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Preferential and Absolute Adsorption to Poly[N^5-(3-hydroxypropyl)-L-glutamine] in Water/2-Chloroethanol Solvent Mixtures

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ABSTRACT: Preferential adsorption to poly[N^5-(3-hydroxypropyl)-L-glutamine] (PHPG) in water/2-chloroethanol solvent mixtures has been determined by measuring density increments at constant concentration and at constant chemical potential. At low alcohol concentrations, decreasing preferential adsorption of 2-chloroethanol is observed. When the organic solvent content is increased, an inversion of preferential adsorption occurs, water being preferentially adsorbed. The inversion zone parallels the conformational transition curve. As an explanation it is proposed that a competition occurs between noninteracting helicogenic 2-chloroethanol, which stabilizes hydrophobic interactions, and the hydrophilic interactions of water with PHPG. The former retains a helical structure, whereas the latter induces a helix-to-coil transition.

It is well known[1-4] that the conformational transition in proteins and synthetic polypeptides is accompanied by changes in preferential and absolute adsorption onto the polymer in water/organic solvent mixtures. To elucidate the mechanisms responsible for the stabilization of protein conformation, it is common to study model synthetic polypeptides. The aim of this work is to study the conformational transition and the interactions of a nonionizable water soluble polypeptide, poly[N^5-(3-hydroxypropyl)-L-glutamine] (PHPG), in the water/2-chloroethanol mixed solvent system. The interactions of proteins with solvent components of mixed water/2-chloroethanol have been extensively studied:[1-6] 2-chloroethanol was shown to be a structure-forming denaturant solvent and was capable of interacting with proteins, probably by way of hydrophobic interactions with the aliphatic side chains. Thus, it seemed to us interesting to study preferential adsorption in the PHPG/water/2-chloroethanol system.

Such a study has been reported for the PHPG/water/dioxane system.[7] The authors indicated that an increasing hydration took place with increasing helicity of the polymer. Very high values of the helical content were not obtained and no preferential solvation of dioxane to the polymer was observed. In a study of the poly(L-glutamic acid)/water/dioxane system, it was noticed[8] that the structural transformation of the polypeptide was preceded by preferential solvation of dioxane and followed by increasing preferential hydration. In counterpart, 2-chloroethanol was shown to be a better helicogenic agent for poly(L-glutamic acid)[9] than dioxane.

Furthermore, we obtained higher values of the helical content of PHPG in water/2-chloroethanol mixtures. Therefore, 2-chloroethanol seems to be of much interest. Knowing that the side chain methylene groups of PHPG are responsible for stabilization of an α-helical secondary structure,[10-12] we expected important hydrophobic interactions enhancing the helical structure of PHPG.

Preferential interactions can be detected by a variety of methods: light-scattering measurements,[5] equilibrium sedimentation measurements,[6,13] isopiestic measurements of vapor pressure,[14] differential refractometry,[1,7,8,13] and density measurements.[13,15] For the water/2-chloroethanol system it is very convenient to use the latter method, and instruments now available allow easy and accurate measurements.

Theoretical

We use the Scatchard-Stockmayer[16,17] notation, in which the polymer is refered to as component 2, water as component 1 (which is considered as the principal solvent), and the organic solvent as component 3. At constant P and T, the preferential adsorption coefficient is related to the density increments $(\partial\rho/\partial c_2)_m{}^0$ and $(\partial\rho/\partial c_2)_\mu{}^0$, respectively at constant molality of component 3 and constant chemical potential of components 1 and 3, at vanishing polymer concentration, by the relation:[13,15,18,19]

$$\left(\frac{\partial g_3}{\partial g_2}\right)_\mu = \frac{(\partial\rho/\partial c_2)_\mu{}^0 - (\partial\rho/\partial c_2)_m{}^0}{1 - \bar{V}_3\rho_0} \qquad (1)$$

where $\bar{v}_3$ represents the partial specific volume of the organic solvent and ρ_0 is the density of the mixed solvents. The preferential adsorption coefficient $(\partial g_3/\partial g_2)_\mu$ represents the preferential binding of component 3 to the polymer, given in grams of component 3 to be added to the solution per gram of component 2, in order to maintain the constancy of the chemical potential of components 1 and 3. Thus, a negative value of $(\partial g_3/\partial g_2)_\mu$ signifies a deficiency of component 3 in the vicinity of the macromolecule, i.e., preferential hydration takes place.

Experimental Section

(A) PHPG Sample. The PHPG sample was prepared from poly(γ-benzyl L-glutamate) by aminolysis[10] with 3-amino-1-propanol and satisfied the purity tests proposed by Lupu-Lotan et al.[10] Viscosity measurements[10] indicate a weight-average molecular weight of 253 000.

(B) Optical Rotatory Dispersion and Circular Dichroism. Optical rotatory dispersion (ORD) measurements were performed on a Perkin-Elmer 141 polarimeter. Reduced residue rotations $[m']$ (deg cm^2 dmol^{-1}) were calculated from the expression

$$[m']_\lambda = \frac{3M_0[\alpha]_\lambda}{100(n^2+2)}$$

where M_0 is the residue molecular weight (186), $[\alpha]_\lambda$ the specific optical rotation defined as usual, n the refractive index of the solvent, and λ the wavelength. The rotatory dispersion parameter b_0 was calculated from the Moffitt and Yang[20] equation.

Circular dichroism (CD) spectra were recorded using a Jobin-Yvon R. J. Mark III dichrograph, flushed with high purity dry nitrogen, with a cell thickness of 0.01 cm. Concentrations of solutions ranged between 0.5 and 2 g/L. The residue ellipticity $[\theta]_\lambda$ was calculated from the relation

$$[\theta]_\lambda = \frac{3300M_0S\Delta A}{lC}$$

where C represents the polypeptide concentration, l the cell length, S the sensitivity of the recording, and ΔA the deviation (in mm) read on the spectrum. Throughout this paper the residue ellipticity $[\theta]_\lambda$ will be expressed in deg cm^2 dmol^{-1}. No precipitation occurred during the time interval needed to make the CD measurements.

Both CD and ORD data were obtained at 25 °C.

(C) Density Measurements. Densities were determined at 25 °C with a digital precision density meter, DMA 02C (Anton Paar, Graz, Austria). The principle of the measurement is the variation of the frequency of a hollow oscillator when filled with liquids of different density. Since this frequency is a function of the density of the liquid, variations of the frequency (or its reciprocal, the period T) can be related to variations in the density of various liquids. The density of an unknown liquid (ρ_1) is measured by reference to a known standard (ρ_2):

$$\rho_1 - \rho_2 = K(T_1^2 - T_2^2)$$

The instrument constant K is obtained from calibration measurements with samples of known density. The instrument constant was obtained by using air and deionized double-distilled water as known standards. The calibration was repeated for every experimental series. The instrument constant did not change significantly within the period needed to complete the density measurements. The cell compartment was maintained at 25 ± 0.01 °C by a Jouan type 01 PT 623 temperature controller.

Once the temperature was stabilized, the cell was washed with three aliquots of 5 mL each of deionized double-distilled water followed by three aliquots of 5 mL each of dry methanol. The cell was then air dried with the instrument pump. A sample was introduced into the cell when air gave a constant reading, which must be the same throughout the experimental series. Stabilization was deemed ready when at least five consecutive readings gave the same period within $\pm 1 \times 10^{-5}$ s at a preset count of 1×10^4. The sample was then removed and the cell washed and dried as described above. Readings with air as standard were run again and when this procedure yielded the same period as previously, the following sample was introduced.

Each experimental series consisted of measurements on the solvent mixture and on four polypeptide solutions within the concentration range of 5–22 mg/mL. Solvents were clarified through a Millipore filter Type LS, ϕ 5 μm, (Millipore Corp.). Solutions were made on a *g/g* basis in order to obtain well-defined values of the concentrations. Dialysis was performed by using dialysis bags (Union Carbide Co.) in vessels sealed with Parafilm wax (Marathon Division, American Can Co.) for a period of 20 h.

The experimental results consisted of: the density of the solvent ρ_0 and of the polypeptide solutions ρ as a function of the polypeptide concentration c_2 (in g/mL) at constant molality of component 3; and the density of the polypeptide solutions, ρ^*, as a function of c_2 at constant chemical potential of components 1 and 3, obtained by dialysis against the solvent mixture.

The density increments $(\partial\rho/\partial c_2)_m{}^0$ at constant molality and $(\partial\rho/\partial c_2)_\mu{}^0$ at constant chemical potential were calculated using the following relations[13]

$$\left(\frac{\partial\rho}{\partial c_2}\right)_m^{\;0} = \left(\frac{\rho - \rho_0}{c_2}\right)_{c_2\to 0}$$

$$\left(\frac{\partial\rho}{\partial c_2}\right)_\mu^{\;0} = \left(\frac{\rho^* - \rho_0}{c_2}\right)_{c_2\to 0}$$

assuming that densities of the solutions are a linear function of c_2, at low polypeptide concentration. Subscript $c_2 \to 0$ represents extrapolation at $c_2 = 0$ of $(\rho - \rho_0)/c_2$. Precision of the measured values of T is $\pm$ 1.10^{-5} s. The uncertainty in the values of the density increments is $\pm 10^{-2}$. But, compared with a second set of solutions, the reproductibility on the value of $(\partial g_3/\partial g_2)_\mu$ is ±10%. The latter is reported in the data plotted in Figure 3. Measurements could not be carried out at high 2-chloroethanol concentrations, precipitation occurring before achievement of dialysis.

Results and Discussion

(A) Conformational Transition of PHPG. In 2-chloroethanol, PHPG exhibits a rich helical content, as shown by the Moffit–Yang[20] parameter b_0 value obtained from the ORD data: $b_0 = -570$. The helical content f_N is given by the relation[10]

$$f_N = \frac{b_0}{b_0{}^H}$$

Assuming[21,22] that $b_0{}^H = -750$ for a fully right-handed α helix of high molecular weight PHPG, it yields: f_N = 76%. CD measurements confirm this result; the high value of the molar residue ellipticity at 222 nm ($[\theta]_{222}$ = 37 352) (Figure 1) indicates a rich helical content. Assuming that

$$f_N = [\theta]_{222}/[\theta]_{222}{}^H \qquad (2)$$

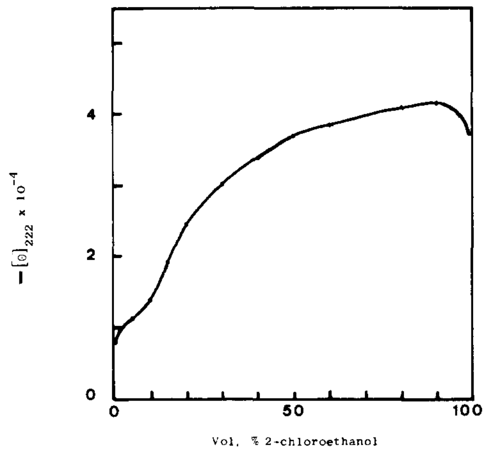

Figure 1. Conformational transition of poly[N^5-(3-hydroxypropyl)-L-glutamine] in water/2-chloroethanol solvent mixtures; molar ellipticity at 222 nm ($[\theta]_{222}$) vs. 2-chloroethanol concentration.

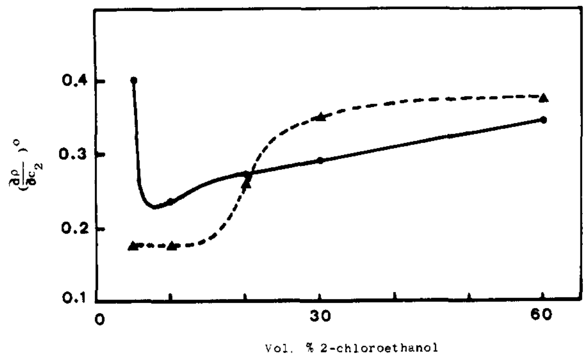

Figure 2. Variation of density increments of poly[N^5-(3-hydroxypropyl)-L-glutamine] vs. 2-chloroethanol concentration at 25 °C at constant molality (triangles, dashed lines) and at constant chemical potential (circles, solid line).

Table I
Preferential and Absolute Adsorption to Poly[N^5-(3-hydroxypropyl)-L-glutamine] (DP_w = 1360) Determined by Measurements of the Density Increments at 25 °C in Water/2-Chloroethanol Solvent Mixtures and Conformational Changes Estimated from Circular Dichroic Measurements

2-Chloroethanol concn, vol %	$\overline{V}^3$,[a] mL/g	$(\partial g_3/\partial g_2)_\mu$, g/g[b]	Helical content,[c] %	g_3, g/g[d]	A_1, g/g[e]	A_3, g/g	A_1[f]	A_3
5	0.7934	+1.14	22	0.063	0.34	1.16	0.32	1.16
10	0.7965	+0.33	26	0.133	0.31	0.371	0.32	0.372
20	0.8001	+0.08	46	0.297	0.28	0.163	0.32	0.187
30	0.8125	−0.43	57	0.505				
60	0.8297	−0.46	73	1.748				

[a] Taken from ref 5. [b] Calculated from eq 1 (reproductibility ± 10%). [c] Calculated from eq 2 with the date plotted on Figure 2. [d] Taken from ref 3. [e] Reference values from ref 7 for the system PHPG/water/dioxane. [f] Reference values from ref 3 for the system β-lactoglobulin A/water/2-chloroethanol.

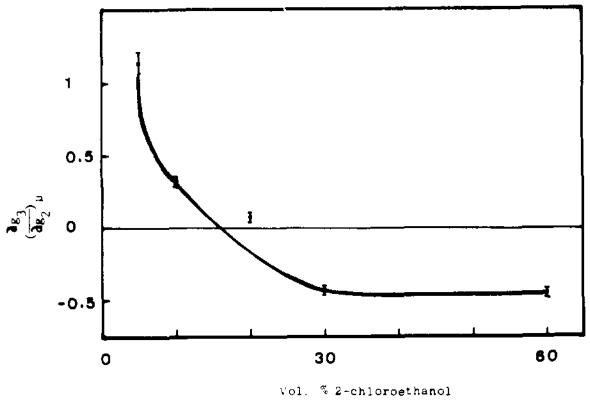

Figure 3. Variation of preferential adsorption (expressed in g/g) on poly[N^5-(3-hydroxypropyl)-L-glutamine] vs. 2-chloroethanol concentration at 25 °C. The error bars indicate the reproductibility in the measurement of preferential adsorption.

with $[\theta]_{222}{}^H = -53\ 000$[10] for a complete α helix of high molecular weight PHPG, we have f_N = 70%. Good agreement in the f_N values is found, giving evidence for a conformation of PHPG in 2-chloroethanol in which the helical structure is largely predominant. The conformational transition in PHPG was followed at 25 °C by the variation of $[\theta]_{222}$ when the water/2-chloroethanol solvent mixture is enriched in its organic solvent component. One can see (Figure 1) that the helical content in the PHPG sample increases rapidly with increasing 2-chloroethanol concentrations, as expected. The transition midpoint is located at a volume concentration of 20% 2-chloroethanol, indicating its good helicogenic properties. In spite of the high values attained by f_N (78% for 90% by volume of 2-chloroethanol), it is seen that the polypeptide did not reach a complete helical conformation, contrary to the case of the methanol-induced transition. The CD spectra of several PHPG/2-chloroethanol/0.1 M NaCl systems showed no difference in the observed molar ellipticity. This suggests that no ionization of the polymer occurs in the solvent mixtures used in this work.

Preferential and Absolute Adsorption to PHPG

(A) Density Increments of PHPG. Figure 2 shows the variation of the density increments at constant molality of component 3 (2-chloroethanol) and at constant chemical potential of the solvent vs. 2-chloroethanol concentration in various water/2-chloroethanol mixtures. Up to 20% 2-chloroethanol concentration, the density increment at constant molality is found to be inferior to the density increment at constant chemical potential, indicating that preferential adsorption of 2-chloroethanol on PHPG takes place, corresponding to positive values of $(\partial g_3/\partial g_2)_\mu$.

The variation of $(\partial \rho/\partial c_2)_m{}^0$ displays a rapid increase at 20% 2-chloroethanol concentration, which is the conformational transition midpoint, and shows the characteristic S-shaped curve which parallels any conformational transition. Such a feature has been noticed[23,24] in the variation of the refractive index increment at constant molality in poly(γ-benzyl L-glutamate)/mixed solvents systems. A pH-induced S-shaped plot of the volume change of a sample of poly(L-glutamic acid) has also been reported.[25]

(B) Preferential Adsorption of 2-Chloroethanol. Table I and Figure 3 give the variation of preferential adsorption of 2-chloroethanol $(\partial g_3/\partial g_2)_\mu$ in the different mixed solvent systems. These data show that a preferential solvation of 2-chloroethanol takes place in the 5–20% organic solvent concentration range. It vanishes rapidly before preferential hydration occurs. The crossover point is located at the conformational transition midpoint (20% vol).

(C) Absolute Adsorption on PHPG. We calculated the absolute adsorption coefficients using the Inoue and Timasheff equation[2,3]

$$\left(\frac{\partial g_3}{\partial g_2}\right)_\mu = A_3 - A_1 g_3 \qquad (3)$$

where A_1 and A_3 represent respectively the number of grams of water and of organic solvent bound to one gram of polymer residue (called respectively absolute hydration and absolute solvation). Here g_3 is the concentration of component 3 in gram per gram of component 1. The parameters A_1 and A_3 are unknown. Making an assumption for A_1 or A_3, it is possible to determine A_3 or A_1. We chose reference values of A_1 in two different systems taken from literature: a protein, β-lactoglobulin A, in water/2-chloroethanol mixed solvents[3] and PHPG in water/dioxane mixtures.[7] To calculate A_3 values, one must know the A_1 values in pure water and how A_1 varies in the solvent mixtures. The method used by Inoue and Izumi[7] (direct measurement of the absolute amount of bound water and dioxane by NMR in the frozen state[26]) is not easily applied to the water/2-chloroethanol systems, the pure solvents having very different freezing points (−67 °C for 2-chloroethanol). Therefore we chose reference values. Inoue and Izumi[7] found A_1 = 0.35 g/g of PHPG (3.5 mol/mol of residue) in pure water. This water is certainly adsorbed on the peptide bond, and the side chain amide bond, and on the terminal alcohol group. Mellon and co-workers[27] found 1–2 mol of water per peptide bond for poly(L-glycine), which is consistent with theoretical work.[28,29] By NMR, Kuntz[30] assigned the hydroxyl group to be hydrated by about 1 mol of water. This indicates for PHPG 3–5 mol of water per residue, which is consistent with Inoue and Izumi's results[7] for PHPG in pure water. For globular proteins, Inoue and Timasheff[3] introduced a correction to the A_1 values, taking account of the fact that new polar sites are exposed to the solvent during the structure-forming denaturation. But in the case of PHPG such a correction is not necessary. Therefore the A_1 values in water/

dioxane mixtures[7] are less than the postulated A_1 value of Inoue and Timasheff.[3] Table I shows the values of A_3 calculated using different reference values of A_1, the choice of which has been discussed above. In spite of the difference between them the calculated values of A_3 are remarkably similar, the difference being small compared to the values of $(\partial g_3/\partial g_2)_\mu$. One can notice a decrease of A_3 with increasing 2-chloroethanol concentrations. This can be related to the fact that the organic solvent has no specific interactions with PHPG. The same conclusion is to be drawn from Inoue and Izumi's results,[7] who found that the amount of bound dioxane remained virtually constant over the transition range. The water/2-chloroethanol system appears to be a much more interesting one.

(D) Relation with the Helix–Coil Transition. Drawing a comparison between the variations of preferential and absolute adsorption and the variation of the helical content f_N (listed in Table I), one can notice the following features:

Up to 20 vol % organic solvent concentration, both preferential and absolute solvation by 2-chloroethanol decrease rapidly.

Around the transition midpoint (20 vol % 2-chloroethanol) the adsorption of the organic solvent vanishes and is replaced by the adsorption of water.

This suggests that a relationship may exist between the interactions of PHPG with solvent components of mixed water/2-chloroethanol and the helix–coil transition. Such a parallel has been pointed out for proteins in water/2-chloroethanol mixed solvents;[3] at low alcohol concentrations, preferential solvation takes place but preferential hydration occurs at higher concentrations of 2-chloroethanol. In the poly(L-glutamic acid)/water/dioxane system such behavior has also been reported,[8] but in the PHPG/water/dioxane system preferential hydration increases monotonously with increasing helicity.[7] More information is needed on the comparison of absolute adsorption with conformational changes. In β-lactoglobulin A/water/2-chloroethanol, it is the variation of the absolute amount of bound organic solvent which parallels almost exactly the conformational transition,[3] indicating that 2-chloroethanol enhances the helical content of the protein by creating hydrophobic domains which favor the formation of α-helical conformations. Inoue and Izumi[7] evaluated the absolute amounts of bound solvents by NMR in the frozen state.[26] They reported that, with increasing helicity, the amount of bound water decreased regularly and the amount of bound dioxane being quasiconstant and small after a brief increase. An explanation may be found in the fact that 2-chloroethanol lowers the activity of water, thus stabilizing the intrachain hydrophobic interactions which increase the helical content of the polypeptide.[10,12]

In this work, values of A_3 could not be obtained over the whole range of solvent mixtures. It is therefore not wise to draw definite conclusions. Nevertheless, it is certain that a parallel between conformational transition of PHPG and decrease of water activity in the solvent mixtures can be deduced. The preferential hydration observed at high volume concentrations of 2-chloroethanol seems to be due to the hydrophilic character of PHPG; water can be retained on the peptide bond, the side chain CO–NH bond, and the terminal hydroxyl group. The 2-chloroethanol stabilizes the helical structure, but remaining water prevents obtaining a full α-helical conformation even when using high molecular weight samples like ours. Study of the interactions of PHPG with water/2-chloroethanol mixed solvents shows that 2-chloroethanol does not bind strongly to the polymer. On the other hand, water seems to be strongly adsorbed onto the polypeptide; preferential hydration occurs when the water content of the solvent mixtures decreases.

The results obtained suggest that a competition takes place between water and 2-chloroethanol, i.e., between the hydrophobic and hydrophilic features of PHPG:2-chloroethanol lowers the activity of water, thus weakening its ability to destroy the intramolecular noncovalent interactions which stabilize the α helix; when the alcohol concentration is increased, the remaining water of the solvent mixture is adsorbed to the hydrophilic sites of PHPG (this is indicated by the inversion of preferential solvation into preferential hydration and is consistent with Inoue and Izumi's observations;[7] when the dioxane concentration is increased, preferential hydration increases too). It gives experimental evidence for the strong hydrophilicity of PHPG, which is balanced by the hydrophobicity of the side chain methylene groups.

Conclusion

The study of preferential and absolute adsorption on poly[N^5-(3-hydroxypropyl)-L-glutamine] in water/2-chloroethanol solvent mixtures emphasizes the fact that water unfolds the helical structure. This work gives experimental support for the assumption proposed by Lupu-Lotan and co-workers;[10,12] in noninteracting solvents (here 2-chloroethanol), a helical structure is attained and stabilized by intrachain interactions. A isothermal coil-to-helix transition is observed in a solvent mixture containing a nonhelicogenic and a helicogenic solvent when the concentration of the latter is increased. We point out the fact that hydrophobic side-chain interactions in PHPG play an important part in the stabilization of ordered structure; these interactions, stabilized in noninteracting 2-chloroethanol, are weakened by interacting water, thus unfolding the α helix. A competition between hydrophobic and hydrophilic interactions in PHPG is also observed.

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