Salt effect on the conformation of an alternating copolymer of L-leucine and L-lysine*

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The 'statistical' copoly(L-leucyl-L-lysine) is shown to undergo a coil to α-helix transition by either adding salt or raising the pH. However, the corresponding alternating copolymer undergoes a conformational transition from a coil to a b-structure, which is accomplished by adding various salts or by raising the pH. The b-structure fraction of the alternating copoly(L-leucyl-L-lysine) is especially enhanced by either water structure, breaking or making anion (e.g., ClO_4^- or SO_4^{2-}), in which the β-structure may be induced by a different molecular mechanism. As a consequence of the strong shielding effect due to the specific binding of ClO_4^- with charged side chain as well as its electrochemical monovalency, an intramolecular cross-β-structure is formed at lower salt concentration (e.g., 0.10 mol l^{-1}). However, in the presence of SO_4^{2-}, due to its divalency, intermolecular β-pleated sheet structure occurs even at very low salt concentration (e.g., 0.002 mol l^{-1}). Furthermore, in both cases the resulting β-structure is stable up to 90°C.

Keywords Salt effect; conformation; L-leucine; L-lysine; alternating copolymer; structure

INTRODUCTION

Alternating copolymers containing hydrophobic and hydrophilic amino acid residues are known to be very important for an understanding of biological structures because they tend to form β-sheets with one hydrophobic and one hydrophilic surface and consequently such sheets stack together to form membrane-like aggregates. Brack and Orgel[1] have found that alternating copoly(L-valyl–L-lysine) undergoes a conformational transition from a disordered structure to a β-structure on raising the pH to 8.8 or by adding NaCl. They suggested that bilayers with a hydrophobic interior and a hydrophilic exterior may be present in aqueous solution. Also Rippon *et al.*[2] have reported that alternating copoly(L-glutamyl–L-alanine) can assume α-helix immediately upon neutralization of ionizable side chains, but after standing for several weeks this copolymer undergoes a helix to β-structure transition. Wagner *et al.*[3] studied in detail the conformation of statistical and alternating copoly(L-lysyl–L-phenylalanine). The alternating copolymer is shown to adopt a β-structure with raising the pH or by adding $NaClO_4$ or methanol. Trudell[4] has reported that alternating copoly(L-tyrosyl–L-glutamic acid) aggregates and changes into antiparallel β-structure below pH 10.5. Recently Goodman *et al.*[5] have obtained similar results in the conformational studies of alternating copoly(L-tyrosyl–L-lysine).

It can be expected that the alternating arrangement of dissimilar residues plays an important role for the formation of such a β-structure. It should be pointed out here that such alternating copolymers can be induced to form a β-structure by adding salt or by changing the pH to a distinct region in which ionizable side chains are neutralized. Therefore, it is required to study in further detail the effect of salt on the conformation of alternating copoly(α-amino acids). The effect of various salts on the conformation of statistical copoly(L-leucyl–L-lysine) was reported previously. In this case the α-helix inducing effect is shown to be dependent on the kind of anions, e.g., ClO_4^-, SO_4^{2-}, Cl^- and F^-. The α-helix fraction of this copolymer is especially enhanced by water structure breaking anions like ClO_4^-. In this work the effect of such anions in the conformation of alternating copoly(L-leucyl–L-lysine) is reported.

EXPERIMENTAL

Materials

N-*Carbobenzoxy(Cbz-)-L-leucine* N-*hydroxysuccinimide ester (I)*

N-Cbz–L-leucine was prepared by the method of Williams and Young[6]. This ester (I) was prepared by the method of Fridkin *et al.*[7]. The yield of the product was 74%; mp, 118°C (lit., yield 80%; mp, 115°–117°C).

Anal. Calcd. for $C_{18}H_{22}N_2O_6$; C, 59.66, H, 6.12, N, 7.73. Found; C, 59.52, H, 6.41, N, 7.93.

N^{ε}-*Trifluoroacetyl–L-lysine (II)*

N^{ε}-Trifluoroacetyl–L-lysine was prepared from L-lysine and ethyl thioltrifluoroacetate[8], in analogy to the synthesis of N^{ε}-trifluoroacetyl–DL-lysine[9]. The yield of the product was 59%; mp, 270°–272°C (lit., yield 53%; mp, 273°–274°C).

Anal. Calcd. for $C_8H_{13}N_2O_3F_3$; C, 39.7, H, 5.4, N, 11.6. Found; C, 39.35, H, 5.09, N, 11.06.

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* Dedicated to Prof. T. H. Müller, Marburg, on his 75th birthday

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N-*Carbobenzoxy*–L-*leucine*–N$^{\varepsilon}$-*trifluoroacetyl*–L-*lysine* (*III*)

This dipeptide was prepared similarly to the synthesis of *N*-Cbz–L-leucine–N^{ε}-trifluoroacetyl–L-ornithine[7]. To a solution of *N*-Cbz–L-leucine *N*-hydroxysuccinimide ester (17.0 g, 46.96 mmol) in dioxane (120 ml) was added a solution of N^{ε}-trifluoroacetyl–L-lysine (11.4 g, 47.11 mmol) and sodium bicarbonate (8.0 g, 95.24 mmol) in water (80 ml). The reaction mixture was stirred for 2 h at room temperature. The mixture was dried in high vacuum and the residue was dissolved in dioxane (10 ml). To the solution was added water (10 ml) and then the solution obtained was acidified (pH 1–2) with 2N HCl, and the oily product extracted into ethyl acetate. The organic solution was washed twice with distilled water, dried over Na_2SO_4, and evaporated to dryness. The product was recrystallized from ethyl acetate and light petroleum. The yield of the product was 78%; mp, 157°–159°C.

Anal. Calcd. for $C_{22}H_{30}N_3O_6F_3$; C, 53.99, H, 6.13, N, 8.59. Found; C, 54.12, H, 6.09, N, 8.42.

N-*Carbobenzoxy*–L-*leucine*–N$^{\varepsilon}$-*trifluoroacetyl*–L-*lysine* N-*hydroxysuccinimide ester* (*IV*)

N-Cbz–L-leucine–N^{ε}-trifluoroacetyl–L-lysine (17.0 g, 34.76 mmol) was coupled with *N*-hydroxysuccinimide (4.2 g, 36.52 mmol) in dioxane (200 ml) with *N*,*N*-dicyclohexylcarbodiimide (7.5 g, 36.41 mmol) as the coupling reagent. After 8 h at room temperature the dicyclohexylurea was filtered off, the solution was evaporated in vacuum and the residue crystallized on addition of ether. After recrystallization from ethyl acetate and light petroleum, the yield of the product was 74%; mp, 124°–129°C.

Anal. Calcd. for $C_{26}H_{33}N_4O_8F_3$; C, 53.24, H, 5.63, N, 9.56. Found; C, 53.48, H, 5.83, N, 9.24.

L-*Leucine*–N$^{\varepsilon}$-*trifluoroacetyl*–L-*lysine* N-*hydroxysuccinimide ester hydrobromide* (*V*)

N-Cbz–L-leucine–N^{ε}-trifluoroacetyl–L-lysine *N*-hydroxysuccinimide ester (14 g, 23.9 mmol) was dissolved in 25% HBr in acetic acid (50 ml), and after 30 min at room temperature the solution was evaporated (25°C/1 mmHg) to dryness. The residue was crystallized on addition of ether and the product was recrystallized from isopropyl alcohol and ether. The yield of the product was 84%; mp, 109°–111°C.

Anal. Calcd. for $C_{18}H_{28}N_4O_6F_3Br$; C, 40.53, H, 5.25, N, 10.51. Found; C, 40.83, H, 5.25, N, 10.12.

Poly(L-*leucyl*–N$^{\varepsilon}$-*trifluoroacetyl*–L-*lysine*) (*VI*)

The dipeptide ester hydrobromide (9.5 g, 17.8 mmol) was dissolved in dimethylformamide (15 ml). Triethylamine (2.47 ml, 17.8 mmol) was added, and the mixture was stirred at room temperature in a round-bottomed flask, protected against moisture with a $CaCl_2$ tube. After 7 days the viscous mixture was diluted with 15 ml of dimethylformamide, and poured into 300 ml of water. The precipitate formed was filtered off and washed with water. The polymeric product was dried *in vacuo* to yield 4.2 g of polymer (72%).

Anal. Calcd. for $C_{14}H_{22}N_3O_3F_3$; C, 49.85, H, 6.53, N, 12.46. Found; C, 50.24, H, 6.48, N, 12.27.

Poly(L-*leucyl*–L-*lysine HCl*) (*VII*)

Poly(L-leucyl–N^{ε}-trifluoroacetyl–L-lysine) (2 g) was suspended in 1 M piperidine in methanol and the mixture was stirred for 2 h. A solution of 1 M piperidine in water (80 ml) was then added to form a homogeneous, fine suspension. After stirring for 2 days at room temperature, the solvents were evaporated *in vacuo*, and the concentrated solution was acidified (pH 1–2) with 2 N HCl. To this solution was added methanol (100 ml) and the fine precipitate was collected by filtration and then the polymeric product was washed with ether and dried over P_2O_5 *in vacuo* to yield 1.2 g of polymer (77%).

Anal. Calcd. for $C_{12}H_{24}N_3O_2Cl$; C, 51.80, H, 8.63, N, 15.11. Found; C, 52.31, H, 8.29, N, 15.46.

N$^{\alpha}$-*DNP-poly*(L-*leucyl*–N$^{\varepsilon}$-*trifluoroacetyl*–L-*lysine*) (*VIII*)

This compound was prepared with a reaction of copolymer (VI) and fluorodinitrobenzene according to a usual method.

Methods

Optical rotatory dispersion (*o.r.d.*)

O.r.d. measurements were made on a JASCO spectropolarimeter Model J-20. The rotation angle χ of a 0.5 g dl^{-1} solution was measured between 320 and 600 nm at 20.0°C with a quartz cell of 1 cm length. The Moffitt parameters a_0 and b_0 were obtained from the usual Moffitt plot[10].

Circular dichroism measurement (*c.d.*)

C.d. measurements were made on a JASCO spectropolarimeter Model J-20. The c.d. spectra were run in 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.

Determination of molecular weight

The molecular weight of dinitrophenylated polymer, N^{α}-DNP-poly(L-leucyl–N^{ε}-trifluoroacetyl–L-lysine) was determined from the quantitative analysis of dinitrophenyl group of the compound on Carl Zeiss spectrophotometers Model DMR-21 with 1 cm quartz cell.

Viscosimetry

Intrinsic viscosity of copolymer was determined in dichloroacetic acid using Ubbelohde viscometer, Viscomatic FICA, at a temperature of 20±0.1°C.

RESULTS

The synthesis of alternating copoly(L-leucyl–L-lysine) is schematically described as follows:

$$\underset{(\mathrm{I})}{\text{Cbz-Leu-OSu}} + \underset{(\mathrm{II})}{N^{\varepsilon}\text{-TFA-Lys}} \rightarrow \underset{(\mathrm{III})}{\text{Cbz-Leu–}N^{\varepsilon}\text{-TFA-Lys}}$$

$$\rightarrow \underset{(\mathrm{IV})}{\text{Cbz-Leu–}N^{\varepsilon}\text{-TFA-Lys-OSu}} \rightarrow \underset{(\mathrm{V})}{\text{HBr·Leu–}N^{\varepsilon}\text{-TFA-Lys-OSu}}$$

$$\rightarrow \underset{(\mathrm{VI})}{(\text{Leu–}N^{\varepsilon}\text{-TFA-Lys})_n} \rightarrow \underset{(\mathrm{VII})}{(\text{Leu–Lys})_n}$$

$$\downarrow$$

$$\underset{(\mathrm{VIII})}{\text{DNP}^{\alpha}\text{–(Leu–}N^{\varepsilon}\text{-TFA-Lys})_n}$$

Cbz, carbobenzoxy; TFA, trifluoroacetyl;
Su, succinimide; DNP, 2,3-dinitrophenyl

Table 1 Moffitt–Yang parameter b_0 and α-helix fraction f_H for the alternating copoly(Leu–N^ε-TFA-Lys)

Solvent	b_0	f_H
DMF	−299	0.48
DMSO	−263	0.42
CH (10% DCA)	−322	0.52
CH (30% DCA)	−217	0.35

DMF; dimethylformamide, DMSO; dimethylsulphoxide, CH; chloroform, DCA; dichloroacetic acid. f_H is α-helix fraction using b_0 = −625 for 100% α-helix[12]

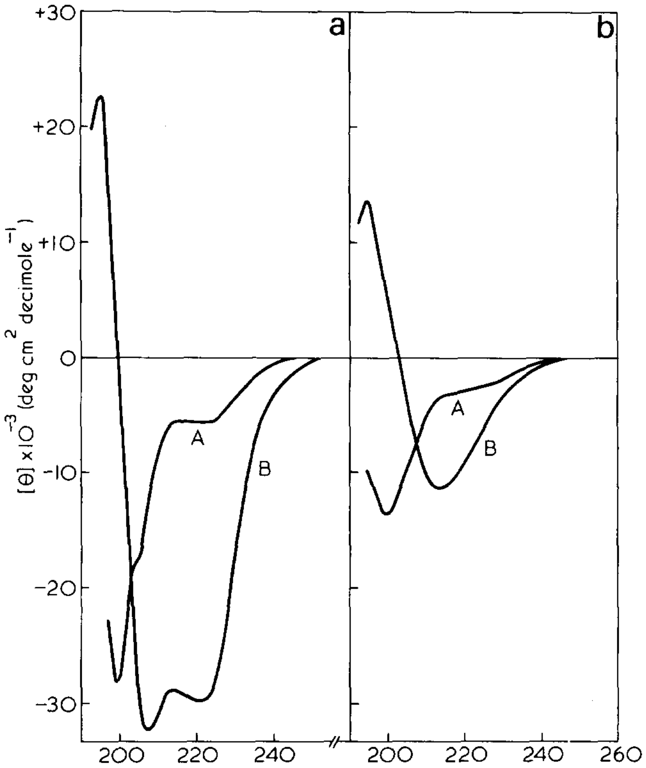

Figure 1 C.d. spectra of statistical and alternating copolymers at 20.0°C and pH 7.0 ± 0.02. A; in salt-free water, B; 0.10 mol l^{-1} $LiClO_4$. (a) 'statistical' copoly(Leu$^{48\cdot3}$, Lys$^{51\cdot7}$); (b) alternating copoly(L-leucyl–L-lysine)

The molecular weight of dinitrophenylated compound (VIII) was determined from the quantitative analysis of dinitrophenyl group by a spectrophotometer. An average molecular weight of this copolymer was calculated to be 5000, corresponding to 14 repeating units of (Leu–Lys). The intrinsic viscosity of copoly(L-leucyl-N^ε-TFA-L-lysine) in dichloroacetic acid at 20.0°C was 0.10 dl g^{-1}.

(*a*) *O.r.d. measurement*

O.r.d measurements of alternating copoly(L-leucyl-N^ε-TFA-L-lysine) were in various solvents at 20.0°C. This copolymer is not soluble in pure chloroform, but on addition of dichloroacetic acid it becomes soluble. *Table 1* shows the Moffitt–Yang parameter, b_0 value of this copolymer. The α-helix fraction of this copolymer is shown to be about 0.5 in dimethylformamide.

(*b*) *C.d. measurement*

C.d. measurements depending on electrolyte concentration. C.d. spectra of 'statistical' and alternating copolymers of L-leucine and L-lysine are shown in *Figure 1*. The c.d. spectra of statistical copolymer are from the previous work. The 'statistical' copolymer has been shown to assume α-helical conformation even at neutral pH by addition of $LiClO_4$. The alternating copolymer cannot form an ordered conformation in salt-free water at neutral pH. However, by addition of $LiClO_4$ the c.d. spectra change and then show a pattern of typical β-structure with a mean residue ellipticity, $-[\theta]_{215}$, of 11 000 at the concentration of 0.10 mol l^{-1}. *Figure 2* shows c.d. spectra of alternating copoly(L-leucyl–L-lysine) in $LiClO_4$ and $NaClO_4$ solutions at various salt concentration. This copolymer is shown to undergo a conformational transition from a disordered to an ordered structure with increasing ClO_4^- concentration, independent of the kind of cation. Above 0.10 mol l^{-1} concentration the polymer solutions become slightly turbid. C.d. spectra of the alternating copolymer in NaF and KF solutions are similar. In this case this copolymer does not form an ordered structure at the salt concentration as low as 0.10 mol l^{-1}. With increasing salt concentration up to 0.5 mol l^{-1}, however c.d. spectra which indicate a β-structure are observed, independent of the kind of cation. In both solutions the value of mean residue ellipticity, $-[\theta]_{215}$, is shown to be 7400–7500 at the concentration of 0.50 mol l^{-1}. In NaCl solution the value of $-[\theta]_{215}$ amounts to 7900 at the same concentration. In those cases the polymer solutions remain clear in all ranges of concentration examined. In Li_2SO_4 and Na_2SO_4 solutions the c.d. spectra indicating a β-structure can be observed already at salt concentration as low as 0.002 mol l^{-1}, and above this concentration the polymer solution becomes slightly turbid.

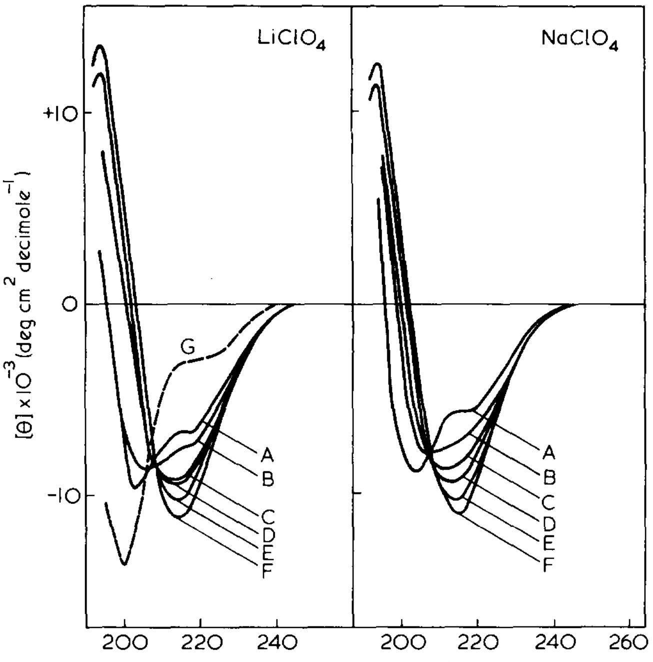

Figure 2 C.d. spectra of alternating copoly(Leu–Lys) in $LiClO_4$ and $NaClO_4$ solutions as a function of salt concentration (mol l^{-1}) at 20.0°C and pH 7.0 ± 0.02. A; 0.005, B; 0.01, C; 0.02, D; 0.03, E; 0.05, F; 0.10, G; in salt-free water

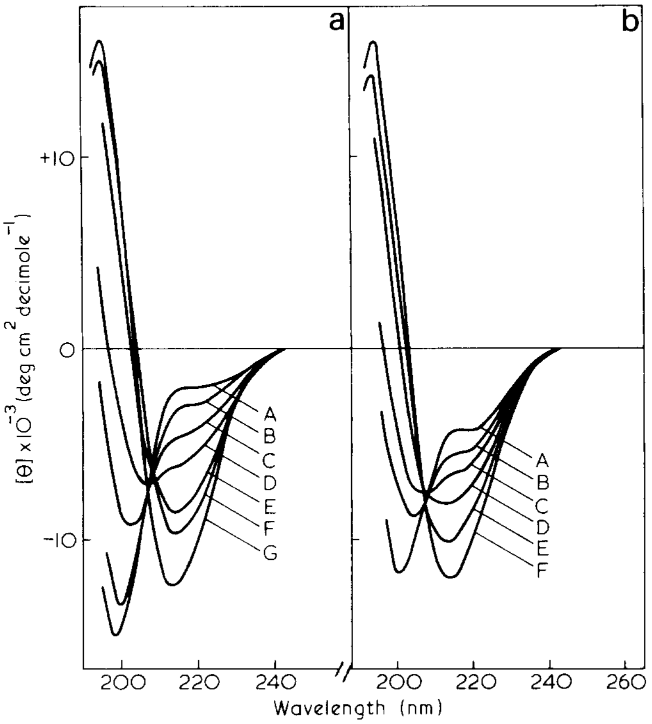

Figure 3 C.d. spectra of alternating copoly(Leu–Lys) as a function of pH at 20.0°C. (a) in salt-free water: A; pH = 3.50, B; pH = 7.08, C; pH = 8.09, D; pH = 8.50, E; pH = 8.76, F; pH = 9.05, G; pH = 9.60. (b) In 0.005 mol l^{-1} NaClO$_4$: A; pH = 3.52, B; pH = 7.04, C; pH = 7.85, D; pH = 8.25, E; pH = 8.49, F; pH = 8.96

C.d. measurements depending on pH. The pH-induced conformational transition of the alternating copoly(L-leucyl–L-lysine) was studied in solutions of various salts, as well as in salt-free water. The c.d. spectra of this copolymer at various pH values in salt-free water as well as in 0.005 mol l^{-1} NaClO$_4$ are shown in *Figure 3* as representative ones. The c.d. spectra of copolymer change with raising the pH, and then show a pattern of β-structure with minimum of mean residue ellipticity at 215 nm. In salt-free water the value of $-[\theta]_{215}$ amounts to 12 200 at pH 9.60, and above this pH the polymer solution becomes slightly turbid. In 0.005 mol l^{-1} NaClO$_4$ the value of $-[\theta]_{215}$ is shown to be 11 900 at pH 8.96, and above this pH a turbidity of the solution is observed. In solutions of NaCl, NaF, and KF the amount of $[\theta]_{215}$ increases with raising the pH, and above pH 9 the solutions become also slightly turbid. In 0.05 mol l^{-1} LiClO$_4$ and NaClO$_4$ solutions this copolymer exists in an ordered structure even at around pH 3.5. The pH of the midpoint at which a conformational transition occurs is shown to be dependent on the kind of anion, and is affected especially by ClO$_4^-$.

C.d. measurement depending on temperature. The thermal stability of an ordered structure of alternating copoly(L-leucyl–L-lysine) was studied in LiClO$_4$ solution. The amount of $[\theta]_{215}$ decreases slightly with raising temperature. The c.d. spectra of this copolymer in LiClO$_4$ solution are that of a β-structure even at higher temperature (90°C).

DISCUSSION

It is shown that the alternating copoly(L-leucyl-N^{ε}-TFA-L-lysine) with a molecular weight of 5000 can assume partially α-helical conformation in dimethylformamide and dimethylsulphoxide, as well as in chloroform containing a small amount of dichloroacetic acid. Also the corresponding alternating copoly(L-leucyl–L-lysine) of a low molecular weight can partially form α-helix in trifluoroethanol as well as in alcohol–water mixture. Therefore it could be considered that this alternating copolymer having a degree of polymerization of about 30 has already α-helix inducing property under distinct conditions.

As it can be seen from *Figure 1*, the alternating copoly(L-leucyl–L-lysine) reveals a tendency to adopt a β-structure. The corresponding 'statistical' copolymer has a very strong tendency to adopt the α-helical conformation. One can explain this observation in the following way. The statistical copolymer does not possess a rigorous structural order along the polymer chain in the sense that both amino acid residues are not regularly located along the chain because this copolymer is prepared via the usual method of NCA copolymerization, and therefore the α-helix which can be induced in the cases of corresponding homopoly(α-amino acids), that is, poly-L-leucine and poly-L-lysine, may be the preferred conformation. The alternating copolymer, however, has a rigorous structural order along the chain, which may produce a β-structure consisting of hydrophobic residues on one side and hydrophilic ones on the other side in water.

From the c.d. spectra, the alternating copoly(L-leucyl–L-lysine) is found to undergo a conformational transition from a disordered to a β-structure by adding various salts. In perchlorate solution (*Figure 2*) this copolymer is able to form a β-structure at the concentration of 0.10 mol l^{-1} to a high extent. This observation can be interpreted in terms of the shielding effect through a specific binding of ClO$_4^-$ anions onto positively charged side groups. In sulphate solution such a salt-induced transition to a β-structure already occurs at a concentration as low as 0.002 mol l^{-1}. It is interesting that the conformational transition in sulphate solution occurs at much lower concentrations, compared with that in perchlorate solution. Water structure making anions like SO$_4^{2-}$ are not specifically bound to the polycation as water structure breaking anions like ClO$_4^-$ (ref. 11). Therefore, in this case one should consider another effect for the conformational transition. However, the hydrophobic interaction among leucine side chains is enhanced by SO$_4^{2-}$ ions via influencing water structure and therefore the formation of a β-structure might become favourable. However, in this case the electrochemical divalency of the SO$_4^{2-}$ anion also plays a very important role.

The polymer solution is found to become turbid above ClO$_4^-$ concentration of 0.10 mol l^{-1} as well as above SO$_4^{2-}$ concentration of 0.003 mol l^{-1}. This observation is similar to that obtained in the case of the alternating copoly(L-leucyl–L-ornithine). It should be pointed out here that ClO$_4^-$ and SO$_4^{2-}$ anions are very similar in respect to structure, size, shape, and polarizability, but they differ in their influence on water structure and in electrochemical valency. In addition, Brack and Orgel[12] reported that the bilayers consisting of a hydrophobic interior and a hydrophilic exterior might be formed in aqueous solution. Taking into account the difference in electrochemical valency of both anions and the existence of such bilayers, the precipitation of polymer in sulphate solution could be interpreted in terms of a ready aggregation of bilayers. That is to say, SO$_4^{2-}$ ions can induce ionic interactions between different bilayers owing to their divalency and therefore the precipitation of polymer is already observed at much lower SO_4^{2-} concentration. However, ClO_4^- ions cannot induce such an ionic intermolecular binding because of its monovalency. As a consequence of the strong shielding effect owing to a specific binding of perchlorate ions with positively charged side chains, probably intramolecular cross-β-structure is formed at lower salt concentrations. Above 0.10 mol l^{-1} ClO_4^- concentration, however, the electrostatic repulsion force among positively charged lysine side chains may be thoroughly cancelled, and consequently an aggregation of polymer chains occurs through the intermolecular binding among hydrophobic parts of lysine side chain[12] as well as the intermolecular interaction among leucine side chains. This is supported by the finding that the precipitation of this alternating copolymer in salt-free water occurs as the positively charged side chains are thoroughly neutralized by raising the pH.

In perchlorate solution the pH of the midpoint at which a conformational transition occurs is shifted to lower values even at 0.1 times as low as concentration of neutral salts like NaCl, NaF, and KF. Furthermore, the pH-induced transition in perchlorate solution is suppressed at the concentration as high as neutral salts, that is, 0.05 mol l^{-1}. One can conclude that the shielding effect through a specific binding of ClO_4^- ions onto the charged groups plays a very important role for the formation of a β-structure.

The pH-induced coil to β transition for the alternating copoly(L-leucyl–L-lysine) occurs in a sharp range from pH 8.0 to 9.0. This cooperative transition for the alternating copolymer may be due to their structural regularity along the polymer chain. The solution of the alternating copolymer becomes slightly turbid above pH 9.6, whereas such a turbidity for the corresponding 'statistical' copoly($Leu^{48.3}$, $Lys^{51.7}$) is observed above pH 10.3. From this finding one can expect that the β-structure bilayer with a hydrophobic interior and a hydrophilic exterior is more favourable for the intermolecular aggregation, compared with an α-helix of the 'statistical' copolymer.

It has become apparent that the salt-induced as well as pH-induced β-structure has a rather high thermal stability. The β-structure of the alternating copolymer formed at pH 9.5 in salt-free water is shown to be kept up to 90°C. It is well known that poly-L-lysine above pH 11 undergoes an α to β transition through an intermediate random coil with raising temperature[12]. This can be interpreted in terms of the intermolecular binding between hydrophobic parts of neutralized lysine side chains. Taking into account such a hydrophobic interaction, the β-structure of the alternating copolymer owes its high thermal stability to the intermolecular hydrophobic interaction between hydrophobic parts of lysine side chains and to some degree to hydrogen bonds between the neighbouring NH_2 groups.

CONCLUSION

The 'statistical' copoly($Leu^{48.3}$, $Lys^{51.7}$) undergoes salt-induced as well as pH-induced conformational transition from coil to α-helix. The alternating copoly(L-leucyl–L-lysine), however, does not show such a conformational transition. In the case of this alternating copolymer, the rigorous structural arrangement along the chain might produce a β-sheet structure consisting of hydrophobic residues on one side and hydrophilic ones on the other side when the repulsion among the positively charged amino groups of lysine side chains is cancelled by adding salts or by raising the pH. The β-structure of the alternating copolymer is especially favoured in the presence of water structure breaking anion like ClO_4^- through a specific binding of ClO_4^- ions onto positively charged side chain groups. Water structure making anions like SO_4^{2-} are not specifically bound to the polycation as ClO_4^- ion. In solutions containing SO_4^{2-}, however, the formation of a β-structure might be favourable due to the intermolecular hydrophobic interactions between the leucyl residues of neighbouring chains and to ionic interactions between one SO_4^{2-} and two NH_3^+ groups of two neighbouring polymer molecules.

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