have rather sizable values which provide the justification for the introduction of the ternary term into the phenomenological description of the ternary system (eq 14). The character of these functions seems to be different for a mixture of two marginal solvents and for a mixture of a poor solvent with a good one. However, measurements on a larger number of ternary systems are needed before any conclusion about these trends can be drawn.

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Preferential and Absolute Adsorption by Poly[N^5-(2-hydroxyethyl)-L-glutamine-*co*-N^5-benzyl-L-glutamine] in Water/2-Chloroethanol Mixtures[1]

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ABSTRACT: The synthesis and characterization of water-soluble random poly[N^5-(2-hydroxyethyl)-L-glutamine-*co*-N^5-benzyl-L-glutamine] and their conformational properties in water and in mixed water/organic solvents are described. Preferential adsorption by the copolymers in water/2-chloroethanol mixtures was determined by the density increment technique. Preferential adsorption by 2-chloroethanol is dependent on copolymer composition. The magnitude of preferential and absolute adsorption increases when the copolymers are enriched in nonpolar monomeric units. The preferential interaction results are also discussed in relation to the solvent-induced coil-to-helix conformational transition. The results are interpreted in terms of hydrophobic interactions.

Preferential and absolute adsorptions by proteins in mixed water/organic solvent systems have been studied in relation to a possible conformational transition.[2-6] In the case of the water/2-chloroethanol solvent system, it was assumed[3-6] that unfolding of globular proteins was accompanied by an increase of preferential and absolute adsorption of 2-chloroethanol by the nonpolar residues buried in the "hydrophobic pocket", which are brought in contact with the solvent during the denaturation of the conformation in water. Timasheff and co-workers[3-6] postulated that the helicogenic power of an organic solvent is related to its ability to interact with hydrophobic residues in globular proteins, thus destroying the native conformation. The study of synthetic polypeptides showed that the helicogenic power of dioxane for aqueous ionized poly(α-L-glutamic acid) is related to adsorption of dioxane by the polymer.[7] The binding of dioxane to the dissociated carboxyl end group weakens electrostatic repulsions, thus allowing a coil-to-helix transition. The study of another polyelectrolyte, poly(L-Lys·HBr), in various water/organic solvent systems, showed[8] that preferential adsorption of the organic solvent is dependent on the nature of the solvent system, but no relation could be found between the variations of preferential interactions and the properties of the organic solvents. The study of a series of nonionizable polypeptides, poly[N^5-(2-hydroxyethyl)-L-glutamine] (PHEG) and poly[N^5-(3-hydroxypropyl)-L-glutamine], in water/2-chloroethanol mixtures emphasized that preferential adsorption of 2-chloroethanol is dependent on the hydrophobic character of the polypeptide and is closely related to the solvent-induced coil-to-helix transition.[9,10] These findings led us to undertake the synthesis of copolymers of variable hydrophobic nature to look for a possible correlation between the interactions of 2-chloroethanol with the copolymers and their hydrophobic character. N^5-(2-Hydroxyethyl)-L-glutamine (HEG) was chosen as one of the monomeric residues, because of its hydrophilic character.[11] In order to counterbalance the effects of the hydrophilic residue, a hydrophobic residue was introduced by statistical substitution of a phenyl group for the hydroxymethyl end group of HEG, which was expected to lead to marked hydrophobic interactions, as was observed for copolymers of L-glutamic acid, with γ-benzyl L-glutamate,[12] and copolymers of L-glutamic acid, with 2-nitro-γ-benzyl L-glutamate.[13] The hydrophobic N^5-benzyl-L-glutamine (BGln) residue was synthesized by

$$\begin{array}{c}[\text{-COCHNH-}]\\ |\\ (\text{CH}_2)_2\text{CONHCH}_2\text{Ph}\end{array}$$

aminolysis of the γ-benzyl L-glutamate side chain of poly(γ-benzyl L-glutamate) (PBLG) with benzylamine (see Experimental Section). Preferential adsorption can be determined by light scattering,[2-4] differential refractometry,[3,4,7,8] or density increments[9,14,15] measurements. For the water/2-chloroethanol mixtures, it is convenient to use

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the last method, since instruments now available allow easy and accurate measurements.

I. Theoretical

A. Preferential Adsorption. We use the notation of Scatchard[16] and Stockmayer,[17] in which the polymer is referred 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 $(\partial\rho/\partial C_2)_{m_3}$ and $(\partial\rho/\partial C_2)_{\mu_1,\mu_3}$, respectively at constant molality of component 3 and at constant chemical potential of components 1 and 3, by the relation[14,15,18,19]

$$\left(\frac{\partial g_3}{\partial g_2}\right)_\mu = \frac{(\partial\rho/\partial C_2)_{\mu_1,\mu_3} - (\partial\rho/\partial C_2)_{m_3}}{1 - \bar{v}_3\rho_0} \quad (1)$$

where $\bar{v}_3$ represents the partial specific volume of component 3 and ρ_0 the density of the mixed solvent. The preferential adsorption coefficient $(\partial g_3/\partial g_2)_\mu$ represents the preferential binding of component 3 to the polymer at constant chemical potential of components 1 and 3, expressed in grams of component 3 per gram of polymer. The concentration C_2 is expressed in g per cm^3 of solution. Equation 1 is exact for an incompressible system dilute in polymer; in practice the neglected terms are insignificant.[18,19]

B. Absolute Adsorption. The preferential adsorption coefficient only gives information on the excess or deficit of component 3 in the solvation layer, in relation to the bulk solvent composition. Knowledge of the exact composition of the solvation layer requires the determination of absolute adsorption numbers A_3 and A_1: A_3, the absolute adsorption of component 3, represents the quantity of component 3 in the solvation layer, expressed in grams per gram of residue; and A_1, the absolute adsorption of component 1 (absolute hydration when water is component 1), represents the quantity of component 1 in the solvation layer, expressed in grams per gram of polymeric residue. A_3 and A_1 are related to preferential adsorption by[6,19,20]

$$\left(\frac{\partial g_3}{\partial g_2}\right)_\mu = A_3 - g_3A_1 \quad (2)$$

where g_3 represents the content of component 3 in grams per gram of component 1 in the solvent mixture. The parameters A_1 and A_3 are unknown. Making an assumption for A_1, it is possible to determine A_3. The values used for A_1 in eq 2 will be given and discussed in section IIIC.

II. Experimental Section

A. Synthesis of the Copolymers. The copolymers were prepared from the same PBLG sample by aminolysis[21] with ethanolamine and benzylamine. In a series of assays, we noted that aminolysis of PBLG by benzylamine could not be achieved completely, and the presence of carboxylic groups was detected. Therefore, we chose to prepare poly[HEG-*co*-BGln] by aminolysis with mixed ethanolamine/benzylamine. We used the same conditions as those used for PHEG synthesis (i.e., in dioxan at 60 °C),[10] to ensure that ethanolamine would react with the γ-benzyl ester groups of starting PBLG which were not yet substituted by benzylamine. After aminolysis for 72 h, the reaction mixture was exhaustively dialyzed against a 1/1 methanol/water mixture and then against pure water. The copolymers were then recovered by lyophilization (90% yield).

B. Characterization of the Copolymers. The ultraviolet spectrum of aqueous benzylamine shows four peaks at 270, 265, 260, and 255 nm,[22] the molar extinction coefficients of which are 110, 160, 210, and 180 L mol^{-1} cm^{-1}, respectively. As we were unable to synthesize poly[N^5-benzyl-L-glutamine] (see section IIA), the molar extinction coefficients of aqueous benzylamine were used in determining the composition of the copolymers, from optical densities at these wavelengths, of aqueous solutions of the copolymers. The composition of the copolymers is given by the mole percent of (N^5-benzyl-L-glutamine) residues (Table I). Designation of the copolymers is also given in Table I.

Table I
Characterization of Copolymers

	$BGln_4$	$BGln_7$	$BGln_{10}$	$BGln_{15}$
mol % of BGln residue	4	7	10	15
partial specific vol in H_2O at 25 °C (mL/g)	0.727	0.727	0.729	0.739
$\bar{M}_w$ (sedimentation equilibrium) × 10^{-4}	9.5	9.2	8.3	8.2
$\overline{DP}_w$	550	530	470	460

Analysis of hydroxyl groups according to Fritz and Shenk[23] gave a weight percent of hydroxyl groups of 8.1 (calcd. 8.9) for copolymer $BGln_4$, 8.6 (calcd 8.6) for copolymer $BGln_7$, 7.7 (calcd 8.2) for copolymer $BGln_{10}$, and 7.1 (calcd 7.7) for copolymer $BGln_{15}$. Potentiometric titration of the copolymers showed no ionizable groups.

To check the copolymers for retention of optical configuration, samples were hydrolyzed in 12 N HCl at 105 °C for 24 h. The optical rotations of the hydrolyzates were compared with the optical rotation of a sample of L-glutamic acid hydrolyzed under the same conditions. The results showed that racemization occurred in less than 1% of the residues.

Weight-average molecular weights were estimated by equilibrium sedimentation in a Beckman Model E ultracentrifuge. The partial specific volumes of the copolymers in water were determined from density measurements (see section D) (Table I). The results are given in Table I. The starting PBLG sample had a weight average degree of polymerization of 1460. Extensive degradation occurred during aminolysis, as is shown by the weight-average degree of polymerization of the copolymers, which are of about one third of that of the initial PBLG.

C. Circular Dichroism. Circular dichroism (CD) spectra were recorded using a Jobin-Yvon R. J. Mark III dichrograph with a 0.01-cm cell. The molar residue ellipticity $[\theta]_\lambda$ is expressed in deg cm^2/dmol. The copolymers were insoluble in anhydrous methanol and 2-chloroethanol. In mixed solvents of low water content, precipitation occurred rapidly, but not before CD measurements could be completed.

D. Density Increments. Densities of polypeptide solutions were determined with a digital precision densitometer DMA 02D (Anton Paar Graz, Austria). Details of the experimental procedure can be found elsewhere.[9,24,25] The cell compartment was maintained at 25 ± 0.01 °C by the use of a LAUDA PTR 20/2 temperature controller. The experimental results consisted of the following: the density of the mixed solvents ρ_0 and of the polypeptide solutions ρ as a function of polypeptide concentration C_2 (in g/mL) at constant concentration of component 3. After 24 h of dialysis against the mixed solvents, the experimental results consisted of the density of the dialyzate, ρ_0', and the polypeptide solutions, ρ', as a function of C_2. The density increments $(\partial\rho/\partial C_2)_{m_3}$ and $(\partial\rho/\partial C_2)_{\mu_1,\mu_3}$ were calculated from the relations:

$$\rho = \rho_0 + C_2\,(\partial\rho/\partial C_2)_{m_3} \quad (3)$$

and

$$\rho' = \rho_0' + C_2\,(\partial\rho/\partial C_2)_{\mu_1,\mu_3} \quad (4)$$

The uncertainty on the values of density increments does not exceed 0.006. Thus, the maximum relative uncertainty in $(\partial g_3/\partial g_2)_\mu$ is 3%. Density measurements could not be carried out at high 2-chloroethanol concentrations, since precipitation occurred during dialysis. Organic solvent concentrations in the mixed solvents are expressed throughout on a volume basis.

III. Results

A. Synthesis and Characterization of Copolymers. Copolymers containing more than 15 mol % BGln residues were insoluble in water. Therefore, 15% was chosen as an upper limit. Nevertheless, the important changes in the

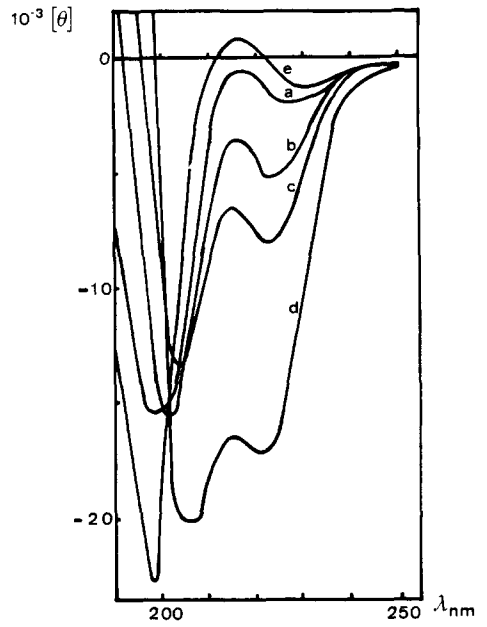

Figure 1. CD spectra for poly[HEG-*co*-BGln] in aqueous solution at room temperature: (a) copolymer $BGln_4$; (b) copolymer $BGln_7$; (c) copolymer $BGln_{10}$; (d) copolymer $BGln_{15}$; (e) PHEG.[10]

properties of the polypeptides induced by the incorporation of BGln residues, even in low amount (see further), justify our choice of copolymer composition, as given in Table I.

The UV spectrum of the benzylamide group overlaps that of the benzyl ester group. Therefore, it is not possible to check the absence of ester. Considering the fact that the aminolyses were run in the same conditions as those used for PHEG, with an excess of ethanolamine in the reaction mixture, we do see no reason to believe that the copolymers would contain more residual γ-benzyl ester groups than PHEG (<0.2% by UV absorption).[10] The good agreement found between the results of UV, elemental, and hydroxyl group analysis leads us to conclude that the use of aminolysis with mixed ethanolamine/benzylamine is a convenient way to synthesize poly[HEG-*co*-BGln].

We could not test the statistical distribution of the BGln residues in the polypeptidic chain. The fact that benzylamine prevents complete aminolysis of PBLG suggests that, after a given extent of substitution, steric factors prohibit further reaction. Therefore, formation of long BGln sequences is not favored. This is an indication, not a proof, that the distribution of BGln residues is more or less random.

B. CD Data for the Copolymers. The CD spectra of poly[HEG-*co*-BGln] in water at room temperature are shown in Figure 1. The spectrum of PHEG ($\bar{M}_w$ 110000)[10] is included for comparison. The CD data are interpretable by linear combination of right-handed, α-helix, and random-coil units. The positive band at 215–216 nm of random-coil PHEG becomes negative, showing a decrease of the random-coil content in the copolymers. The negative band at 222 nm indicates a right-handed, α-helical conformation, and its increasing intensity shows that incorporation of BGln increases the helical content of the polymer. The melting curves (Figure 2) demonstrate that, at low temperature, the incorporation of BGln increases the helical content, whereas, at high temperatures, the α-helix is destablized by BGln residues.

In mixed water/organic solvent, the coil-to-helix transition was followed by measuring the ellipticity $[\theta]_{222}$ as a function of organic solvent content in the mixed

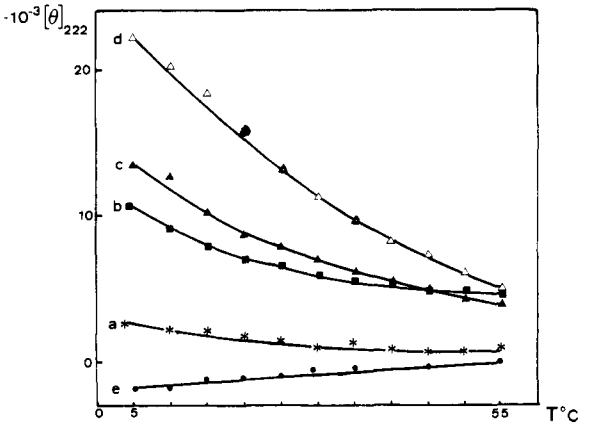

Figure 2. Temperature dependence of $-[\theta]_{222}$ for poly[HEG-*co*-BGln] in water (sample identification as in Figure 1).

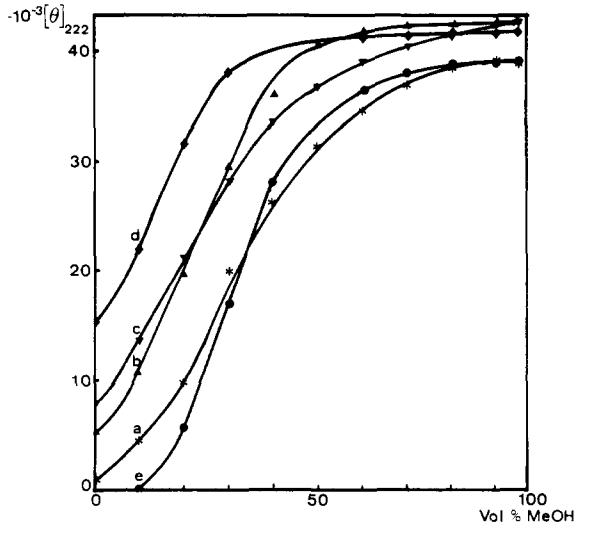

Figure 3. Variation of $-[\theta]_{222}$ for PHEG and poly[HEG-*co*-BGln] with solvent composition of mixed water/methanol at room temperature (sample identification as in Figure 1).

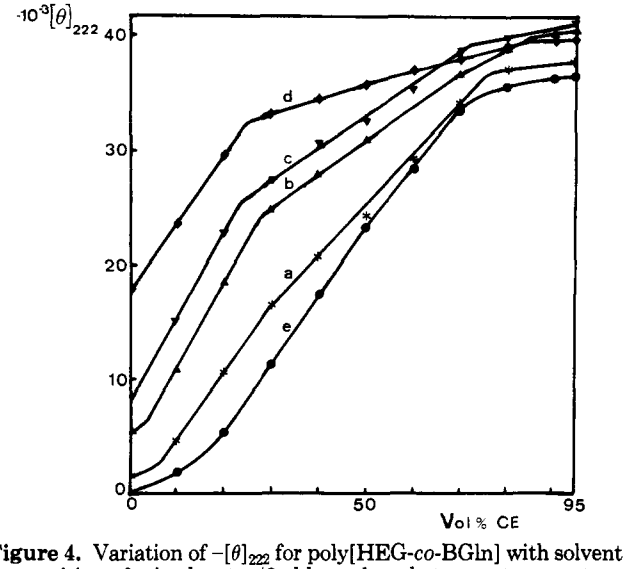

Figure 4. Variation of $-[\theta]_{222}$ for poly[HEG-*co*-BGln] with solvent composition of mixed water/2-chloroethanol at room temperature (data on PHEG[10] are included for comparison) (sample identification as in Figure 1).

solvents. The results show that methanol (Figure 3) and 2-chloroethanol (Figure 4) are efficient helicogenic agents. The values obtained for $[\theta]_{222}$ of the copolymers in 95% organic solvent mixture (−38000 to 43000) are higher than

Table II
Conformational Properties of Poly[HEG-*co*-BGln] in Solution
(Data for PHEG and PHPG are Included for Comparison)

	PHPG	PHEG	copolymer $BGln_4$	copolymer $BGln_7$	copolymer $BGln_{10}$	copolymer $BGln_{15}$
f_H in water at rt	0.20[a]	0[b]	0.07	0.16	0.22	0.40
vol % of organic solvent						
water/methanol[d]	15[a]	35	30	21	19	9
water/2-chloroethanol[e]	25[c]	45[b]	39	22	17	5

[a] From ref 21. [b] From ref 10. [c] From ref 9. [d] Solvent induced transition: vol % of organic solvent at $f_H = 0.5$ in water/methanol. [e] The same as footnote *d* but in water/2-chloroethanol.

Table III
Preferential Adsorption $(\partial g_3/\partial g_2)_\mu$ to Poly[HEG-*co*-BGln] in Mixed Water/2-Chloroethanol at 25 °C[a]

	$(\partial g_3/\partial g_2)_\mu$, g/g				
			copolymer		
vol % of 2-chloroethanol	PHEG	$BGln_4$	$BGln_7$	$BGln_{10}$	$BGln_{15}$
10	0.022	0.032	0.085	0.133	0.007
20	0.086	0.134	0.129	0.174	0.205
30	0.077	0.211	0.201	0.260	0.512
40	0.037	0.046	0.082	0.293	0.415
50	−0.269	−0.204	0.029	0.192	0.336
60	−0.273	−0.341	−0.080	−0.146	0.170
80	−0.065	−0.674	−0.957		

[a] Maximum relative uncertainty 3%. Data for PHEG from 10 are included for comparison.

those reported for PHEG (−42 000 in 95% methanol,[26] −36 600 in 95% 2-chloroethanol,[10] and −34 000 in tetrafluoroethanol[27]). Therefore, the highest observed value was used for $[\theta]_{222}$ of the complete α helix:

$$[\theta]^{H}_{222} = -43000$$

For the completely random coil, the value $[\theta]^{RC}_{222} = +2000$ attained by PHEG in water at low temperature (Figure 2) was chosen. Thus, the helix fraction f_H, defined as

$$f_H = \frac{[\theta]^{RC}_{222} - [\theta]_{222}}{[\theta]^{RC}_{222} - [\theta]^{H}_{222}} \quad (5)$$

can be calculated from the experimental results by the relation

$$f_H = \frac{2000 - [\theta]_{222}}{45000} \quad (6)$$

The helix fraction of the copolymers in water at room temperature and the solvent composition at the transition midpoint ($f_H = 0.5$) thus determined are given in Table II. Although the helical fraction of a polypeptide, in a given solvent and at a given temperature, also depends on molecular weight, the $\bar{M}_w$ of the copolymers (Table I) are close enough so that the differences can be neglected.

C. Preferential Adsorption in Water/2-Chloroethanol Solvent Mixtures. $\bar{v}_3$ values were taken from literature data.[4] The values of $(\partial g_3/\partial g_2)_\mu$ are given in Table III. Plots of $(\partial g_3/\partial g_2)_\mu$ as a function of 2-chloroethanol concentration in water/2-chloroethanol mixtures show (Figure 5) similar variations for the different copolymers (which are also similar to the behavior of PHEG[10]). First preferential adsorption by 2-chloroethanol is indicated by a positive $(\partial g_3/\partial g_2)_\mu$. The preferential solvation of the organic solvent passes through a maximum at 30–40 vol % of 2-chloroethanol, then decreases and becomes negative (except for copolymer $BGln_{15}$) as preferential hydration takes place. The values of preferential solvation, at a given solvent composition, increase as the BGln content of the copolymers is increased; this suggests that 2-chloroethanol–poly[HEG-*co*-BGln] interactions are dependent

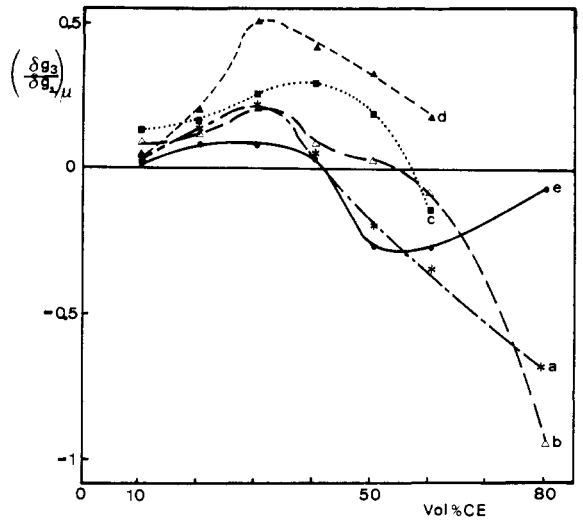

Figure 5. Variation of preferential adsorption $(\partial g_3/\partial g_2)_\mu$ with solvent composition in mixed water/2-chloroethanol for poly[HEG-*co*-BGln] at 25 °C. Data on PHEG[10] are included for comparison (sample identification as in Figure 1).

on copolymer composition: the incorporation of BGln hydrophobic residues enhances 2-chloroethanol adsorption. We were unable to obtain a linear relation between $(\partial g_3/\partial g_2)_\mu$ and copolymer composition at each solvent composition. However, the maximum positive value of $(\partial g_3/\partial g_2)_\mu$ shows a regular upward variation with BGln content (Figure 6). The inversion point (i.e., the vol % of 2-chloroethanol at which $(\partial g_3/\partial g_2)_\mu = 0$) shows a linear dependence with copolymer composition (Figure 6), with a positive slope; when the BGln content is increased in the copolymers, the preferential adsorption inversion point is located at high 2-chloroethanol concentrations.

D. Absolute Adsorption in Water/2-Chloroethanol Solvent Mixtures. In a study of synthetic polypeptides in aqueous solutions, Kuntz[28,29] showed that a polar side chain is hydrated by 2–4 mol of water per mol of residue and that the hydration number of an amide or a hydroxyl group is 1 mol/mol, in good agreement with theoretical

Table IV
Absolute Adsorption, A_3, to PHEG and to Poly[HEG-*co*-BGln] in Mixed Water/2-Chloroethanol at 25 °C[a]

vol % of 2-chloroethanol	A_1, g/g	A_3, g/g, for the copolymers				
		PHEG	$BGln_4$	$BGln_7$	$BGln_{10}$	$BGln_{15}$
10	0.31	0.06	0.07	0.13	0.17	0.05
			(0.07)	(0.12)	(0.17)	(0.04)
20	0.28	0.17	0.22	0.21	0.26	0.29
			(0.21)	(0.21)	(0.25)	(0.28)
30	0.25	0.20	0.34	0.33	0.39	0.64
			(0.31)	(0.32)	(0.37)	(0.62)
40	0.24	0.23	0.23	0.27	0.48	0.60
			(0.23)	(0.26)	(0.46)	(0.58)
50	0.23	0.03	0.06	0.29	0.46	0.61
			(0.06)	(0.28)	(0.43)	(0.57)
60	0.21	0.09	0.03	0.28	0.22	0.54
			(0.01)	(0.26)	(0.18)	(0.48)
80	0.20	0.86	0.25			
			(0.22)			

[a] Data in parentheses were calculated with the A_1 values of eq 7.

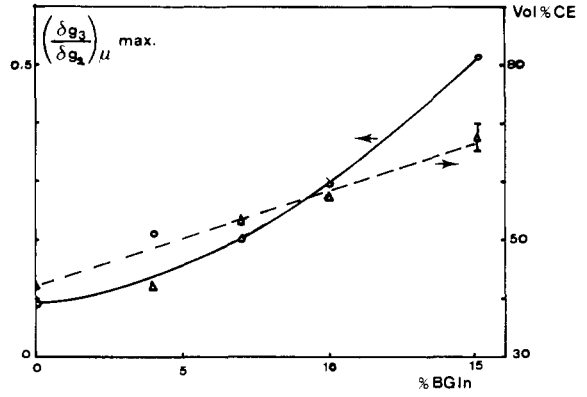

Figure 6. Variation of the maximum positive value of $(\partial g_3/\partial g_2)_\mu$ (left ordinate) and the vol % of 2-chloroethanol at the inversion point (right ordinate) for poly[HEG-*co*-BGln]. Extrapolated value for copolymer $BGln_{15}$. Data of PHEG[10] are included.

work.[30] Thus, A_1 for poly[N^5-(3-hydroxypropyl)-L-glutamine] (PHPG) should be 3 mol of water/mol of residue (0.31 g/g), which is close to the value found by Inoue and Izumi in pure water.[31] For PHEG, this hydration number also yields A_1 = 0.31 g/g. When dioxane was added to aqueous PHPG, the absolute hydration measured by NMR by Inoue and Izumi[31] was decreased to 0.20 g/g at 60 wt % of dioxane. This can be attributed to helix formation, which involves intramolecular hydrogen bonding and consequent decreased hydration of peptide linkages. Thus, the reference A_1 values we used to determine A_3 were lowered as the organic solvent content was increased in the solvent mixture to 0.20 g/g for 80 vol % of 2-chloroethanol. Reasonable variations in those values of A_1 do not affect A_3 critically. First, the A_3 values were calculated for the copolymers using the same values of A_1 as for PHEG (see Table IV). Kuntz[28,29] showed that nonpolar side-chain groups are hydrated by less than 1 mol of water per mol of residue. Thus the introduction of the BGln residue lowers the absolute hydration of the copolymer as compared to PHEG. New reference A_1 values were then calculated using the linear relation

$$A_1 = A_1'(1 - x) + A_1''x \qquad (7)$$

where A_1' is the absolute hydration of the HEG residue. A_1'' is the absolute hydration of the BGln residue, and x is the weight fraction of BGln. A_1'' was assumed to be 1 mol/mol of water smaller than A_1'. The A_3 values thus calculated (Table IV) appear only slightly different from those calculated first. When plotted as a function of

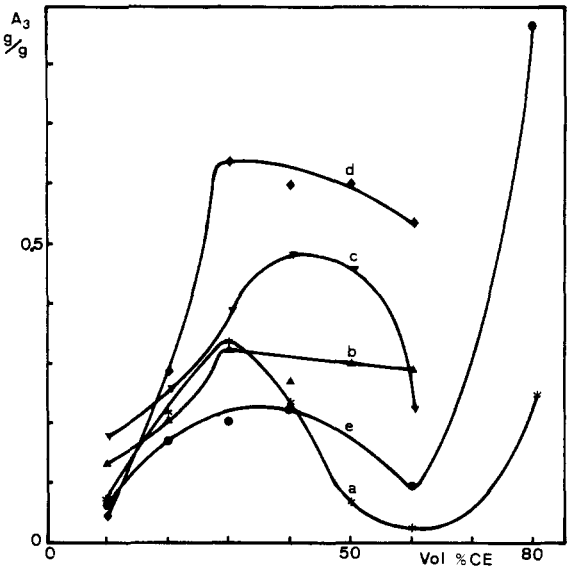

Figure 7. Variation of absolute adsorption A_3 for PHEG and poly[HEG-*co*-BGln] with solvent composition in mixed water/2-chloroethanol (sample identification as in Figure 1).

2-chloroethanol content in the solvent mixtures, the absolute adsorption of 2-chloroethanol shows the following features (Figure 7): when the organic solvent content is increased, A_3 increases, reaches a maximum, and then decreases; beyond a minimum (not observed for copolymers $BGln_7$, $BGln_{10}$, and $BGln_{15}$), A_3 increases again, as was observed for $(\partial g_3/\partial g_2)_\mu$, at each solvent composition. The values of A_3 increase as the BGln content in the copolymers is increased, indicating that nonpolar solvent/hydrophobic polypeptide interactions are enhanced.

IV. Discussion

A. Helix–Coil Transition and Copolymer Composition. As shown in Figure 1 and Table II, in aqueous solution, the increase of the α-helical content of the copolymers, with increasing BGln content, is not a linear function of copolymer composition. One can see that the values of $-[\theta]_{222}$ or $-[\theta]_{216}$ increase rapidly as BGln is introduced in the polymer chain, even in small amounts. The stabilization of the α helix in aqueous solution obviously has its origin in specific interactions between the nonpolar side chain groups of the copolymers, which are called hydrophobic interactions.[32-34] Although a matter of controversy,[35] hydrophobic interactions are known to stabilize the helical conformation of polypeptidic chains

in aqueous solution.[32-34] Experimentally, this was demonstrated with the series of poly(N^5-alkyl-L-glutamines)[11,36] and with copolymers of variable hydrophobic character.[12,13,37-39] The temperature-induced conformational transitions of poly[HEG-*co*-BGln] are of the "normal" type (Figure 2); this demonstrates that the stabilizing effects of the hydrophobic interactions, which are strengthened when the temperature is increased,[33,34] on the helical conformation are counterbalanced by the destruction of intrapeptide hydrogen bonds at high temperature.[33,34] The results in Figures 3 and 4 show that methanol and 2-chloroethanol are efficient helicogenic solvents. From the composition of the solvent mixture at the transition midpoint (f_H = 0.5) (Table II), one can see that less organic solvent is needed when the copolymers have a greater hydrophobic character. At high organic solvent composition, hydrophobic interactions have a less marked effect in stabilizing the α-helical conformation. This can be attributed to disruption of hydrophobic interactions when an aqueous medium is replaced by a less polar medium.[33,34,40,41]

The isothermal solvent-induced coil-to-helix transition can be interpreted in terms of competition between an "active" solvent, water, which destabilizes the α helix, and an "inactive" solvent, which enhances the "structuring" of the polypeptide chain.[36] The addition of organic solvent lowers the activity of water and contributes to form a solvent medium in which intrapeptide hydrogen bonds replace the hydrogen bonds of water with peptide groups thus enhancing the α-helix stabilization. The role of many helicogenic organic solvents has been interpreted in the same terms.[5,9,31,36,42,43] In addition, hydrophobic interactions between nonpolar side chains, here BGln residues, contribute to the stabilization of the α-helical conformation, especially when the solvent mixture is essentially aqueous.

B. Preferential and Absolute Adsorption. We have seen (section IIIB) that the variations of $(\partial g_3/\partial g_2)_\mu$ with the solvent composition are qualitatively similar for PHEG and poly[HEG-*co*-BGln]. The fact that we could obtain no linear relation between $(\partial g_3/\partial g_2)_\mu$ and copolymer composition, at each solvent composition, excludes the possibility of a simple correlation between these parameters. Interactions between identical side chains and between HEG and BGln side chains, differences in f_H, and interactions between the polypeptides and the solvent components cannot be taken into account independently. Nevertheless, the main feature is that the preferential and absolute adsorptions of 2-chloroethanol by the copolymers and by PHEG are dependent on copolymer composition: both increase regularly when the hydrophobic character of the copolymers is increased. This is experimental support of the assumption that 2-chloroethanol can interact with hydrophobic residues in globular proteins.[5,6]

It is not possible to compare our results with those of other water/organic solvent/copolymer systems, since no similar work has been published on synthetic polypeptides. Nevertheless, it is valuable to compare these results to those obtained for PHPG, the higher homologue of PHEG.

Preferential adsorption of PHPG from mixed water/2-chloroethanol was studied in the laboratory.[9] At low organic solvent concentration, preferential adsorption by 2-chloroethanol is observed, which decreased monotonically. It was followed by increasing hydration, when the solvent mixture was enriched in 2-chloroethanol. $(\partial g_3/\partial g_2)_\mu$ is greater for PHPG (1.14 g/g) than for PHEG, which is a less hydrophobic polypeptide. This is consistent with the above assumption. For poly[HEG-*co*-BGln], the inversion point is shifted to higher 2-chloroethanol concentration when the polypeptide becomes more hydrophobic (see Figure 6). In the case of PHPG, the inversion point is located at lower 2-chloroethanol concentrations than for PHEG (20 vol % and 42%, respectively). It appears that the additional methylene or PHPG, compared to PHEG, if it increases the hydrophobic character, contributes in another way to the interactions of the polypeptide with the solvent components. An explanation could be that the increase of 2-chloroethanol adsorption on the [HEG-*co*-BGln] copolymers is due to specific interactions between 2-chloroethanol and the BGln residue. Further study, of poly[N^5-(4-hydroxybutyl)-L-glutamine], for instance, would be necessary to confirm this. For PHPG, the absolute adsorption of 2-chloroethanol reaches A_3 = 1.16 g/g, or 2 mol/mol of monomeric unit.[9] In PHEG, A_3 has a maximum value at 40% 2-chloroethanol of A_3 = 0.23 g/g, or 0.4 mol/mol. (The values attained by A_3 at 80% of 2-chloroethanol were not taken into account for because they were not observed for copolymers $BGln_7$, $BGln_{10}$, and $BGln_{15}$.) Again, the polypeptide/nonpolar solvent interactions are weaker for the more hydrophilic polypeptide. This is confirmed for PHEG and poly[HEG-*co*-BGln]; the maximum values of A_3 attained at 30–40 vol % of 2-chloroethanol are a linear function of copolymer composition (Figure 8).

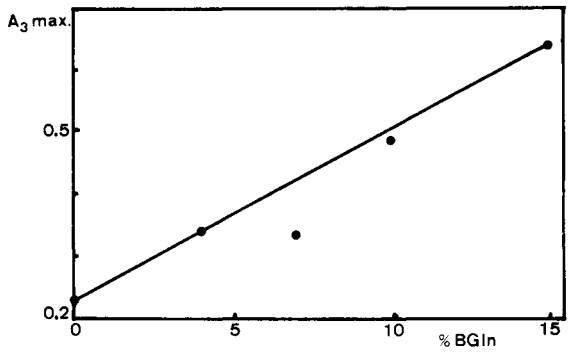

Figure 8. Variation of maximum value of A_3, attained at 30–40 vol % of 2-chloroethanol, with copolymer composition.

One would then have the relation

$$A_3^{\text{exptl}} = A_3^{\text{HEG}}(1 - x) + A_3^{\text{BGln}}x \qquad (8)$$

where A_3^{exptl} is the experimental A_3 for the copolymer, A_3^{HEG} and A_3^{BGln} are respectively the maximum absolute adsorption values of 2-chloroethanol on the HEG and BGln residues, and x is the weight fraction of BGln residues. Graphically (Figure 8), this yields $A_3^{\text{BGln}} \simeq 2$ g/g, or 5 mol/mol. This indicates a strong affinity of 2-chloroethanol for BGln residues. 2-Chloroethanol is adsorbed to a tenfold greater extent by BGln residues than by HEG residues. Figure 8 suggests that the two residues act independently for the maximum absolute solvation at least in the range of copolymer composition studied. The fact that such a linear relationship is not observed at all solvent compositions indicates the limits of this assumption. It must be pointed out that the value of A_3^{BGln} so determined corresponds to a residue in a fully helical conformation, since in eq 8 A_3^{BGln} is obtained by extrapolation to x = 1.

C. Relation to Solvent-Induced Coil-to-Helix Transition. In the case of proteins, it has been shown[3-6] that the adsorption of chloroethanol by the protein parallels its helicogenic effects. A parallel can also be drawn between the preferential and absolute adsorptions of 2-chloroethanol and its helicogenic effect on PHPG[9] and PHEG:[10] a greater helicogenic efficiency of 2-chloroethanol (transition midpoint at 20 vol % and 45 vol % of 2-

chloroethanol, respectively) is accompanied by higher values of $(\partial g_3/\partial g_2)_\mu$ and A_3. Moreover, the inversion point parallels the transition midpoint:[9,10] addition of 2-chloroethanol favors the stabilization of the α helix, which is greater the more hydrophobic the polypeptide is. Dioxane, which is less helicogenic than 2-chloroethanol (f_H = 0.5 at 40% of dioxane) for PHPG,[31] is less adsorbed on PHPG (A_3 = 0.05 g/g, or 0.1 mol/mol).[31]

All these results favor a parallel between the polypeptide/nonpolar solvent interactions and the following: (1) the helicogenic efficiency of the nonpolar solvent, and (2) the hydrophobic character of the polypeptide. We have seen that a linear relationship holds between the copolymer composition and both (1) the inversion point (Figure 6) and (2) the maximum value of A_3 (Figure 8). Specific interactions between 2-chloroethanol and the BGln residue could be an explanation for this. The inversion point no longer parallels the midpoint of the conformational transition, but varies inversely; when the BGln content is increased in the different copolymers, less 2-chloroethanol is needed to make f_H = 0.5 (see Table II), and the helix fraction at which $(\partial g_3/\partial g_2)_\mu = 0$ is shifted to higher values, contrary to what was observed for PHEG[10] and PHPG.[9] The fact that preferential hydration occurs to higher 2-chloroethanol concentrations and higher f_H's can be interpreted as the result of strong hydrophobic interactions, which supplant hydrophilicity of HEG residues, even though they are numerous in the polypeptide chain (the mole fraction of BGln residues does not exceed 0.15!). Thus, it appears that the nonpolar BGln residues are responsible for the strong copolymer/2-chloroethanol interactions. Such hydrophobic interactions between a nonpolar solvent molecule and a nonpolar residue were observed recently by Pekary[44] in the system pyridine/water/poly[α-L-glutamic acid^{50}-*co*-L-tyrosine^{50}].

D. Chemical Potential. The effect of hydrophobic interactions must be a stabilization of the system when the copolymers are introduced in the solvent mixture; the perturbation of the chemical potential of 2-chloroethanol by addition of the macromolecule can be expressed by[18]

$$\left(\frac{\partial \mu_3}{\partial m_2}\right)_{m_3} = -\left(\frac{\partial m_3}{\partial m_2}\right)_{\mu_3}\left(\frac{\partial \mu_3}{\partial m_3}\right)_{m_2} \qquad (9)$$

where $(\partial m_3/\partial m_2)_{\mu_3}$ is the molar preferential adsorption coefficient, which can be approximated[45] by RT/m_3. The results show (Figure 9) that introduction of poly[HEG-*co*-BGln] causes the chemical potential of 2-chloroethanol to become more negative, at low organic solvent concentrations, indicating no repulsion between the copolymers and 2-chloroethanol. At high 2-chloroethanol content in the solvent mixture, $-(\partial \mu_3/\partial m_2)_{m_3}$ decreases and reverses sign, indicating repulsion between the copolymers and 2-chloroethanol. This is consistent with our experimental findings: we observed (see sections IIC and IID) that the copolymers were insoluble in mixtures of low water content.

V. Conclusions

Copolymers containing N^5-benzyl-L-glutamine and N^5-(2-hydroxyethyl)-L-glutamine were synthetized and characterized. In aqueous solution and in mixed water/2-chloroethanol, it is shown that the helical conformation is stabilized by hydrophobic interactions between the hydrophobic BGln residues. Adsorption of 2-chloroethanol to the copolymers is dependent on copolymer composition; when the hydrophobic BGln residues content is increased, preferential and absolute adsorption of 2-chloroethanol increases too. The existence of specific interactions between these two hydrophobic species is deduced from the results.

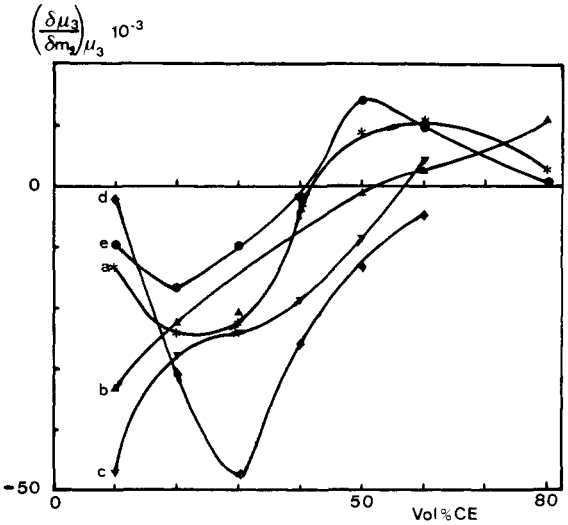

Figure 9. Variation of $(\partial \mu_3/\partial m_2)_{m_3}$ in cal mol^{-1}/1000 g of H_2O, with 2-chloroethanol concentration in mixed water/2-chloroethanol, for PHEG and poly[HEG-*co*-BGln] copolymers (sample identification as in Figure 1).

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Linear Oligopeptides. 57.[1] A Circular Dichroism Study of α-Helix and β-Structure Formation in Solution by Homooligo-L-methionines

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ABSTRACT: The conformational properties of poly(ethylene glycol)-bound, monodispersed *N-tert*-butyloxycarbonylhomooligo-L-methionines to the pentadecapeptide were examined in solvents of high polarity (a variety of alcohols, haloalcohols, and water) using circular dichroism. The corresponding N-deblocked derivatives were also investigated. This study shows that the L-methionine peptides may exist in right-handed α-helical, β, and statistical coil conformation depending upon chain length, solvent polarity, temperature, concentration, pH, ionic strength, and presence of the N-blocking group.

The possibility of investigating the conformation of solubilized oligopeptides through attachment to a suitable polymer, e.g., poly(ethylene glycol) (PEG), has recently opened the door to a number of circular dichroism (CD) studies aiming to provide a deeper insight into the relation between conformation and chain length, primary sequence, protecting group, solvent, concentration, ionic strength and temperature.[3-10]

A CD investigation of PEG-bound ACTH peptides revealed a distinct tendency for ordered structures at the N-terminal part of the hormone.[10] A significant increase in helical structure was noted when the helix-promoting sequence L-Pro-L-Ala-L-Ala was inserted at the N terminus. Transitions from statistically unordered to partially helical structures at chain lengths of about eight to ten residues were observed in 2,2,2-trifluoroethanol (TFE) during the stepwise liquid-phase synthesis[11] of the myoglobin fragment 66–73,[4] the hormone substance P,[3-4] and model peptides for the antibiotic alamethicin.[9]

The formation of the α helix at acidic pH for PEG-bound homooligopeptides derived from L-Glu(OH) starts at $n = 7$, at $n = 14$ the helix content amounts to about 35%, and at $n = 20$ the helix content amounts to about 60%.[5,6] A helix $\rightarrow$ coil transition is induced by the ionization of the carboxyl groups of the lateral chains. TFE stabilizes the α helix more than water does; the addition of increasing amounts of trifluoroacetic acid resulted in the expected disruption of the helical structure. Also, the solvent-depended conformational properties were not changed by the anchor polymer. The homooligopeptide series derived from L-Glu(OBzl) exhibits in TFE higher helicities than the corresponding L-Glu(OH) analogues, although the onset of the α helix is still observed at $n = 7$.

In the context of the collaborative program which is underway between the Tübingen and Padova laboratories, the following series of PEG-bound *N-tert*-butyloxycarbonyl (*t*-Boc) apolar homooligopeptides have been synthesized by the liquid-phase method[4,10,12] and their conformational preferences investigated by CD:[4,5,7,8,10] *t*-Boc-(L-Ala)$_n$-Gly-OPEG ($n = 1$–8), *t*-Boc-(L-Ala)$_n$-OPEG ($n = 1$–10), *t*-Boc-(L-Val)$_n$-Gly-OPEG ($n = 1$–7), *t*-Boc-(L-Val)$_n$-Gly-OPEG-M ($n = 2$–8) (PEG-M, poly(ethylene glycol) monomethyl ether), and *t*-Boc-(L-Ile)$_n$-OPEG ($n = 1$–8).

In water the peptides from L-Ala and L-Val with $n = 6$, 7 adopt a β conformation. High concentration, temperature, ionic strength, and absence of the N-terminal ammonium group favor the formation of the ordered structure. The β structure of L-Val peptides is more stable than that of L-Ala peptides. In TFE only the L-Ala peptides assume an α-helical conformation (starting at about $n = 8$). More polar solvents, e.g., 1,1,1,3,3,3-hexafluoropropan-2-ol (HFIP), and high temperatures hamper the formation of the α-helical structure in the L-Ala peptides. The absence of the *N-t*-Boc group and the use of less polar solvents, e.g., methanol (MeOH), favor the formation of associated species (β structure). In general, the effect brought about the polymeric support, and its molecular weight, even if not negligible, should be considered as a minor one. In TFE the order of stability of the β structure of the apolar homooligopeptides appears to be L-Ile > L-Val > L-Ala.

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