Macromolecules **1994,** *27,* 1455-1460

Polymers with Amino Acids in Their Side Chain: Conformation of Polymers Derived from Glycylglycine and Phenylalanine

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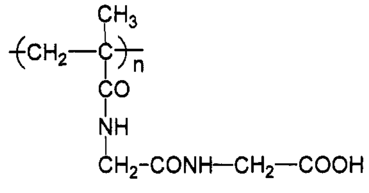
Received July 22, 1993; Revised Manuscript Received November 22, 1993[●]

ABSTRACT: Two polymethacrylamides bearing glycylglycine (PGlyGly) and phenylalanine (PPhe) in the side chain were synthesized by free radical polymerization of the corresponding monomers (general formulas: $-[CH_2-C(CH_3)-CO-NH-CH_2-CO-NH-CH_2-COOH]-_n$ and $-[CH_2-C(CH_3)-CO-NH-CH(COOH)-CH_2-C_6H_5]-_n$, respectively). PGlyGly behaves as a normal polyelectrolyte in relation with its hydrophilic nature. On the contrary PPhe takes a compact conformation in water. The free enthalpy of stabilization of this conformation is high as revealed by potentiometric titrations. The influence of some additives (metal ion, urea, organic solvent) on the compact conformation and the pH-induced conformational transition was investigated. It was concluded that the main factor responsible for the existence of this structure consists of hydrophobic domains with nonpolar interactions between the aromatic side chains.

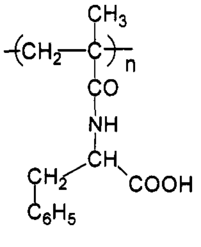
Introduction

In previous papers,[1-5] some polymers with various natural amino acids (Ala, Glu, Asp, Asn, Lys) in the side chain were synthesized in order to study their complexing properties toward divalent metal ions. The conformation of these polymers in water is that of normal polyelectrolytes, in relation with the hydrophilic nature of the amino acids. On the contrary, when using much more hydrophobic amino acids such as phenylalanine, for example, it is expected that the polymers could take a compact conformation in water, as poly(methacrylic acid) or copolymers containing hydrophobic residues, with possible effects on the metal coordination. This paper described the conformational properties of poly(*N*-methacryloyl-L-phenylalanine) in water as a function of ionization and the influence of some added molecules like neutral salts, metal ions, organic solvents, or urea. The behavior of this polymer is compared to that of a polymer derived from glycine–glycine, expected to have a normal behavior.



PGlyGly

PPhe



Experimental Section

Materials. The monomer *N*-methacryloyl-L-phenylalanine was prepared from L-phenylalanine and methacryloyl chloride according to a process already described[1] ($[\alpha]_D$ = +16.88° in water). It is soluble in water, dioxane, alcohols, and dimethyl sulfoxide (DMSO). The IR spectrum exhibits the usual bands characteristic of the amide group (1650 and 1520 cm^{-1}) and of the acid group (1730 cm^{-1}) and NH vibration at 3370 cm^{-1}. The polymerization was carried out in dioxane at 65 °C, using AIBN as the initiator. The polymer named thereafter PPhe was recovered by precipitation, filtered, and washed. PPhe was further purified by dialysis against dioxane and water using Visking dialysis tubing with a cut-off limit of about $(8-10) \times 10^3$ ($[\alpha]_D$ = +16.45° in water). The model molecule (MPhe) was prepared in the same way, using isobutyroyl chloride in place of methacryloyl chloride, and purified by recrystallization ($[\alpha]_D$ = +22.02° in water, mp = 110 °C). The same procedure was used for the synthesis of the monomer, polymer, and model molecule from glycine–glycine (PGlyGly and MGlyGly, respectively): monomer, mp 193 °C, soluble in water, alcohols, and DMSO; MGlyGly, mp 159 °C, soluble in water and alcohols.

All samples were also characterized by IR and ^{13}C NMR spectroscopy.

The weight average molecular weight of the polymers was determined by light scattering in dioxane solutions (M_w = 112 000 ± 10 000 for PGlyGly and M_w = 95 000 ± 10 000 for PPhe).

Methods. Preparation of the Solutions. As recovered from the polymerization, PPhe is not directly soluble in water. Thus the sample was first dissolved in aqueous sodium hydroxide and the solution was treated with a cationic exchange resin (Amberlite IR 120 in the acid form) by the batch method. Solutions prepared in this way looked very slightly turbid but were stable during many weeks in the refrigerator. The maximum polymer concentration attainable by this method was about 4×10^{-3} $mol \cdot L^{-1}$. To check the absence of intermolecular aggregates, light scattering was done on PPhe solutions in dioxane and in water at low pH. No significant difference was found between the M_w values in these two solvents, indicating that no intermolecular aggregation occurs at low pH in water. Solutions of PGlyGly or the model molecules were prepared in the usual way by weighing and dissolution in the appropriate amount of solvent. In all cases, the exact concentration of the solutions was determined by conventional acid–base titration, monitored by potentiometry (in 0.5 M $NaClO_4$) or conductometry (no added salt), and expressed on the basis of repeat units.

Potentiometric titrations were performed under dry nitrogen flow, using a Radiometer pHM 64 pHmeter equipped with a Schott N 65 combined electrode. Standardization was done with phthalate (pH 4.01) and borate (pH 9.18) buffers.

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[●] Abstract published in *Advance ACS Abstracts,* February 15, 1994.

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In the pH range where the compact to coil transition occurs for PPhe, the stabilization of the pH needed a few minutes and further addition of the titrant was delayed unit a constant value of pH.

Viscosity measurements were performed at 25 ± 0.01 °C with a Schott AVS 400 viscosimeter. Ubbelohde tubes of diameter ranging between 0.84 and 0.53 mm were used in order to adjust the flow time t_0 of the solvent to about 100 s. No kinetic energy correction was necessary. The reduced viscosity was calculated from $(t - t_0)/(t_0 c)$.

Conductometric titrations were done with a Tacussel CDRV 62 conductometer fitted with a double platinum electrode.

Azobenzene solubilization measurements were carried out in order to estimate the ability of the compact structure of PPhe to solubilize water-insoluble nonpolar compounds in its hydrophobic domain. An excess of azobenzene crystals (about 50 mg) was added to 5 mL of the polymer solution ($C = 30 \times 10^{-3}$ mol^{-1}) in a small flask, at room temperature. The mixture was left about 1 week, under shaking, to ensure equilibrium. Then it was carefully filtered with Millipore filters (0.1 μm pore diameter) and the spectrum of the aqueous solution was recorded. Then the aqueous solution was diluted with an equal amount of ethanol, allowing the unfolding of the compact conformation, and the absorption spectrum was recorded. This procedure was also applied to a blank mixture in the absence of polymer to make the necessary corrections for the solubility s of azobenzene in pure water. The result was $s = 4.6 \times 10^{-5}$ mol·L^{-1} in agreement with previous works.[6] The solubility Sp of azobenzene in the presence of polymer was expressed in moles of azobenzene per equivalent of polymer.

Spectrophotometric measurements were made at $\lambda = 316$ nm with a Kontron Uvikon 930 spectrophotometer. Calculations were made using $\epsilon = 16\,500$ L·mol^{-1}·cm^{-1} for the molar absorption coefficient of azobenzene in the water/ethanol mixture.

Absorption spectra of pure polymer solutions as a function of pH were also recorded in order to study the effect of the compact conformation on the absorption properties of the phenyl group of PPhe.

The surface tension of polymer solutions was determined at room temperature with a Dognon Abribat tensiometer (Prolabo, France) using the method of pulling out a platinum thin plate from the sample solution.

Fluorescence measurements were made with a Perkin-Elmer LS 50 luminescence spectrophotometer equipped with 1-cm cells.

Light scattering (Macrotron, France) and specific refractive index measurements (Brice Phoenix) were made at $\lambda = 5460$ Å.

Results and Discussion

The reduced viscosity of the two polymers versus ionization is plotted in Figure 1. Though the two polymers do not have the same molecular weight, the way in which their viscosities change upon ionization can be compared. The ratio between the maximum and minimum values for PPhe is about 40 against 2.5 for PGlyGly. This fact and the very low absolute value for PPhe at $\alpha = 0$ suggest that a peculiar conformation exists for this polymer at low pH. Light scattering measurements which give close M_w values in water and dioxane indicate that intermolecular aggregation does not occur. Thus the low viscosity value is likely due to intramolecular interactions between the phenyl groups.

The existence of a compact conformation in the case of PPhe can also been demonstrated using potentiometric titrations. In Figure 2 are plotted the titration curves of PPhe, PGlyGly, and the model molecule MPhe in solutions of ionic strength 0 and 0.1 M in NaClO$_4$. When PGlyGly is titrated in pure water or in the presence of salt (Figure 2a), the pH increases regularly, but in the latter case the pH at a given α value is always lower. Thus, PGlyGly behaves as a stronger acid in the presence of salt (shielding effect). At a given α value the difference in pH of the two solutions can reach 1.5 pH units.

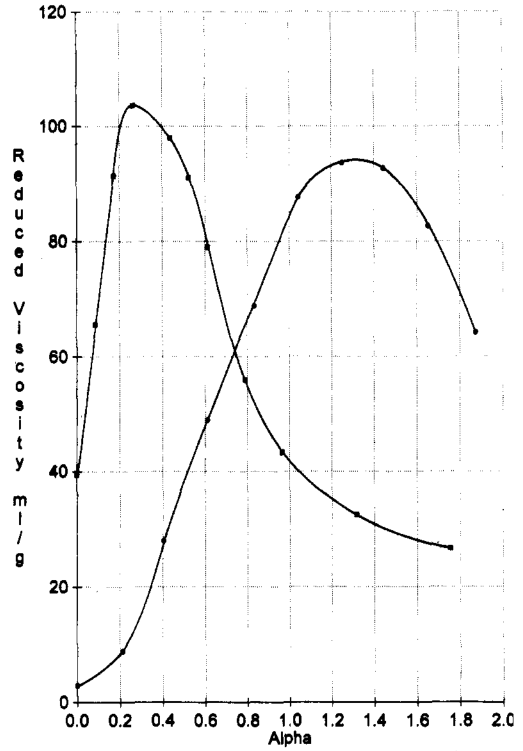

Figure 1. Variation of the reduced viscosity of 3×10^{-3} mol·L^{-1} solutions of PGlyGly (0.6 g·L^{-1}) (■) and PPhe (0.7 g·L^{-1}) (●) versus the neutralization coefficient α.

The titration curve of PPhe in the absence of salt is very different (Figure 2b). In this case, the pH increases strongly in the first part of the titration curve, between $\alpha = 0$ and 0.2. This is typical of the titration of a very tight compact conformation in which the cohesive forces (hydrophobic interactions between the phenyl groups) keep the carboxyl groups close together thus increasing the pK. Beyond $\alpha = 0.2$, the electrostatic repulsions begin to exceed the cohesive forces and the compact conformation begins to unfold with a decrease of the pK. At this stage, the titration curve looks like that of PGlyGly. Nevertheless, the pH is always higher for PPhe, reflecting the presence of the phenyl groups which decrease the local dielectric constant and increase the pK of the carboxyl groups. In the presence of salt, the pH is lower as for PGlyGly and the conformational transition occurs later (around $\alpha = 0.3$). The titration curve of the model molecule MPhe is not affected by the presence of salt, as expected (Figure 2c), and no special behavior is observed in the range $\alpha = 0$ to 0.3. The titration curves of MGlyGly are quite similar to those of MPhe.

The data of potentiometric titrations were plotted as Henderson–Hasselbach graphs using the general equation.[7-9]

$$\mathrm{pH} = \mathrm{p}K_{1/2} + n \log[\alpha/(1-\alpha)] \qquad (1)$$

where p$K_{1/2}$ and n are constants for a given polymer concentration and ionic strength. These two parameters reflect the strength of the electrostatic interactions between the ionizable groups and thus are dependent on the ionic strength and on the conformation of the polymer. In the case of a small molecule p$K_{1/2}$ is equal to pK_0 and $n = 1$. Figure 3 shows typical H–H plots for PGlyGly and PPhe in the absence of salt. With PGlyGly, a straight line is obtained as expected. For PPhe, on the contrary, the H–H plot consists of three parts corresponding to the titration of the compact conformation at low α values (high

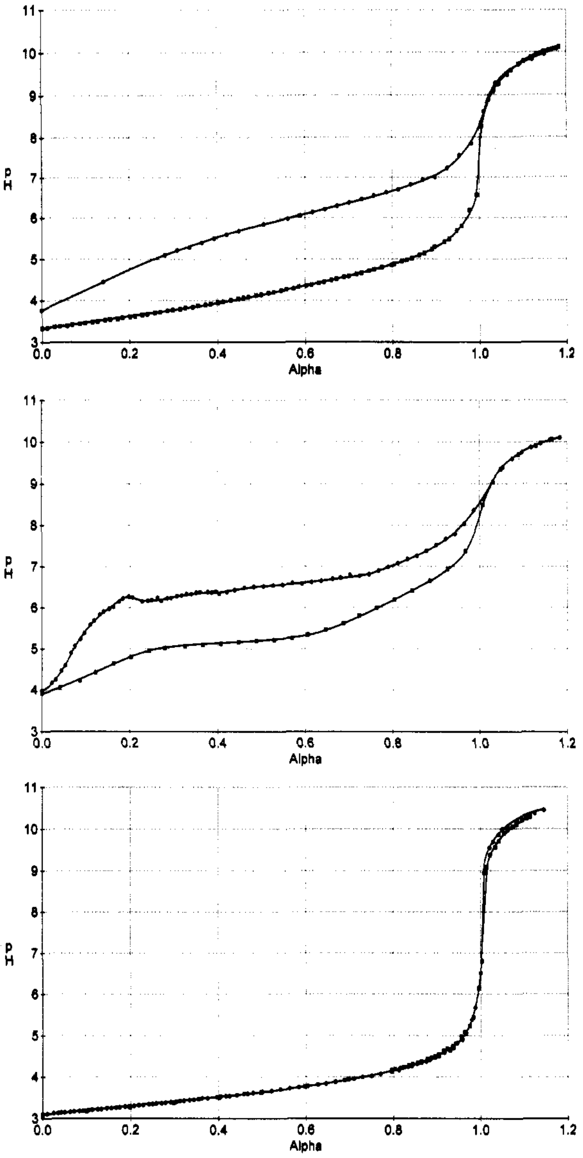

Figure 2. Titration curve in pure water (●) and $NaClO_4$ 0.1 M (■): (a) PGlyGly (0.6 g·L^{-1}); (b) PPhe (0.7 g·L^{-1}); (c) MPhe (0.7 g·L^{-1}).

log values), to the titration of the extended conformation at high α values, and to a transition region between $\alpha = 0.16$ and $\alpha = 0.75$ where the conformational transition occurs. The transition domain is large and indicates the importance of the cohesive forces.

The n and p$K_{1/2}$ values for the different polymers and model molecules at different ionic strength are collected in Table 1. Literature data taken from previous works on polymers derived from alanine and aspartic and glutamic acids are also given.[5] First it has been checked that the n values for the model molecules are close to unity as expected. Their p$K_{1/2}$ value is slightly lower in 0.1 M $NaClO_4$ due to the salt effect. The p$K_{1/2}$ values for the two polymers are much higher than those of the model molecules due the interactions between the charged groups and these values are decreased, as expected, in the presence of salt. The values for PGlyGly are close to those of PAla which has also only one carboxyl group in each side chain. The value for PPhe is higher and similar to those of PGlu and PAsp which both have two carboxyl groups in their side chain instead of one. This is obviously due to the conformation of PPhe and could indicate that the average distance between the carboxyl groups belonging to two adjacent side chains of PPhe is nearly the same as the distance between two groups of the same side chain in PAsp or PGlu. The values of n for PGlyGly are somewhat lower than for PAla due to the difference in the length of the side chain. For PPhe, the n value of the compact conformation in pure water is intermediate between the corresponding values for PAla and PAsp or PGlu and decreases by addition of salt. The H–H plot for PPhe in the presence of salt gives a transition domain ranging from $\alpha = 0.26$ to 0.58, i.e. much narrower than in pure water ($\alpha = 0.16$ to 0.75; $\Delta\alpha = 0.59$).

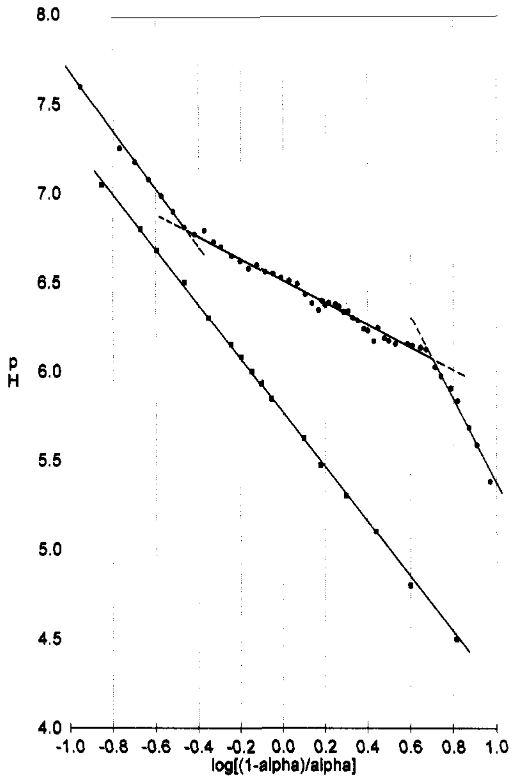

Figure 3. Henderson–Hasselbach plot for PGlyGly (0.6 g·L^{-1}) (■) and PPhe (0.67 g·L^{-1}) (●) in pure water.

Table 1. p$K_{1/2}$ and n Values for Compact and Extended Conformations of Various Polymers

	$I = 0$			$I = 0.1$ M $NaClO_4$		
sample	n_c	p$K_{1/2}$	n_e	n_c	p$K_{1/2}$	n_e
MAla					3.57	1
MGlu					4.05	1
MAsp					4.05	1
MGlyGly		3.65	0.99		3.55	1
MPhe		3.65	0.97		3.62	0.97
PAla[a]		5.75	1.85		4.44	1.39
PGlu[a]		6.7	2.55		5.05	2.34
PAsp[a]		6.7	1.7		5.05	2.49
PGlyGly		5.82	1.72		4.15	1.22
PPhe	2.2	6.52	1.5	1.45	5.2	2.26

[a] Reference 5.

An other usual way to plot the data of potentiometric titrations of polyelectrolytes is by calculating the apparent pK, pK_{app}[10,11]

$$\mathrm{p}K_{app} = \mathrm{pH} - \log[\alpha/(1-\alpha)] = \mathrm{p}K_0 + 0.434\,F(\alpha)/kT \quad (2)$$

where pK_0 is the intrinsic dissociation constant of one ionizable group and $F(\alpha)$ a function of α reflecting the electrostatic interactions and depending on conformation.

Figure 4 shows typical curves p$K_{app} = f(\alpha)$ for PGlyGly and PPhe in pure water. The curve for PGlyGly steadily increases from about 5 to 6, whereas the curve for PPhe is more complex reflecting the conformational transition between $\alpha = 0.18$ and 0.75 in agreement with the H–H

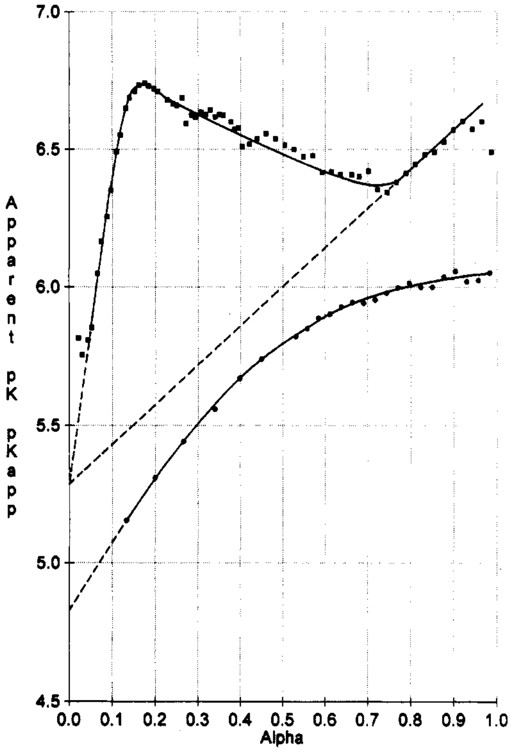

Figure 4. Variation of the apparent pK versus α for PGlyGly (0.6 g·L^{-1}) (●) and PPhe (0.7 g·L^{-1}) (■) in pure water.

Table 2. Values of ΔG at 298 K for Different Polymers and Copolymers Having a Compact Structure at low pH

polymer[a]	ΔG, cal/mol
poly(methacrylic acid)[b]	170
methacrylic acid/benzyl methacrylate (75/25) copolymer[c]	175
methacryloylalanine/phenylmethacrylamide (27/63) copolymer[d]	670
maleic acid/butyl vinyl ether alternating copolymer[e]	308
maleic acid/hexyl vinyl ether alternating copolymer[e]	1130
PPhe	650 ± 30
PPhe in 7.2 M urea	640 ± 30
PPhe in 0.1 M NaClO$_4$	620 [illegible] 30

[a] In pure water, unless otherwise indicated. [b] Reference 14. [c] Reference 15. [d] Reference 15. [e] Reference 16.

plots in Figure 3. The unitary free enthalpy ΔG of the conformational transition between the uncharged forms of the polymer can be obtained from the plot of pK_{app} versus α using the relation[12,13]

$$\sum_{\alpha=0}^{\alpha=1} (\mathrm{p}K_{app} - \mathrm{p}K_{coil})\, d\alpha = 0.434\Delta G/RT \qquad (3)$$

where pK_{coil} is the apparent dissociation constant of the coil conformation. ΔG may be determined graphically from the area A between the experimental curve (Figure 5) and the extrapolated curve corresponding to the hypothetical titration of the coil; i.e. $\Delta G = 2.3RTA$.

Values of ΔG for PPhe as determined in this way are reported in Table 2.

The value found for ΔG in pure water is rather high if compared to data of literature concerning polymers in compact conformation.

According to Tanford[17] and Crescenzi,[18] the ΔG value can be considered as the sum of the contributions of the main chain Δg_1, the hydrophilic parts of the side chain Δg_2, and the hydrophobic part of the side chain Δg_3. The side chain of PPhe contains one amide, one CH, one CH$_2$ and one phenyl group more than the side chain of poly(methacrylic acid) (PMA). The contribution of one methylene or methyl group to the stability of the compact conformation has been estimated to +400 cal/mol (favorable contribution)[16] and that of the amide group to −1100 cal/mol (unfavorable).[1] From these data, the contribution of a phenyl group can be estimated to about +800 cal/mol, that is only about 2 times that of a methylene group. The ΔG value is nearly insensitive to the temperature (ΔG = 630 ± 30 at 323 K). The entropy change ΔS during the conformational transition is nearly zero. The enthalpy change ΔH is about +650 cal/mol, of the same order as the enthalpy of transfer of a benzene molecule to an aqueous solution (+500 cal/mol).[19]

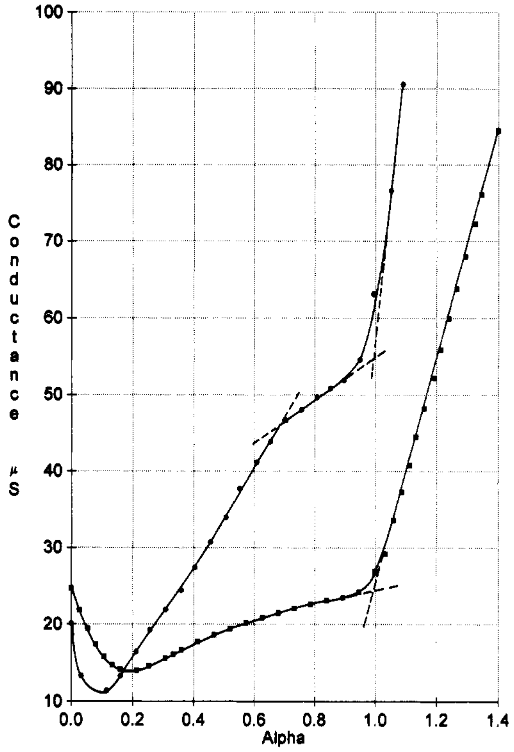

Figure 5. Conductometric titrations of PGlyGly (0.6 g·L^{-1}) (■) and PPhe (0.7 g·L^{-1}) (●) in pure water.

The existence of a conformational transition for PPhe can be shown by other methods, among which is conductometric titration.[20] Figure 5 gives the titration curves of PGlyGly and PPhe. In the first case, the conductance increases regularly up to the end point except at the very beginning of the curve which is typical of the titration of a weak acid. For PPhe, on the contrary, a distinct break is seen around α = 0.68 at the end of the conformational transition. The slope decreases, indicating a more expanded conformation, in agreement with viscosimetric data.

Dubin and Strauss[16] have shown that the hydrophobic domains of compact conformations are able to dissolve compounds that are otherwise insoluble or very sligtly soluble in water. They have demonstrated, for example, that the solubility of Yellow OB is linearly increasing with the length of the alkyl side chain in alternating copolymers of maleic acid and alkyl vinyl ethers. In a previous work,[15] we have shown that, on the contrary, the solubility of azobenzene in copolymers of methacrylic acid and benzyl methacrylate decreases when the amount of benzyl groups increases. In the case of PPhe, the solubility Sp at low pH, corrected for the blank (see Experimental Section) is equal to 7.3 × 10^{-3} mol of azobenzene/repeat unit, which

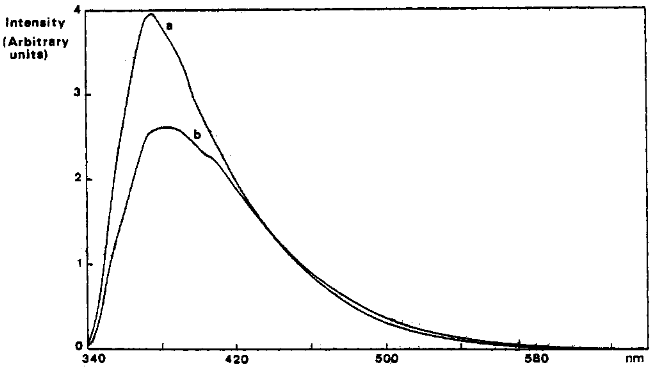

Figure 6. Fluorescence spectra of PPhe at low (a) and high pH (b).

Table 3. Surface Tension γ in dyn·dm^{-1}

medium	pH 4.3	pH 10.0
water	71.9	71.9
PGlyGly C = 8 × 10^{-3} mol/L	67.7	65.6
PPhe C = 3 × 10^{-3} mol/L	62.1	59.8

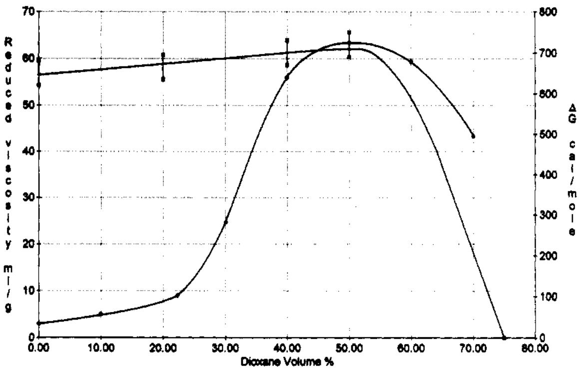

Figure 7. Reduced viscosity change of un-ionized PPhe upon addition of dioxane (left scale) (●) and variation of the free enthalpy ΔG of conformational transition for PPhe with the composition of the water/dioxane mixture (right scale) (■).

is a rather high value compared to literature data. In the high pH range where the conformation is unfolded, the solubility decreases to 2 × 10^{-3} but is not null, indicating that some interaction remains between azobenzene and the phenyl groups. For poly(methacrylic acid), on the contrary, the solubility in basic medium is zero. These data indicate that rather large hydrophobic domains exist in PPhe.

Fluorescence spectra of PPhe in the compact and extended conformation were also recorded (Figure 6). The intrinsic fluorescence intensity of the phenyl group is affected by pH, indicating a higher mobility of this group in the extended conformation. In addition, the shift of the emission wavelength to higher value on increasing pH shows that the phenyl groups are exposed to a more polar environment upon unfolding of the conformation.

In Table 3 are reported the surface tension values γ for PGlyGly and PPhe in acidic medium. The decrease of γ compared to pure water is larger for PPhe in relation with its hydrophobic nature.

If it is assumed that the compact conformation of PPhe is stabilized by hydrophobic interactions between the side chains, the compact conformation should be destabilized, even destroyed by the addition of an organic solvent. Figure 7 shows the variation of the viscosity of un-ionized PPhe upon addition of dioxane. The shape of the curve is similar to that in Figure 1 and indicates that the addition of an organic solvent is also able to unfold the compact conformation. From viscosimetric measurements, it appears that about 50% of dioxane in the solvent mixture is needed for this purpose.

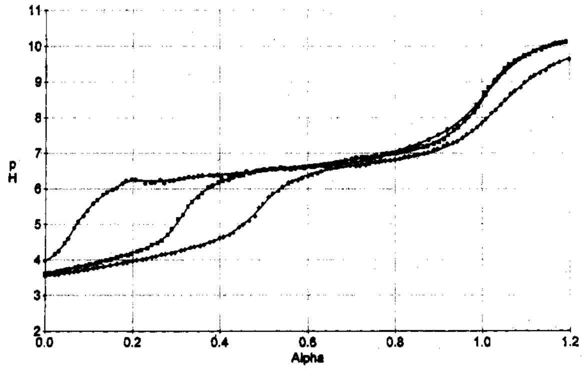

Figure 8. Potentiometric titrations of PPhe in the presence or absence of metal ion: (●) no metal; (■) with copper, [P]/[Cu] = 8.6; (◆) with copper, [P]/[Cu] = 4.9.

Potentiometric titrations have been done using water/methanol or water/dioxane mixtures of various compositions as the solvent. In each case, the free enthalpy of transition ΔG was determined (Figure 7). In the first part of the curve, the value of ΔG is not very sensitive to the presence of dioxane (ΔG = 670 cal/mol in 50 vol % dioxane) and then decreases very rapidly to be nearly zero in a solvent containing 75 vol % of dioxane. This behavior is very different from that of other polymers in compact conformation for which ΔG decreases nearly linearly with the amount of organic solvent.[1,21]

In dioxane-rich solutions, the plot of pK_{app} versus α looks like that of PGlyGly in water; i.e. no transition is observed.

The above results support the assumption that the compact conformation of PPhe is stabilized by hydrophobic interactions. Nevertheless a contribution of hydrogen bonds to the stabilization of this conformation cannot be excluded. These hydrogen bonds could bind amide and carboxyl groups. To check this, potentiometric titrations were also carried out in the presence of increasing amounts of urea. Urea is known to affect the way in which solute–solute and solute–water hydrogen bonds are formed. In fact, the value of ΔG is unaffected by the presence of urea (640 cal/mol in 7.2 M urea). This is a confirmation that the stabilization of the compact conformation is mainly due to hydrophobic interactions as indicated above.

The unfolding of the compact conformation occurs when the electrostatic repulsions overcome the cohesive forces. Thus a decrease of the overall electrostatic interactions should stabilize the compact conformation and delay the transition. It has been shown above that addition of a neutral monovalent salt has this effect. Addition of a divalent metal ion which will form a 1:2 complex with the carboxylate groups is expected to further stabilize the compact conformation. The potentiometric titration in the presence of increasing amounts of copper clearly shows that the transition is shifted to higher α values (Figure 8).

This shift is in proportion of the concentration of metal ion and calculations indicate that a 2:1 COO/Cu complex is formed in the lower pH range. The decrease of pH at a given α value indicates that formation of this complex makes easier the ionization of the carboxyl groups. Another interesting feature is that the equivalent point is increased on increasing copper concentration. This indicates the titration of protons other than those of the carboxyl groups and suggests that the deprotonation of the amide group occurs. A detailed study of the nature of the complexes existing in these systems will be the subject of a further paper.

The above results clearly show that the stabilization of the compact conformation of PPhe at low pH has its origin in the hydrophobic interactions between the aromatic side chains. We are currently studying polymers derived from other amino acids. Preliminary results show that, with methionine for example, a peculiar conformation also exists at low pH, but in this case the mechanism of stabilization seems to be quite different.

Acknowledgment. The authors thank Mrs. Anne-Marie Caze for technical assistance during this work, Dr. A. Pollet for fluorescence measurements, and P. Fasquel for preliminary experiments.

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