metal salts investigated. On the other hand when viscosity changes ensue from a build-up in the ion cloud, as with weakly bound ions such as NaCl and guanidine salts, major viscometric changes can occur without concomitant effects on optical rotatory behavior.

The fact that the linear relationship between specific rotation and degree of ionization extends over most (20–100 % ionization) but not all of the curve, suggests that some structural factor may be influential in modifying the rotational properties in the first 20 % of ionization of GP. A similar disparity is evident in the viscosity curves shown in Fig. 5. The rise in viscosity that occurs between 0 and 20 % ionization is decidedly less than the change observed in a similar portion of the remainder of the curve. In fact, above ~ 30 % ionization there is a linear increase in specific viscosity with ionization whereas, below, it is convex towards the abscissa. KATCHALSKY[38] has reported similar viscometric behavior with synthetic α-polyaspartic acid. He attributed the initial lag in viscosity to the inability of the electrostatic field produced by the low degree of ionization to overcome intramolecular cohesive forces due to hydrogen bonding between segments of the polymeric chain. If this type of cross-linking is appreciable, the configuration of GP at low degrees of ionization would assume that of a hypercoiled form. As the charge increases, the electrostatic repulsive forces outweigh the cohesive forces and the molecule expands rapidly. The expansion now depends on the balance between electrical stretching forces and Brownian contractile forces.

The increase in viscosity of HGP found in urea and guanidine solutions suggests an unfolding of the HGP molecule which depends on the rupture of hydrogen bonds. The failure of the specific rotation at low values of ionization, to fit the linear relation observed above 20 %, suggests a gradual modification in the structure of GP. As the net

charge of GP is reduced by acid, the nature of the solute–solvent interaction is altered in accord with the concept of water acting as a good solvent at high degrees of ionization and as a poor solvent below ~20%. Direct thermodynamic support is provided by light scattering data of the fully ionized and un-ionized forms of GP. In the absence of salt the second virial coefficient is very large for NaGP and very small for HGP[6]. When HGP was dissolved in a good solvent, such as a 5.0 *M* guanidine solution, the specific rotation increased to the value HGP presumably would have if water had remained an effective solvent for HGP. Similar conclusions have been reached by SILBERBERG *et al.*[39], who have presented evidence that (un-ionized) polymethacrylic acid is hypercoiled in dilute HCl solutions as a result of intramolecular hydrogen bonding between carboxyl groups. MOREWETZ[40] has presented data on styrene–methacrylic acid copolymers in organic solvents which shows that the intrinsic viscosity is inversely related to the degree of hydrogen bonding.

The optical rotatory and solubility behavior indicate that NaGP in 8 *M* LiBr is partially ionized. From the rotational data it cannot be ascertained with any large degree of confidence whether the observed changes are due to charge neutralization effects or to other characteristics of LiBr. When GP is 65% ionized both $[\alpha]$ and λ_c in water are about the same as the values observed for NaGP in 8 *M* LiBr. The properties of the acidified 8 *M* LiBr solution corresponds approximately to GP at 50% ionization.

The similarity in rotatory constants does not however carry over to the viscometric behavior. The intrinsic viscosity of NaGP in 8 *M* LiBr is substantially less than that of 65% ionized GP even when in 1 *M* NaCl. It would appear therefore that intramolecular hydrogen bond formation is enhanced in 8 *M* LiBr. The indifference of $[\alpha]$ to temperature in this solvent lends further support to this point of view. Consequently it seems reasonable to assume that both HGP in water and NaGP in 8 *M* LiBr have hypercoiled forms. They do not however approach the highly condensed forms of many globular proteins.

REFERENCES

1 J. T. YANG AND P. DOTY, *J. Am. Chem. Soc.*, 79 (1957) 761.
2 P. DOTY AND R. D. LUNDBERG, *Proc. Natl. Acad. Sci., U.S.*, 43 (1957) 213.
3 C. SCHELLMAN AND J. A. SCHELLMAN, *Compt. rend. trav. lab. Carlsberg, Sér. chim.*, 30 (1958) 463.
4 D. D. FITTS AND J. K. KIRKWOOD, *Proc. Natl. Acad. Sci., U.S.*, 43 (1957) 1046.
5 J. A. SCHELLMAN, *Compt. rend. trav. lab. Carlsberg, Sér. chim.*, 30 (1958) 363, 395, 415, 429, 439, 450.
6 H. EDELHOCH AND J. B. BATEMAN, *J. Am. Chem. Soc.*, 79 (1957) 6093.
7 L. H. KENT, B. R. RECORD AND R. G. WALLIS, *Phil. Trans. Roy. Soc., London, Ser. A*, No. 972, 250 (1957) 1.
8 J. APPLEQUIST AND P. DOTY, *Abstracts, Am. Chem. Soc. Meeting, San Francisco, April 7-11, 1958.*
9 J. F. FOSTER AND J. T. YANG, *J. Am. Chem. Soc.*, 76 (1954) 1588.
10 R. B. SIMPSON AND W. KAUZMANN, *J. Am. Chem. Soc.*, 75 (1953) 5139.
11 H. K. FRENSDORFF, M. T. WATSON AND W. KAUZMANN, *J. Am. Chem. Soc.*, 75 (1953) 5157.
12 F. S. STEVENS AND G. R. TRISTRAM, *Biochem. J.*, 73 (1959) 86.
13 B. JIRGENSONS, *Arch. Biochem. Biophys*, 48 (1954) 154.
14 J. T. YANG AND J. F. FOSTER, *J. Am. Chem. Soc.*, 77 (1955) 2374.
15 P. DOTY, A. WADA, J. T. YANG AND E. R. BLOUT, *J. Polymer Sci.*, 23 (1957) 851.
16 R. E. WEBER AND C. TANFORD, *J. Am. Chem. Soc.*, 81 (1959) 3255.
17 W. F. HARRINGTON, *Nature*, 181 (1958) 997.
18 W. E. HANBY AND H. N. RYDON, *Biochem. J.*, 40 (1946) 297.
19 V. BRUCKNER, J. KOVACS AND G. DENES, *Nature*, 172 (1953) 508.
20 W. J. WILLIAMS AND C. B. THORNE, in W. D. MCELROY AND B. GLASS, *Amino Acid Metabolism*, The Johns Hopkins Press, Baltimore, 1955, p. 107.

[21] J. R. HUIZENGA, P. F. GRIEGER AND F. T. WALL, *J. Am. Chem. Soc.*, 72 (1950) 2636.
[22] F. T. WALL AND R. H. DOREMUS, *J. Am. Chem. Soc.*, 76 (1954) 1557.
[23] R. MACFARLANE, JR. AND R. M. FUOSS, *J. Polymer Sci.*, 23 (1957) 403.
[24] C. TANFORD, *J. Am. Chem. Soc.*, 76 (1954) 945.
[25] W. F. HARRINGTON AND J. A. SCHELLMAN, *Compt. rend. trav. lab. Carlsberg, Sér. chim.*, 30 (1957) 167.
[26] H. EDELHOCH AND R. E. LIPPOLDT, unpublished experiments.
[27] P. J. FLORY AND J. E. OSTERHELD, *J. Phys. Chem.*, 58 (1954) 653.
[28] N. S. SCHNEIDER AND P. DOTY, *J. Phys. Chem.*, 58 (1954) 762.
[29] W. KAUZMANN AND H. EYRING, *J. Chem. Phys.*, 9 (1941) 41.
[30] F. R. N. GURD AND P. WILCOX, *Advances in Protein Chemistry*, 11 (1956) 311 (Table VII).
[31] B. JIRGENSONS, *Arch. Biochem. Biophys.*, 39 (1952) 261; *Arch. Biochem. Biophys.*, 74 (1958) 70.
[32] G. TOENNIES, T. F. LAVINE AND M. A. BENNETT, *J. Biol. Chem.*, 112 (1936) 493.
[33] R. R. BECKER AND M. A. STAHMANN, *J. Am. Chem. Soc.*, 74 (1952) 38.
[34] J. LIQUIER-MILWARD, *Trans. Faraday Soc.*, 26 (1930) 390.
[35] A. KATCHALSKY, *J. Polymer Sci.*, 12 (1954) 195.
[36] F. E. HARRIS AND S. A. RICE, *J. Phys. Chem.*, 58 (1954) 725.
[37] S. A. RICE AND F. E. HARRIS, *J. Phys. Chem.*, 58 (1954) 733.
[38] A. KATCHALSKY, *J. Polymer Sci.*, 7 (1951) 393.
[39] A. SILBERBERG, J. ELIASSAF AND A. KATCHALSKY, *J. Polymer Sci.*, 23 (1957) 259.
[40] H. MORAWETZ, *J. Polymer Sci.*, 23 (1957) 247.
[41] H. EDELHOCH AND R. E. LIPPOLDT, *Biochim. Biophys. Acta*, 30 (1958) 657.