Salt effects on the conformation of a 'statistical' copolymer of L-leucine and L-lysine*

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For studying the influence of water structure breaking and making anions on the conformation of poly(α-amino acids) containing hydrophobic and ionic side chains, c.d. measurements on 'statistical copolymers of L-leucine and L-lysine were carried out in $LiClO_4$, $NaClO_4$, Li_2SO_4, Na_2SO_4, KF, NaF, and NaCl solutions and also in salt-free water. Copolymers with 50 mol% L-leucine do not form α-helices in pure water at pH 7 (20°C); however $LiClO_4$ and $NaClO_4$ at concentrations as low as 0.003 mol l^{-1} induce α-helix formation almost independent of cation. Surprisingly, not only ClO_4^- but also SO_4^{2-} has an α-helix inducing effect in this case, in contrast with basic homopoly(α-amino acids), where such an effect of SO_4^{2-} was not observed. Therefore the presence of L-leucyl residues seems to be responsible for this α-helix inducing effect of sulphate anions. This agrees with the fact that sulphate stabilizes the ordered periodic conformation of native proteins as the increase of denaturing temperature shows. It can be assumed that the results of these measurements support the assumptions on the influence of water structure in making SO_4 anions on hydrophobic interactions, as well as of water structure breaking ClO_4 anions.

Keywords Salt effects; conformation; L-leucine; L-lysine; perchlorate; sulphate

INTRODUCTION

Neutral salts change the stability of the conformation of proteins and polypeptides[1,2] depending on the kind of cation and especially on the kind of anion. Some anions—at constant cation—lower the temperature T_m. (This is the temperature of the mid-point of the S-shaped conformation transition curve obtained by plotting the specific rotation or ellipticity against temperature.) However, this influence of anions on the conformational stability of polypeptides depends on the chemical composition of the polypeptide. In the case of native proteins, e.g., keratins[1], ribonuclease[2], etc., perchlorate anions, which are used here as representative for one group of anions, lower the stability of the native conformation to a rather high extent. Sulphate anions representing the other type of anion increase T_m and therefore the thermal stability of the native proteins or polypeptides. Considering the conformation destabilization or stabilization power of the anions in general indicates a strong correlation between this property and their influence on water structure[1]. The higher the water structure breaking effect of an anion, the higher its denaturing power. Water structure makers like SO_4^{2-} increase the stability of the native conformation as one can see from the increase of T_m with increasing concentration[1,2].

It was supposed that these effects are due to changing the stability of the hydrophobic interactions of the amino acid side chains induced by the anions via influencing water structure. If water structure breaking anions are brought near apolar groups involved in hydrophobic interaction, the entropy of the water molecules surrounding them is increased. Therefore the decrease in entropy concomitant with the opening of hydrophobic bonds should be compensated to some degree, and as a consequence, the stability of this kind of intermolecular interaction should be lowered. Water structure making anions like SO_4^{2-}, therefore, should increase their stability.

In contrast to native proteins, the influence of the anions mentioned above on the conformation of basic poly(α-amino acids) like poly(L-ornithine) $(Orn)_n$, poly(L-lysine) $(Lys)_n$, etc. is the reverse[3-11]. In the case of these homopolyelectrolytes a pH-induced conformational change from α-helix to a nonperiodic conformation occurs. The electrostatic repulsion forces of the positively charged basic groups are responsible for the conformational change from α-helix to a nonperiodic or to an extended helical conformation[12]. This pH-induced conformational change is suppressed completely in distinct temperature and concentration ranges, e.g. by perchlorate anions almost independent of the cation[7,9] and other water structure breaking anions[2,8]. This means that the ordered periodic α-helical conformation of basic poly(α-amino acids) is stabilized by water structure breaking anions, contradictory to their destabilizing effect on the conformation of native proteins. The water structure making SO_4^{2-} anions, on the other hand, do not stabilize the α-helix of basic poly(α-amino acids) at all[7,9-11,13]. This inverse effect in respect of the influence

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on stabilizing and destabilizing the periodic conformation of ordinary native proteins and basic homopoly(α-amino acids) is obviously due to affecting different kinds of molecular interactions in both cases. In the latter, electrostatic repulsion forces responsible for the pH-induced conformation change have to be neutralized for stabilizing the α-helix in the protonated state. Therefore, a strong specific interaction of the anions with the protonated side chains is necessary. In any case the anions are situated in the immobile part of the electric double layer as shown recently by ultracentrifuge measurements[10,11,13]. According to these experiments, SO_4^{2-} ions are present only in the mobile part of the diffuse double layer. Summarizing these previous results one can conclude that the effects of the anions on the conformation of polypeptides may differ depending on their chemical composition because at least two kinds of conformation stabilizing intermolecular interactions are affected by them:

(a) hydrophobic interactions,

(b) electrostatic forces.

For studying these effects in detail using model substances, experiments on the conformation of copoly(α-amino acids) containing basic and apolar side chains as well as various electrolyte solutions are desirable. For this reason we used copolymers of L-leucine and L-lysine for experiments on the influence of some water structure breaking or making anions on their conformational stability.

EXPERIMENTAL

Materials

N-Carboxy amino acid anhydrides (NCA). NCA of N^{ε}-carbobenzyloxy(Cbz)-L-lysine (I) was prepared by the reaction of phosgene with N^{ε}-Cbz-L-lysine[14] in dioxane by the method similar to that of Fasman *et al.*[15]; mp 100°C (Lit., mp 101°–101.5°C). NCA of L-leucine (II) was obtained in an analogous way according to Fasman *et al.*[16]; mp 76°C).

Copoly(L-leucyl-L-lysine · HBr) (IV). This copolymer was prepared by the method similar to that of Snell and Fasman[17]. The mixture of (I), (II), and n-butylamine in freshly distilled dioxane was allowed to react by stirring at 20°C for 7 days in a flask with a $CaCl_2$ drying tube. The total initial concentration of (I) and (II) was 5 g dl^{-1}. The anhydride to initiator ratio (A/I) was 200. The molar ratio (II/I) of (II) to (I) was set to be 1, 2 and 3, respectively. In another copolymerization triethylamine was used as initiator. In this case, the value of A/I was set at 40 and other conditions were similar to that described above. After copolymerization of NCAs, the resulting viscous solution was diluted to the low concentration of 1 g dl^{-1} with dioxane, and then hydrogen chloride gas was bubbled through the solution for 30 min followed by dry hydrogen bromide gas for 1 h. The solution was stirred for 90 min and then dry nitrogen gas was bubbled through the solution for 90 min. The solvent was evaporated on a rotary evaporator and the resultant solid was washed twice with ether and dissolved in water. In practice, however, the copolymer which was prepared at the initial molar ratio (II/I) of 3 was not soluble in water. Therefore, this copolymer was washed with water and dried. Other soluble copolymers were treated as follows. The pH of aqueous solution was adjusted to 5 with 1 M sodium hydroxide solution and the solution dialysed against 0.01 M hydrochloric acid. The final solution was filtered to remove any undissolved material, lyophilized and dried.

Methods

Amino acid analysis. The amino acid compositions of copolymers were obtained by amino acid analysis on a BIOCAL BC 201 analyser, after complete hydrolysis in 6 N HCl. The hydrolysis of copolymers prepared at the initial molar ratio (II/I) of 1 was carried out for 48 h at 105°C. The complete hydrolysis of other copolymers was carried out for 72 h at 105°C.

Viscosimetry. Intrinsic viscosities of copolymers were determined in dichloroacetic acid using a Ubbelohde viscometer, VISCOMATIC FICA, at a temperature of 20° ±0.1°C.

Circular dichroism measurement. C.d. measurements were made on a Jasco spectropolarimeter Model J-20. The c.d. spectra were run in a quartz cell with 0.1 cm path length at a polymer concentration of 0.05 g dl^{-1}. The wavelength range covered was 190–300 nm. The pH of the polymer solution was adjusted with 0.1 M KOH. The mean residue ellipticity, $[\theta]_\lambda$, expressed in deg cm^2 $dmol^{-1}$ at a given wavelength λ, was calculated from the following equation:

$$[\theta]_\lambda = \frac{\theta_\lambda \times MRW}{10 \times l \times C}$$

where θ_λ is the ellipticity in degrees at the wavelength λ, MRW is the mean residue molecular weight obtained from an average of the residue weights calculated from the amino acid composition, l is the cell path length in cm and C is the polymer concentration in grams per ml.

RESULTS

Preparation of copolymer

The results of copolymerization are summarized in *Table 1*. The sample code for IV prepared with n-butylamine or triethylamine as an initiator is IV_{B-m} or IV_{E-m}, where m is the initial molar ratio of II to I in the copolymerization. The amino acid composition of IV was determined by an amino acid analyser. The value of molar ratio (r) of leucyl residues to lysyl residues in IV is not so different from the value of initial molar ratio (m) of II to I. Mean residue weight (M_0) of IV was calculated from the following equation:

$$M_0 \text{ of IV} = (131r + 209)/(1 + r)$$

where 131 and 209 are the formula weights of L-leucine and L-lysine·HBr residues, respectively. Conversions of I and II were calculated from the yield of IV, M_0 and r as follows:

$$\text{Conversion of I} = \frac{P}{Y_{lys}} \times \frac{1}{1+r} \times \frac{306}{M_0 \text{ of IV}} \times 100(\%)$$

$$\text{Conversion of II} = \frac{P}{Y_{leu}} \times \frac{r}{1+r} \times \frac{157}{M_0 \text{ of IV}} \times 100(\%)$$

where P is yield (g) of copolymer IV, Y_{lys} and Y_{leu} are the amounts (g) of I and II used, respectively; and 306 and 157

Table 1 Copoly(L-lysine . HBr, L-leucine)

Sample code	Initial molar ratio, Leu/Lys	Conversion of NCA		Amino acid content		Molar ratio Leu/Lys (r)	$[\eta]$ in DCA at 20.0°C
		Leu (%)	Lys (%)	Leu (%)	Lys (%)		
IV_{B-1}	1.0	75	75	48.3	51.7	0.93	0.54
IV_{B-2}	2.0	74	71	66.0	34.0	1.94	0.49
IV_{B-3}	3.0	82	87	72.6	27.6	2.65	0.39
IV_{E-1}	1.0	76	77	49.8	50.2	0.99	1.34
IV_{E-2}	2.0	69	76	62.9	37.1	1.70	1.36
IV_{E-3}	3.0	78	89	71.0	29.0	2.45	1.69

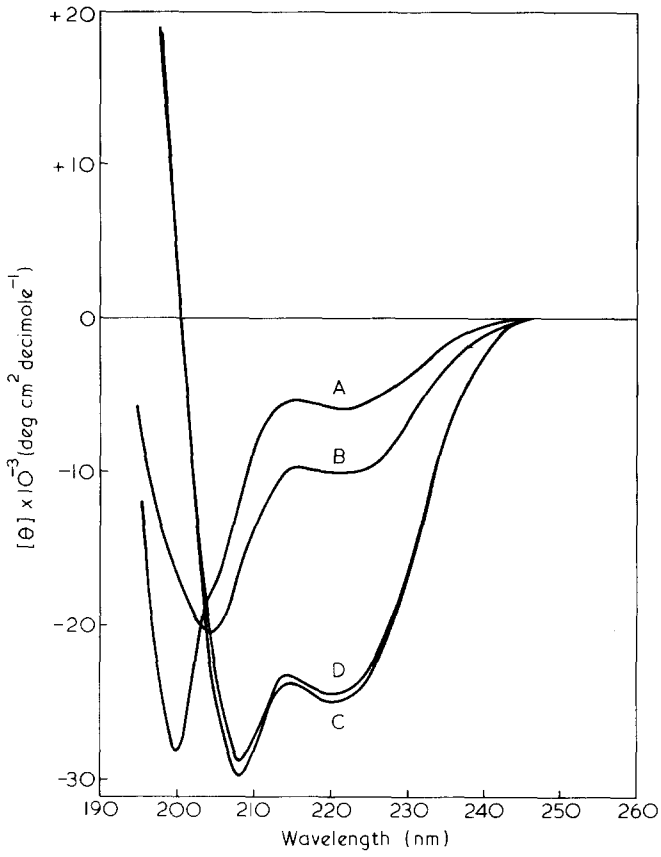

Figure 1 C.d. spectra of copoly(Leu^x, Lys^y)s in salt-free water at 20.0°C and pH 7.0 ± 0.02. A: IV_{B-1}; copoly(Leu$^{48.3}$, Lys$^{51.7}$); B: IV_{E-1}; copoly(Leu$^{49.8}$, Lys$^{50.2}$); C: IV_{B-2}; copoly(Leu$^{66.0}$, Lys$^{34.0}$); D: IV_{E-2}; copoly(Leu$^{62.9}$, Lys$^{37.1}$)

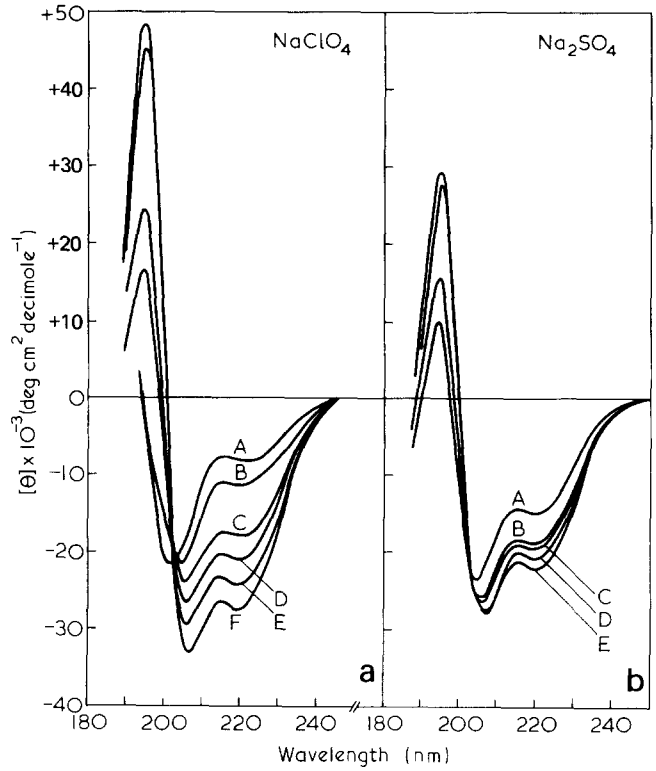

Figure 2a C.d. spectra of copolymer IV_{B-1} as a function of ClO_4^- concentration at 20.0°C and pH 7.0 ± 0.02. A; 0.0005 mol l^{-1}, B; 0.001 mol l^{-1}, C; 0.003 mol l^{-1}, D; 0.005 mol l^{-1}, E; 0.01 mol l^{-1}, F; 0.02 mol l^{-1}

Figure 2b C.d. spectra of copolymer IV_{B-1} as a function of SO_4^{2-} concentration at 20.0°C and pH 7.0 ± 0.02. A; 0.0003 mol l^{-1}, B; 0.0005 mol l^{-1}, C; 0.001 mol l^{-1}, D; 0.01 mol l^{-1}, E, 0.02 mol l^{-1}

are molecular weights of I and II, respectively. The intrinsic viscosities $[\eta]$ in dichloroacetic acid of the copolymers, IV_B, prepared with n-butylamine ranged between 0.39 and 0.54. In the case of the copolymers, IV_E, obtained with triethylamine, the value of $[\eta]$ ranged between 1.34 and 1.69, indicating a much higher molecular weight compared with IV_B.

C.d. measurement

The conformation of the copolymers with different amino acid composition was studied in salt-free water. The higher the leucine content of the copolymer, the lower is the solubility in water. The leucine content of copolymers represented by IV_{B-3} and IV_{E-3} was more than 70 mol%. In practice, these copolymers were not soluble in water. Therefore, other copolymers of lower leucine content (less than 70 mol%) were used for the c.d. studies. The c.d. spectra of copoly(Leu^x, Lys^y)s in salt-free water at neutral pH are shown in *Figure 1*. Copolymers like IV_{B-2} and IV_{E-2} having ~65 mol% leucine content can be assumed to have α-helical conformation, whereas copolymers like IV_{B-1} and IV_{E-1} having ~50 mol% leucine content cannot be found in the ordered conformation. Because the results obtained are almost independent of the molecular weight, in general only that of IV_{B-1} is shown in this paper.

C.d. measurements depending on electrolyte concentration

The c.d. spectra of copolymer IV_{B-1} in the solution of $NaClO_4$ and Na_2SO_4 at neutral pH are shown in *Figures 2a* and *b*. The stability of the α-helical conformation of copolymer IV_{B-1} can be enhanced with increasing ClO_4^- or SO_4^{2-} ions concentration. Copolymer IV_{B-1} can be found in the α-helical conformation at concentration

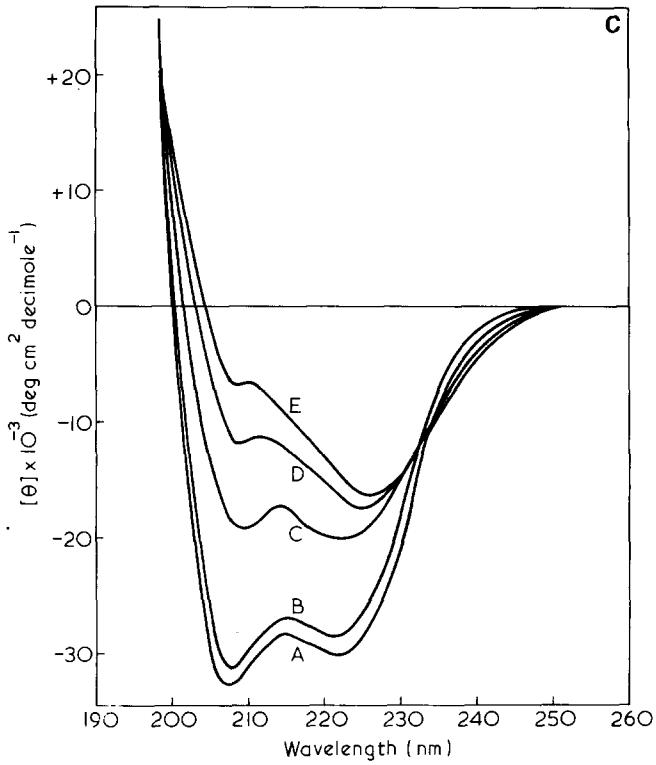

Figure 2c C.d. spectra of copolymer IV_{B-1} in $LiClO_4$ solution as a function of salt concentration at 20.0°C and pH 7.0 ± 0.02. A; 0.20 mol l^{-1}, B; 0.25 mol l^{-1}, C; 0.30 mol l^{-1}, D; 0.325 mol l^{-1}, E; 0.35 mol l^{-1}

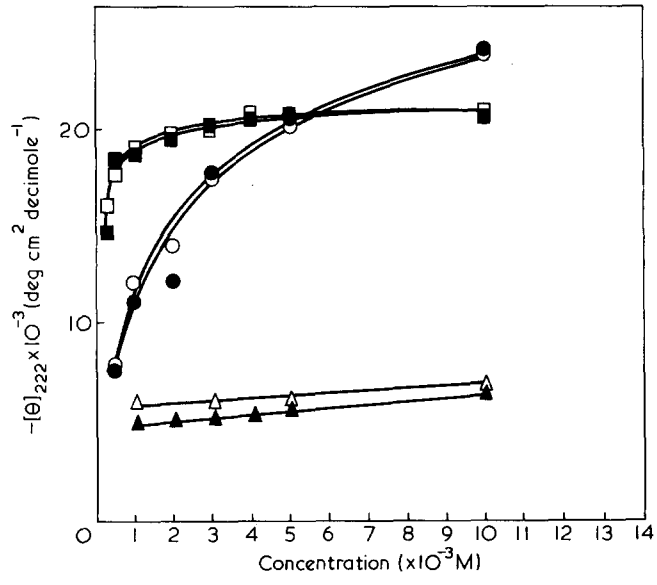

Figure 3 Plot of mean residue ellipticities, $-[\theta]_{222}$, of copolymer IV_{B-1} as a function of salt concentration at 20.0°C and pH 7.0 ± 0.02. (○); $LiClO_4$, (●); $NaClO_4$, (□); Li_2SO_4, (■); Na_2SO_4, (△); NaF, (▲); KF

above 0.003 mol l^{-1} ClO_4^-, as well as above 0.0005 mol l^{-1} SO_4^{2-}. *Figure 3* shows the plot of mean residual ellipticities, $-[\theta]_{222}$, as a function of concentrations of various salts at neutral pH. Copolymer IV_{B-1} cannot be found in the ordered conformation at concentrations less than 0.01 mol l^{-1} of NaCl, NaF, or KF. However, copolymer having ~50 mol% leucine content becomes partly α-helical at a concentration above 0.3 mol l^{-1} KF. A turbidity of polymer solutions cannot be observed in the KF solutions examined. However, the solution of copolymer IV_{B-1} becomes slightly turbid with increasing $LiClO_4$ concentration more than 0.30 mol l^{-1}. In the case of copolymer IV_{B-2}, such a turbidity occurs already at concentrations above 0.15 mol l^{-1}. In Li_2SO_4 solution such a turbidity was observed at concentrations as low as 0.01 mol l^{-1}. Similar c.d. spectra were obtained with copolymer IV_{B-2} in $LiClO_4$ or Li_2SO_4 solution. The c.d. spectra of these copolymers have a minimum around 226–228 nm and a shoulder at 208 nm at higher Li_2SO_4 or Li_2SO_4 concentration at which the solutions become slightly turbid (*Figure 2c*). The relations of the mean residual ellipticities, $-[\theta]_{222}$, as a function of salt concentration are shown in *Figure 4*. The molecules of copolymer IV_{B-1} aggregate at $LiClO_4$ concentration more than 0.3 mol l^{-1}. However, the turbidity of solution owing to such an aggregation disappears with rising temperature. The temperature at which such a disaggregation occurs is shifted to higher values with increasing $LiClO_4$ concentration. The molecules of copolymer IV_{B-2} aggregate at $LiClO_4$ concentrations more than 0.15 mol l^{-1}. However, on the contrary to copolymer IV_{B-1}, the resulting aggregation does not disappear even at high temperatures.

C.d. measurements depending on pH

The pH-induced conformational change of copolymers was studied in salt-free water, in $LiClO_4$ and KF solutions, respectively. The polymer solutions become slightly turbid with an increase in pH value. Similar to increasing salt concentration mentioned above, these c.d. spectra also have a minimum around 226–228 nm and a shoulder at 208 nm in the region of higher pH at which the turbidity of solution appears. Also in this case the

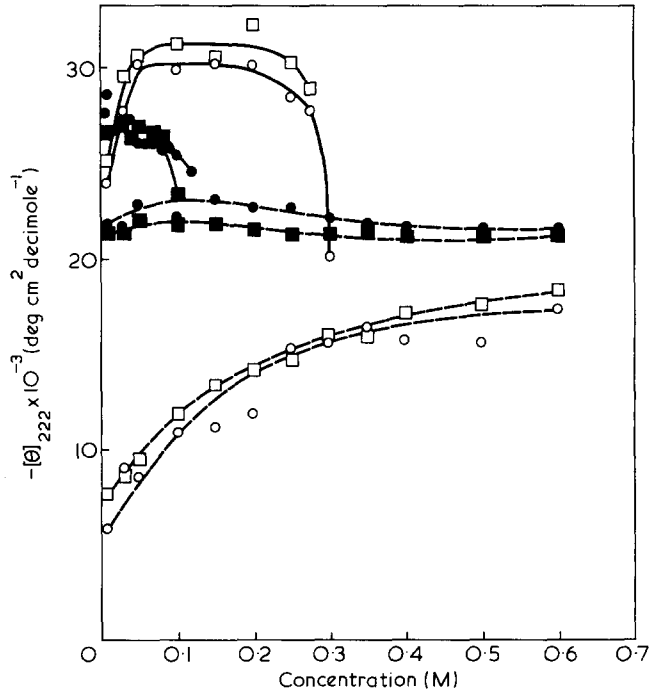

Figure 4 Plot of mean residue ellipticities, $-[\theta]_{222}$, as a function of $LiClO_4$ of KF concentration for the copolymers IV_{B-1}, IV_{E-1}, IV_{B-2}, and IV_{E-2}. (○—○); IV_{B-1} in $LiClO_4$, (□—□); IV_{E-1} in $LiClO_4$, (●—●); IV_{B-2} in $LiClO_4$, (■—■); IV_{E-2} in $LiClO_4$, (○- - -○); IV_{B-1} in KF, (□- - -□); IV_{E-1} in KF, (●- - -●); IV_{B-2} in KF, (■- - -■); IV_{E-2} in KF

turbidity is due to an aggregation of molecules of copolymer IV_{B-1} or IV_{B-2}. In the case of copolymer IV_{B-1}, the turbidity is observed above pH 9.45, 9.78, and 10.18 in $LiClO_4$ and KF solutions, and salt-free water, respectively. In the case of copolymer IV_{B-2}, such a turbidity appears above pH 8.29, 8.87, and 9.48 in $LiClO_4$ and KF solutions, and salt-free water, respectively. The pH-region at which the aggregation of molecules occurs is shifted to lower pH values with higher content of leucine in various solvent systems.

DISCUSSION

Conformation of polypeptides and c.d. spectra

Polypeptides in the α-helical conformation show a c.d. spectrum characterized by a typical negative double bond (trough) at 222 nm due to the n–π^* transition of the amide group and at 208 nm (*Figures 1* and *2*) which is one of the π–π^* transitions of the C=O group. The other one is responsible for the positive c.d. absorption peak at 198 nm (*Figures 2* and *3*), which cannot be observed in the presence of air due to the u.v. absorption of oxygen.

As shown below one can estimate the α-helix content from the specific ellipticities at these wavelengths.

The β-conformation leads to a broad negative band with only one minimum between 226 and 228 nm.

In the case of a mixture of α- and β-conformation the peak at 208 nm decreases with decreasing α-helix content whereas the peak at 222 nm is shifted to 226–228 nm.

If the polypeptide attains the random coil-conformation a c.d. spectrum like that in *Figures 1a* and *b* with a broad shallow negative band between ~210 and 230 nm and another one at 200–210 nm occurs.

Conformation in salt-free water

As one can see from *Figure 1*, copolymers with a L-leucine content of ~50 mol% (IV_{B-1} and IV_{E-1}) are not α-helical in pure water at neutral pH like $(Lys)_n$; however at a L-leucine content of ~65 mol% (IV_{B-2} and IV_{E-2}) α-helices are formed under the same conditions to a high extent. The molar ellipticities of these two samples are $[\theta]_{208} = -30\,000$ deg cm^2 dmol^{-1} and $[\theta]_{222} = -25\,000$ deg cm^2 dmol^{-1}. From these values one can estimate the α-helix content in the following way. According to Greenfield and Fasman[18] the ellipticity of β-structure and nonperiodic conformation in the case of $(Lys)_n$ amounts to -4000 deg cm^2 dmol^{-1} at 208 nm, whereas that of the α-helix is $[\theta]_{208} = -33\,000$ deg cm^2 dmol^{-1}. Therefore they have evaluated the α-helix content f_H to a first approximation by

$$f_H(\%) = \frac{-[\theta]_{208} - 4\,000}{33\,000 - 4\,000} \qquad (1)$$

Using this relation f_H of the samples with 65 mol% L-leucine content is 90%. This rather high α-helicity may be explained (1) by a dilution effect of the L-leucyl residues situated between the positively charged L-lysyl residues increasing the distance of the basic groups repulsing one another by electrostatic forces. However, it should be pointed out that the moderate increase in L-leucine content from 50 mol% to 65 mol% is accompanied by a rather high conformational change. Therefore (2) the stabilization of the α-helix by hydrophobic interactions among the L-leucyl residues and between them and the apolar part of the L-lysyl side chains probably plays a very important role.

Conformation in salt solutions containing water structure breaking or making anions

The conformational stability change of proteins and polypeptides induced by neutral salts can be classified in two ways.

(1) The neutral salt interacts with the macromolecules non-specifically, e.g. by changing the diffuse part of the electric double layer (Gouy–Chapman layer).

(2) There is a specific interaction of the salt ions with the macromolecules, e.g. by a preferentially binding (adsorption) in the Stern layer by which a conformational change may be induced.

In general, NaCl and similar neutral salts belong to the first group. The ions of these electrolytes do not change the water structure very much, in contrast to those of the second group. To this belong salts containing big anions like ClO_4^-, I^-, SCN^-, etc., which are strong water structure breakers.

Sulphate anions are water structure makers like F^- and it has been shown as mentioned in the introduction that they do not undergo specific binding[10,11,13]. However, due to their divalency and size they may influence the water structure in the diffuse double layer around the macromolecules much more than the F^- ions do. Therefore, ClO_4^- and SO_4^{2-} ions, in spite of the fact that they are very similar in respect to size, shape and polarizability, behave differently against water structure and their influence on the stability of polypeptide and protein conformation. This has some importance for the phenomena discussed here for two reasons:

(1) In the case of basic homopoly(α-amino acids) like $(Lys)_n$ the water structure breaking effect of ClO_4^- favours the specific interaction with the positively charged side-chains because the water molecules around this anion can easily be stripped off[8]. As a consequence of such a specific binding of ClO_4^- and also other water structure breaking anions which was shown convincingly by several methods[8,10,11,13], an electrostatic shielding effect against the repulsing electrostatic forces of the charged groups of $(Lys)_n$ occurs and therefore induce α-helix formation. Sulphate anions, as water structure makers, undergo no specific binding to the polycation[10,11,13] and have no α-helix inducing effect.

(2) If hydrophobic interactions are involved in stabilizing the α-helix to a high extent as in the copolymer IV_{B-2} or in native proteins, one has also to consider the influence of water structure affecting anions on the hydrophobic interactions.

Therefore, in the case of copolymers with 50 mol% L-leucine these two effects may overlap. This would explain why $LiClO_4$ and $NaClO_4$ on the one hand and Li_2SO_4 and Na_2SO_4 on the other induce α-helix formation as shown in *Figure 3*: the sulphate anions, enriched in the diffuse part of the double layer, enhance the intensity of hydrophobic interactions by decreasing the entropy of water molecules near the apolar groups and concomitantly, α-helix formation occurs. Perchlorate anions stabilize the α-helix by an electrostatic shielding effect as a consequence of their binding. These findings seem to confirm what is claimed about the effect of water structure breaking and making anions on α-helix stabilization. *Figure 5* shows that at higher concentration

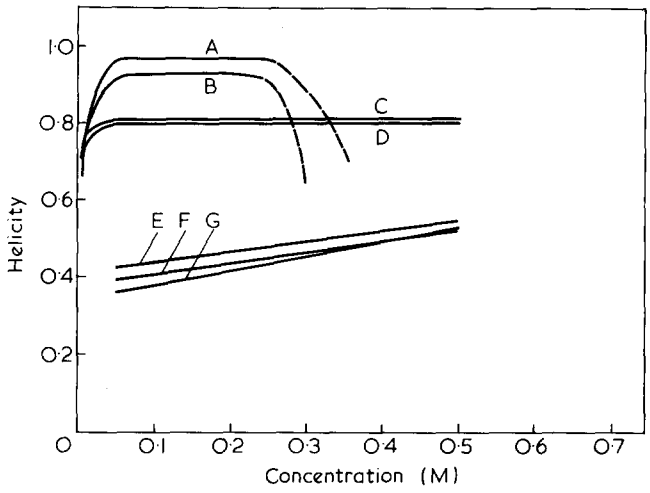

Figure 5 Helix content of copolymer IV_{B-1} as a function of salt concentration at 20.0°C and pH 7.0 ± 0.02. A; $NaClO_4$, B; $LiClO_4$, C; Na_2SO_4, D; Li_2SO_4, E; NaCl, F; NaF, G; KF

KF also has a α-helix inducing effect. This may be due to the water structure making character of F^-. However it is much lower than that of SO_4^{2-}, probably because of its monovalency and f_H amounting to ~53% only in 0.5 molar KF solution. (This concentration is much higher than in *Figure 3*.) The monovalency of the F^- may be responsible for a lower concentration and a lower water structure making effect in the diffuse double layer compared with SO_4^{2-}.

In the Li and Na sulphate solutions the α-helix content is lower (80%) than in the Li and Na perchlorate solutions (92 and 98%) and constant up to 0.95 mol l^{-1}. The helicity of copolymer in both ClO_4^- solutions seems to decrease at ~0.3 mol l^{-1} concentration (dashed lines). At this concentration a slight turbidity of the solution occurs as a consequence of aggregation of the polymer. With increasing temperature these c.d. spectra change to the well-known c.d. pattern of the α-helix and simultaneously the turbidity disappears. The c.d. spectra may be due to aggregated α-helices, as supposed by Cassim and Yang, who found similar c.d. spectra in the case of aggregated poly(L-glutamic acid)[19]. Similar c.d. spectra were found by Kim[20] and by Ganados and Bello[21] in the case of the aggregation of poly(N^{ε}-trimethyl-L-lysine). However, as mentioned above, these spectra seem to be those of a mixture of α-helix and β-conformation, which is probably responsible for the aggregation of the polymer molecules. If this is true, this would be an interesting example for a salt induced α→β transition.

Temperature dependence of α-helix content

The relation between α-helix content and temperature is shown in *Figures 6a* and *b*. The transition curves are rather broad, indicating that the temperature induced conformational change has no or at least a low cooperative character. In 0.05 mol l^{-1} ClO_4 solution up to 70°C about 80% helix of this copolymer IV_{B-1} is kept. In the case of copolymer IV_{B-2} its α-helical conformation is stable up to 80°C in salt-free water. Ostroy and coworkers[22] have reported that the α-helical conformation of the copolymer, $(D,L\text{-Lys})_m$–$(L\text{-Leu})_n$–$(D,L\text{-Lys})_m$, ($n = 56$) is stable up to 90°C in salt-free water at neutral pH, and this α-helix content is ~70% in the whole range of temperature. From these findings, therefore, one should expect from the very high helix stabilizing power of leucyl residues the high α-helical stability of our copolymers. It should be noted that the random copolymers examined in this work contain a portion of local blocking sequence of leucyl residues. This may be necessary for the stabilization of α-helix conformation, because the alternating copoly(L-Leu, L-Lys) reveals a tendency to adopt a β-structure with increasing salt concentration or the pH as well (Ebert and Kuroyanagi, following paper).

pH-dependence of conformation

The influence of leucyl residues on the conformation of copoly(L-Leu^x, L-Lys^y) becomes apparent when the data obtained are compared to those reported by Snell and Fasman[17]. This is illustrated in *Figure 7*. It is clear that the pH at the midpoint of the coil to helix transition is lowered with increasing leucine content. This influence can be interpreted as the result of an increase of the hydrophobicity of environment of the amino group due to the presence of the leucyl residues. The increasing amount of hydrophobic interactions with increasing L-leucyl content favours the α-helix stability at lower pH values.

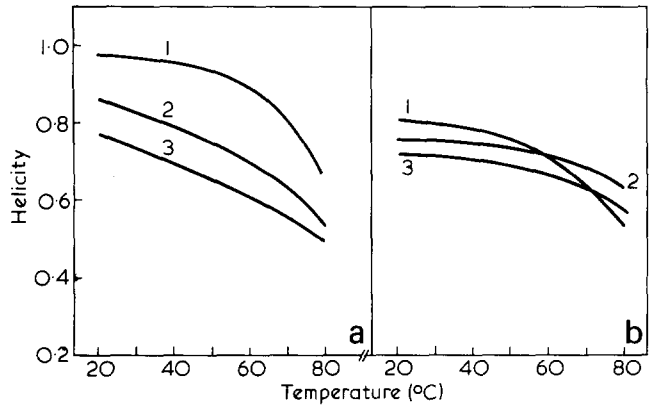

Figure 6 Helix content of copolymer IV_{B-1} as a function of temperature at pH 7.0 ± 0.02. (a); in $LiClO_4$ solution, (b) in Li_2SO_4 solution, (1) 0.05 mol l^{-1}, (2) 0.01 mol l^{-1}, (3) 0.005 mol l^{-1}

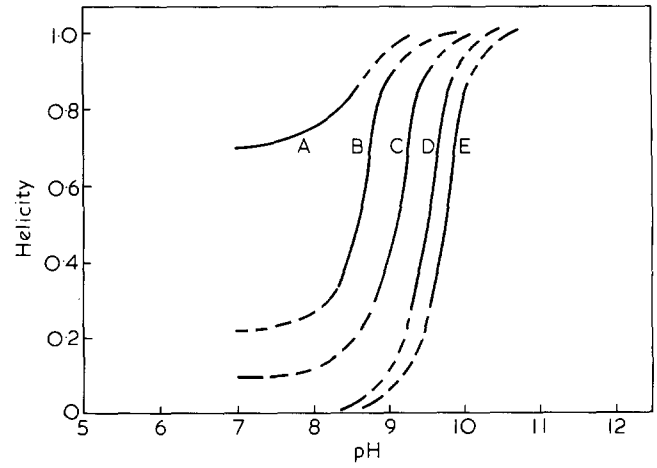

Figure 7 Helix content of copoly(Leu^x, Lys^y) in 0.05 mol l^{-1} KF solution as a function of pH. A; copoly($Leu^{66.0}$, $Lys^{34.0}$), IV_{B-2} in this work, B; copoly($Leu^{48.3}$, $Lys^{51.7}$), IV_{B-1} in this work, C; copoly($Leu^{41.0}$, $Lys^{59.0}$), by Snell and Fasman, D; copoly($Leu^{32.0}$, $Lys^{68.0}$), by Snell and Fasman, E; copoly($Leu^{16.0}$, $Lys^{84.0}$), by Snell and Fasman

The turbidity at higher pH values (>10.1 for IV_{B-1} and >9.48 for IV_{B-2}) can be discussed in the same way mentioned above.

CONCLUSION

Summarizing the discussion given above it follows that:

(1) α-helix formation of $(Lys)_n$ in the protonated state is induced by strong water structure breaking anions like perchlorate, but not by structure making anions like sulphate.

(2) In the case of random copoly(L-Leu, L-Lys), perchlorate and sulphate anions induce α-helix formation at pH 7 (protonated lysyl side groups).

(3) Therefore perchlorate and sulphate anions (i.e., water structure breaking and making anions) influence different kinds of intermolecular interactions of the amino acid side chains. Perchlorate cancels the electrostatic repulsive forces (shielding effect) as a consequence of its strong specific interactions with the protonated side chains, whereas sulphate anions which interact non-specifically with the basic groups obviously enhance the hydrophobic interactions of the apolar leucyl side chains.

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REFERENCES

1 Ebert, G., Ebert, Ch. and Wendorff, J. *Kolloid Z. u. Z. Polymere* 1970, **237**, 229
2 von Hippel, P. H. and Schleich, T. in 'Structure and Stability of Biological Macromolecules' (Eds. S. N. Timasheff and G. D. Fasman), Marcel Dekker Inc., New York, 1969, pp. 417–574
3 Rifkind, J. M. *Biopolymers* 1969, **8**, 685
4 Cassim, J. Y. and Yang, J. T. *Biopolymers* 1970, **9**, 1475
5 Ebert, Ch., Ebert, G. and Werner, W. *Kolloid Z. u. Z. Polymere* 1973, **251**, 504
6 Conio, G., Trefiletti, V., Bodria, F., Troglia, C. and Patrone, E. *Biopolymers* 1974, **13**, 1483
7 Ebert, Ch. and Ebert, G. *Progr. Colloid Polym. Sci.* 1975, **57**, 100
8 Murai, N., Miyazaki, M. and Sugai, S. *Nippon Kagaku Kaishi* 1976, **4**, 659
9 Ebert, Ch. and Ebert, G. *Colloid Polym. Sci.* 1977, **255**, 1041
10 Ebert, G., Ebert, Ch. and Paudjojo, L. *Progr. Colloid Polym. Sci.* 1978, **65**, 60
11 Ebert, G. and Paudjojo, L. in 'Dynamic Aspects of Biolectrolytes and Biomembranes', (Ed. F. Oosawa), Kodansha, Tokyo, 1981
12 Tiffany, M. L. and Krimm, S. *Biopolymers* 1969, **8**, 327
13 Paudjojo, L. *Thesis* Marburg(L), F. R. G., 1979
14 Okuda, T. and Zahn, H. *ber. Bunges* 1965, **98**, 1164
15 Fasman, G. D., Idelson, M. and Blout, E. R. *J. Am. Chem. Soc.* 1961, **83**, 709
16 Fasman, G. D., Lindblow, C. and Bodenheimer, E. *Biochemistry* 1964, **3**, 155
17 Snell, C. R. and Fasman, G. D. *Biopolymers* 1972, **11**, 1723
18 Greenfield, N. and Fasman, G. D. *Biochemistry* 1969, **8**, 4108
19 Cassim, J. Y. and Yang, J. T. *Biochem. Biophys. Res. Comm.* 1967, **26**, 58
20 Kim, Y. H. *Thesis* Marburg(L), F. R. G., 1978
21 Granados, E. N. and Bello, J. *Biopolymers* 1979, **18**, 1479
22 Ostroy, S. E., Lotan, N., Ingwall, R. T. and Scheraga, H. A. *Biopolymers* 1970, **9**, 749