Anion-induced conformational transition of poly(L-arginine) and its two homologues

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The interaction of various anions with poly(L-arginine) and its two homologues, poly(L-homoarginine) and poly(L-α-amino-γ-guanidinobutyric acid), has been studied in the neutral pH region. These polypeptides were found to change their conformations from coil to helix due to I^-, ClO_4^- and SCN^-, and among them poly(L-homoarginine) and poly(L-arginine) were found to change conformation at smaller concentrations of the anions than poly(L-lysine). The helix of poly(L-homoarginine) was induced in the lyotropic series of the counteranions. Using the van't Hoff enthalpies for the transition of the polypeptides and the transition enthalpies obtained from calorimetry, the various thermodynamic parameters of the transitions were calculated by use of a theory based on the non-specific and the specific binding interactions of the anions with the charged sites on the polypeptides. The binding constants of ClO_4^- and SCN^- with poly(L-homoarginine) and poly(L-arginine) were found to be four times as large as those with poly(L-lysine). The free energy changes of the transitions from coil to helix of poly(L-homoarginine) and poly(L-arginine) were found to be more negative than that of poly(L-lysine). From these results, the guanidinium ion can be concluded to form easily the ion pair with the anions on the polymer surface, thus allowing poly(L-homoarginine) and poly(L-arginine) to change their conformations at smaller concentrations of the anions than poly(L-lysine).

INTRODUCTION

The interaction of ionizable polypeptides with neutral salts has been investigated by many workers[1]. Poly(L-glutamic acid) is only contracted in volume upon addition of monovalent cations such as Na^+ and K^+ (ref 2). On the other hand, poly(L-lysine) (PLL) is effectively transformed from coil to helix by I^-, ClO_4^- and SCN^- at relatively low concentrations of the anions in acidic and neutral pH regions, while Cl^- and Br^- scarcely induce the conformational change at all[3-5]. As I^-, ClO_4^- and SCN^- are known to be 'water structure breaking anions' and to disrupt the secondary structures of proteins, the coil to helix transition of PLL induced by these anions had been considered to be curious.

Some authors have assumed interactions of these anions with the charged amino groups in the side chain of PLL through specific binding, which reduces the charge density on the polymer and induces the helix formation[3-5]. Recently, Conio *et al.*[6] have estimated the intrinsic binding constant of SCN^- with the charged amino group of PLL by potentiometric titration measurements of poly(L-ornitine) (PLO) in aqueous KCl and KSCN solutions and with the results successfully explained the salt-induced conformational change of PLL in terms of non-specific and specific interactions.

On the other hand, the hydrophobic interaction is an important factor in stabilizing the ordered structures of polypeptides in aqueous solution. In studies on PLL, PLO and poly(L-α,γ-diaminobutyric acid) (PLDAB), the helix stability increases with an increase in number of methylene groups in the side chain[7,8]. Such a dependence of the helical stability on the side chain length was also shown in a study on a series of polymers of N^5-(ω-hydroxyalkyl)-L-glutamine by Lotan *et al.*[9] and in our previous study on polycarboxylic acids with various lengths of side chain[10].

In contrast to PLL and PLO, few thermodynamic studies on the conformational stabilities of poly(L-arginine) (PLA) and its homologues have been performed, because the high pK value of guanidino groups in the side chains makes pH titration measurements difficult. Rifkind[11] has reported that PLA undergoes the conformational transition from coil to helix in aqueous $NaClO_4$ solution at lower salt concentration than PLL.

Here the interactions of various anions with poly(L-homoarginine) (PLHA), for which the number of methylene groups in the side chain is same as that of PLL, were studied qualitatively in the neutral pH region by measuring o.r.d. and conductivity and by observing the salting-out phenomenon. Further quantitative studies on the anion-induced conformational transition of PLHA, PLA and poly(L-α-amino-γ-guanidinobutyric acid) (PLAGB) in the same pH region were carried out by means of c.d., calorimetry and equilibrium dialysis. These results were interpreted in terms of the non-specific and the specific interactions of the anions with the charged groups in the polymers. The results were also compared with those of PLL and PLO.

EXPERIMENTAL

Materials

PLL and PLDAB were prepared by decarbobenzyloxylation of poly(N^ϵ-carbobenzyloxy-L-lysine) and poly(N^γ-carbobenzyloxy-L-diaminobutyric acid), respectively, which were obtained by polymerization of the corresponding *N*-carboxyamino-acid anhydrides in dioxane with sodium methoxide as an initiator. Degrees of polymerization (*DP*) of PLL and PLDAB, determined by viscosity measurement[12], were 1500 and 1060, respectively. Another PLL sample

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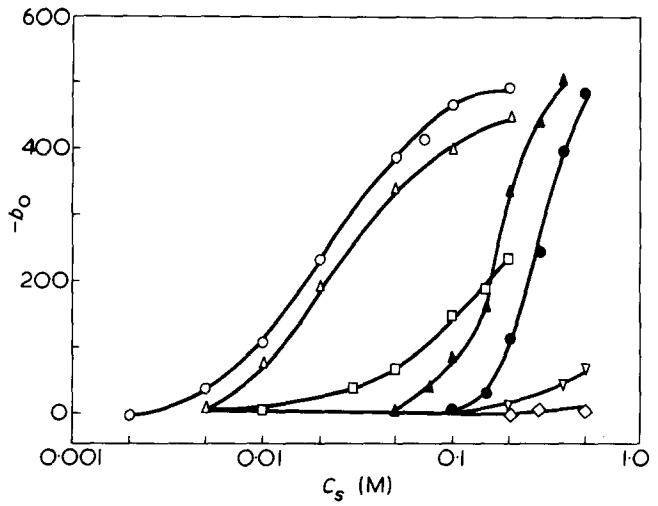

Figure 1 b_0 values of aqueous salt solutions of PLHA (*DP* = 1500) and PLL (*DP* = 1500) at 25.0°C. (○, △, □, ▽, ◇) and (●, ▲) refer to PLHA and PLL, respectively. ○, ●, NaSCN; △, ▲, $NaClO_4$; □, NaI; ▽, NaBr; ◇, NaCl

(*DP* = 450) was purchased from Protein Research Foundation, Osaka, and PLO (*DP* = 104) from Miles–Yeda Ltd.

PLHA, PLA and PLAGB were prepared by guanidization of the corresponding parent polymers with 1-guanyl-3,5-dimethylpyrazol nitrate according to the method of Ariely *et al.*[13]. After the polymer solutions had been dialysed against distilled water for a week, the polypeptides were converted into the hydrochloride form by passing through an Amberlite IRA-400 column in chloride form and then lyophilized. The remaining amino groups in the polypeptides were found by quantitation of the free amino groups with 2,4,6-trinitrobenzenesulphonic acid[14] to be 1.6 mol % for PLHA and 2.8 mol % for PLA and PLAGB. Other salt forms of the polypeptides were also prepared by the ion exchange column procedure. The pH values of aqueous solutions of polypeptides thus obtained were in a range 5.0 to 6.5.

Inorganic salts used here were the guaranteed reagents of Nakarai Chemicals Ltd, Kyoto.

Methods

O.r.d. and conductivity measurements were carried out at 25.0°C in the same manner as reported previously[15] with solutions of polymer concentration (C_p) of 0.01 monomol/l. C.d. measurements were performed with a Jasco J-20 spectropolarimeter with cells of 1, 5 and 10 mm length and the polymer solutions of C_p in a range 3×10^{-4} to 1×10^{-2} monomol/l.

Calorimetric studies were made at 25.0°C with a twin batch heat-burst microcalorimeter (RCM-1F, Oyo Denki Kenkyuzyo, Tokyo)[16]. Equal volumes (~5 ml) of the polypeptide solution of C_p in a range 5×10^{-3} to 3×10^{-2} monomol/l and of deionized water were mixed with about 0.5 ml of the salt solutions in the sample and the reference cells, respectively. Difference in the heat liberated between the cells was measured.

The extent of SCN^- binding with PLHA was estimated by the equilibrium dialysis method. A number of solutions of the polymer of different C_p in dialysis bags (Visking Co.) were equilibrated against an external salt solution in a container for 48 h with shaking at 25.0°C. In each run a dialysis bag containing no polymer was included as a reference. Before the dialysis experiments, the bags were boiled and washed with water. After the equilibration, the concentrations of SCN^- in the internal and the external solutions were determined by conductometric titration with $AgNO_3$[17].

Salting-out behaviours of the polypeptides were observed by addition of the aqueous salt solution (3 M) with a microsyringe to the polypeptide solutions (0.01 monomol/l) and stirring vigorously at 25.0°C until precipitation was found.

C_p was determined by the micro-Kjeldahl method and colloidal titration with potassium poly(vinyl sulphonate) and toluidine blue as an indicator[18].

RESULTS

Optical rotatory dispersion (o.r.d.)

Figure 1 shows the o.r.d. parameter, b_0, of PLL (*DP* = 1500) and PLHA (*DP* = 1500) in the aqueous salt solutions against the anion concentration. I^-, ClO_4^- and SCN^- evidently induce the conformational change of coil to helix with increase of the anion concentration. On the other hand, Cl^- and Br^- scarcely induce the transition. The anion series for inducing the helix is:

$$Cl^- \lesssim Br^- < I^- < ClO_4^- < SCN^- \quad (1)$$

It is noteworthy that PLHA is transformed by SCN^- or ClO_4^- at about 1/10 of the anion concentration required for the transition of PLL.

Salting-out

The precipitation of PLHA observed at salt concentrations of 0.29, 0.34, 0.34, 0.62 and 0.92 M for $NaClO_4$, NaSCN, NaI, NaBr and NaCl, respectively. Thus, the anion series for salting-out of PLHA is:

$$Cl^- < Br^- < I^- \sim SCN^- < ClO_4^- \quad (2)$$

Specific conductance

The dependence of the specific conductance, κ, of solutions of the various salts of PLHA on the added salt concentration are shown in *Figure 2*, where κ values of the added

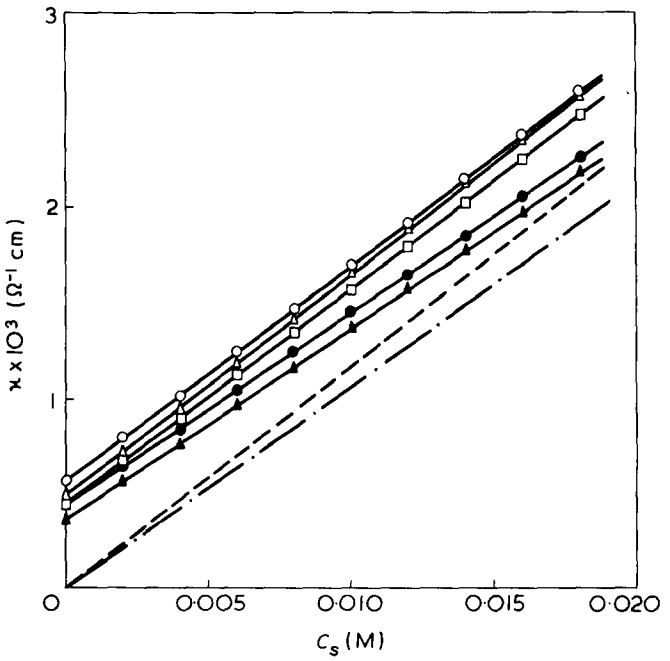

Figure 2 Specific conductivities of aqueous salt solutions of PLHA and of inorganic salts at 25.0°C. ○, PLHA–NaCl; △, PLHA–NaBr; □, PLHA–NaI; ●, PLHA–$NaClO_4$; ▲, PLHA–NaSCN; – – – –, NaCl, NaBr and NaI; – · – · – ·, $NaClO_4$ and NaSCN

Table 1 $\Delta\kappa$ values of aqueous salt solutions of PLHA and PLL at 25.0°C

Polymer	Counterion				
	Cl^-	Br^-	I^-	ClO_4^-	SCN^-
PLHA	0.038	0.038	0.049	0.065	0.067
PLL	0.037	–	–	0.049	0.052

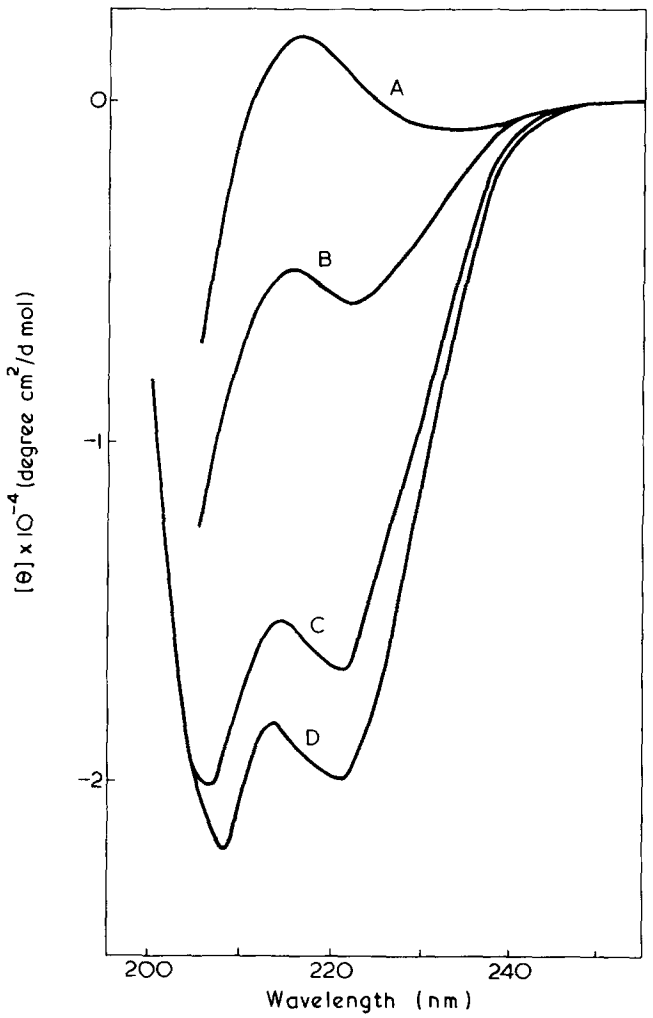

Figure 3 C.d. spectra of PLHA in aqueous solutions of $NaClO_4$ at 25.0°C. Concentrations of $NaClO_4$ are: A, 0 M; B, 0.01 M; C, 0.05 M; D, 0.10 M

salts are also shown. As a measure of deviation from the additivity law for conductivity, $\Delta\kappa$ was defined as[19,20].

$$\Delta\kappa = \frac{\kappa_p + \kappa_s - \kappa_{ps}}{\kappa_{ps}} \quad (3)$$

where κ_{ps}, κ_p and κ_s are the specific conductances of the polymer solution with the added salt, the salt-free polymer solution and the inorganic salt solution, respectively. For PLHA and PLL, the values are listed in *Table 1*. They increase in the order:

$$Cl^- \sim Br^- < I^- < ClO_4^- \sim SCN^- \text{ for PLHA} \quad (4)$$

and

$$Cl^- < ClO_4^- \sim SCN^- \text{ for PLL} \quad (5)$$

The value is considered to relate to the extent of counterion binding to the polyion through the site specific binding[19]. The above series are in agreement with those observed for other basic polyelectrolytes[19,21,23] and with the series for inducing the helical conformations of PLHA and PLL as shown in *Figure 1*.

Circular dichroism (c.d.)

To study in more detail the conformational transitions, c.d. measurements of the polypeptides in aqueous $NaClO_4$ solution were performed. *Figure 3* shows the spectra of PLHA in aqueous $NaClO_4$ solutions at 25.0°C. The double negative peaks at 222 nm and 207–208 nm indicate the α helical conformation. The spectra of PLHA and PLAGB were observed to be similar to those of PLHA. The residue ellipticities at 222 nm, $[\theta]_{222}$, of the polypeptides are plotted against the $NaClO_4$ concentration in *Figure 4*.

The conformational transition of PLHA estimated from the c.d. measurements agrees well with that from o.r.d. It appears that the transition of PLHA is not remarkably affected by the difference in *DP*, when *DP* is above 450. Also, the transition of PLA induced by $NaClO_4$ reported here is in agreement with that reported by Rifkind[11].

The helical conformations of these polypeptides are induced by $NaClO_4$ in the order: PLAGB < PLA < PLHA. However, the difference in the helical stability between PLHA and PLA is much smaller than that between PLL and PLO[5,6].

The temperature dependence of $[\theta]_{222}$ for PLHA is shown in *Figure 5*. A similar dependence for PLA was also observed. The helical structures of the two polypeptides become more stable with decrease of temperature. However, the polypeptides used here aggregate in the concentrated salt solutions at the lower temperature without attaining the fully helical conformation. For example, PLHA in aqueous $NaClO_4$ of 0.2 M did aggregate below 13°C.

As Cassim and Yang[23] have observed the fully helical PLL with $[\theta]_{221} = -35\,800$ degree cm²/dmol in aqueous $NaClO_4$ of 0.5 M at pH 6.30 and 25.0°C, the values of

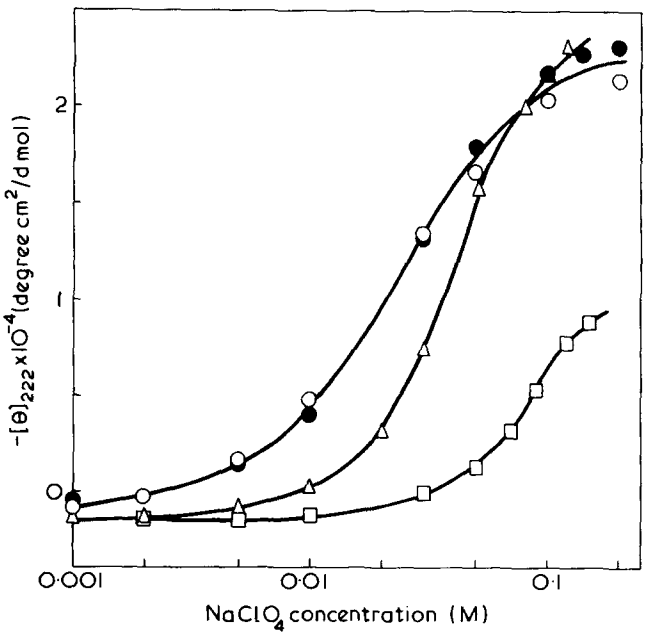

Figure 4 $[\theta]_{222}$ of the polypeptides in aqueous solutions of $NaClO_4$. ○, PLHA (*DP* = 450); ●, PLHA (*DP* = 1500); △, PLA; □, PLAGB

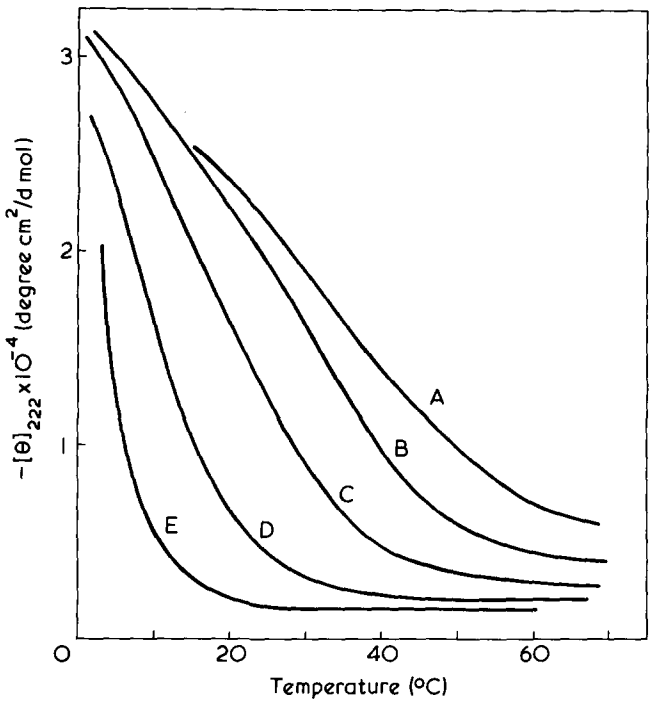

Figure 5 Temperature dependence of $[\theta]_{222}$ of PLHA at several concentrations of $NaClO_4$. Concentrations of $NaClO_4$ are: A, 0.20 M; B, 0.10 M; C, 0.03 M; D, 0.01 M; E, 0.005 M

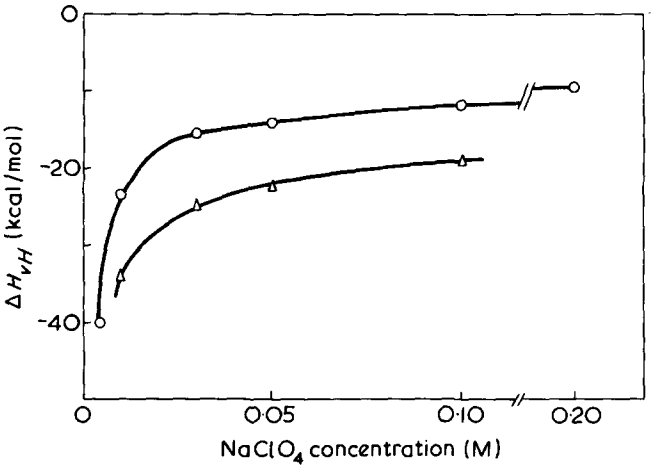

Figure 6 van't Hoff enthalpy changes of PLHA (○) and PLA (△) in aqueous solutions of $NaClO_4$

$[\theta]_{222}$ for the fully helical PLHA and PLA were assumed to be −35 000 degree cm^2/dmol in this study[24,25], and the helical contents of the polypeptides were calculated. With increase of $NaClO_4$ concentration, the melting temperature increases and the helical conformations of the polypeptides become more stable. The midpoints of the transition at 25.0°C correspond to 0.050 and 0.055 M of $NaClO_4$ for PLHA and PLA, respectively.

The van't Hoff enthalpy change of the transition, ΔH_{vH}, from coil to helix calculated from the temperature dependence of the helical content is shown in *Figure 6*, in which it steeply increases with the increase of $NaClO_4$ concentration. A similar change in ΔH_{vH} has been observed for PLL in an aqueous solution of $NaClO_4$[26].

Calorimetry

The transfer enthalpy, ΔH_t, of the polypeptide from water to the aqueous solution of $NaClO_4$, obtained by calorimetry, is shown in *Figure 7*. The change in ΔH_t seems to correspond to the conformational change shown in *Figure 4*. As the dilution enthalpy of the salt was experimentally compensated, the observed ΔH_t contains the transition enthalpy change, ΔH_0, from uncharged coil to uncharged helix and the binding enthalpy of the salt with the polypeptide, ΔH_0^b, as:

$$\Delta H_t = f_h \Delta H_0 + \gamma \Delta H_0^b \tag{6}$$

where f_h is the helical content and γ the degree of association of ClO_4^- with the polypeptide. On the other hand, binding of an anion (A^-) with a fully charged residue in a basic polypeptide (P^+) is expressed as[6]:

$$P^+ + A^- \rightleftarrows P^+A^- \tag{7}$$

and

$$K_{app} = \frac{\gamma}{(1-\gamma)C_s f_\pm} = K_0^b \exp[2w(1-\gamma)] \tag{8}$$

where K_{app} is the apparent binding constant, K_0^b is the intrinsic binding constant and C_s is the added salt concentration. w is the electrostatic interaction parameter for coil or helical form of the polypeptide and $f_\pm$ the mean activity coefficient of the salt. Conio *et al.*[6] have assumed proportionality of w to $\ln C_s$ and determined the w values for both forms of any polypeptide. Here, we used values of w_c (coil) and w_h(helix). At the low concentration of the salt where $f_h = 0$, an approximate relation can be derived from equations (6) and (8) as:

$$\frac{1}{\Delta H_t} = \frac{1}{K_0^b \Delta H_0^b \exp(2w_c) C_s f_\pm} + \frac{1}{\Delta H_0^b} \tag{9}$$

In *Figure 8*, the plots of $1/\Delta H_t$ against $1/[\exp(2w_c)C_s f_\pm]$ are shown for PLHA and PLAGB at concentrations of $NaClO_4$ of 0.001–0.005 and 0.01–0.04 M, respectively. K_0^b determined from the Figure is 1.2 ± 0.2 M^{-1} and ΔH_0^b -850 ± 150 cal/mol. The scattering of the values results from the small values of ΔH_t at low salt concentrations.

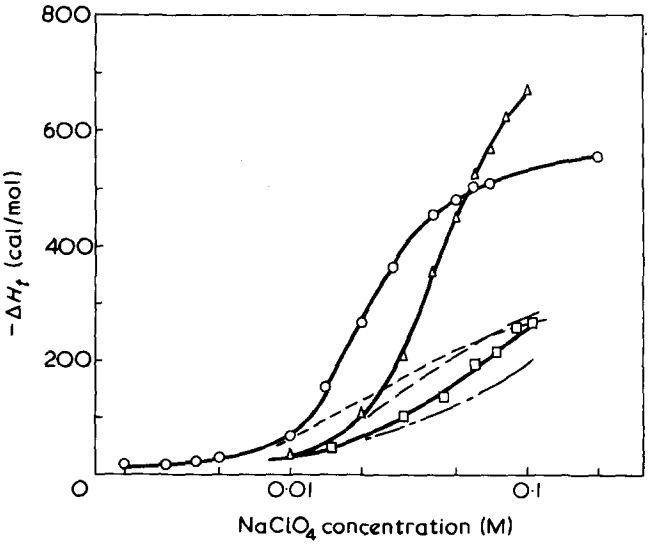

Figure 7 Transfer enthalpy of PLHA (*DP* = 450) (○), PLA (△) and PLAGB (□) in aqueous solutions of $NaClO_4$ at 25.0°C

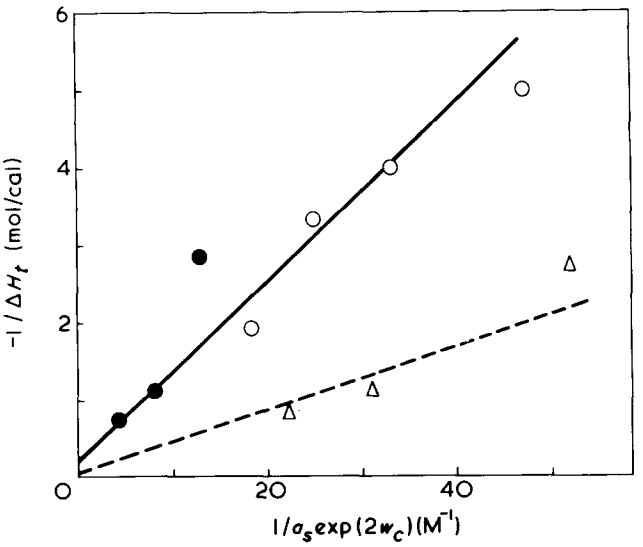

Figure 8 The plot of $1/\Delta H_t$ *versus* $1/[a_s \exp(2w_c)]$. ○, PLHA–$NaClO_4$; ●, PLAGB–$NaClO_4$; △, PLHA–NaSCN

Table 2 Thermodynamic parameters for the coil to helix transition of PLHA and PLA at 25.0°C

Polymer	ΔG_0 (cal/mol)	ΔH_0 (cal/mol)	ΔS_0 (e.u.)	$\sigma \times 10^3$
PLHA	−180 ± 20	−540 ± 40	−1.2 ± 0.2	1.5 ± 0.2
PLA	−170 ± 20	−625 ± 50	−1.5 ± 0.2	0.85 ± 0.13

In the same Figure, the plot for the PLHA–NaSCN system is also shown. K_0^b for binding of SCN^- with PLHA is found to be about 1.2 M^{-1}, which is more uncertain than the value with ClO_4. These values of K_0^b are about four times as large as that obtained for binding of SCN^- with PLO and PLL (K_0^b = 0.23 M^{-1})[6]. This may suggest that the guanidino group binds more strongly with the anions than the amino group. By using these values of K_0^b, the contribution of ΔH_0^b to ΔH_t was calculated as shown by the broken lines in *Figure 7*. ΔH_0 was determined to be −540 ± 50 and −620 ± 50 cal/monomol for PLHA and PLA, respectively.

Furthermore, the initiation parameters, σ, for the helix formation in the polypeptides were calculated from the well-known relation[27]:

$$\sigma^{1/2} = \Delta H_0 / \Delta H_{vH} \tag{10}$$

the values are given in *Table 2*. The value for PLA, 8.5 × 10^{-4}, agrees well with the theoretical value for arginine in protein, 7 × 10^{-4}, reported by Finkelstein *et al.*[28], although PLA used in this study has a small *DP*.

Equilibrium dialysis

The equilibrium dialysis allows the amount of anion binding to be determined directly. As the concentration of SCN^- can be accurately determined by a simple chemical procedure, the dialysis measurements were performed with the PLHA–NaSCN system. The molarity of SCN^-, (SCN^-), can be determined with the errors less than 0.1% in the absence of polymer and less than 0.5% in the presence of polymer.

The total molarity in the inner solution, $(SCN^-)_t$, relates to the molarity of the free ion, $(SCN^-)_f$, as:

$$(SCN^-)_t = (SCN^-)_f + C_p\gamma \tag{11}$$

Considering the Donnan effect and the electroneutrality, the following relations are obtained:

$$(SCN^-)_f(Na^+) = [(SCN^-)']^2 \tag{12}$$

and

$$(SCN^-)_f = (Na^+) + (1-\gamma)C_p \tag{13}$$

where $(\,)'$ refers to the outer solution or to the polymer-free solution. From these equations, one obtains:

$$(SCN^-)_t = (SCN^-)' + \frac{1+\gamma}{2} C_p + (- - -)^2 C_p^2 + \ldots \tag{14}$$

A plot of $(SCN^-)_t$ against C_p would permit determination of γ from the slope at $C_p = 0$. The plots were found to be linear in a range of C_p less than half of C_s. Dependence of γ on $(SCN^-)_f$ is given in *Figure 9*, in which the theoretical curves calculated from equation (8) by assuming K_0^b = 0.9 and 1.0 M^{-1} are also shown. The experimental results may show that K_0^b is 0.9 to 1.0 M^{-1}, which coincides pretty well with the value from calorimetry. γ is also found to increase with an increase in C_s, which may indicate that PLHA transforms from coil to helix through reduction of its charge density by binding of SCN^-.

DISCUSSION

The conformational effects reported in this paper are considered to involve the random coil, α helix equilibrium. However, the existence of a true coiled conformation for highly charged polypeptides at low ionic strength has been questioned[29]. The c.d. spectra support the presence along the chain of locally ordered structures. Therefore, the expression 'coil' is used in this paper to indicate the state that undergoes the transition to α-helix without any specification of its detailed conformational properties.

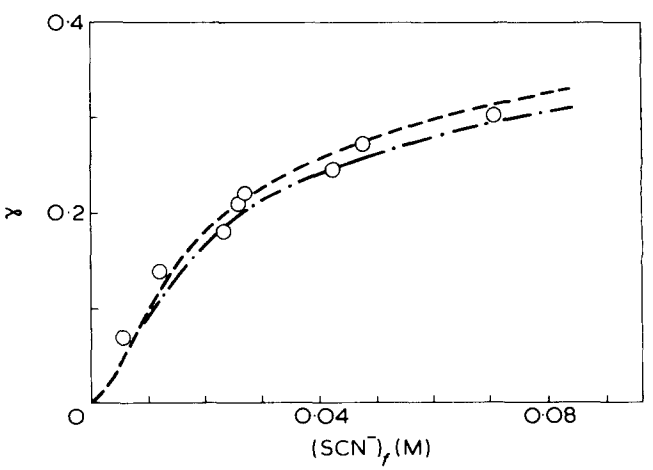

Figure 9 Degree of the binding of SCN^- with PLHA (*DP* = 450) at 25.0°C. Two lines are calculated by assuming K_0^b = 1.0 M^{-1} (– – – –) and 0.9 M^{-1} (– · – ·)

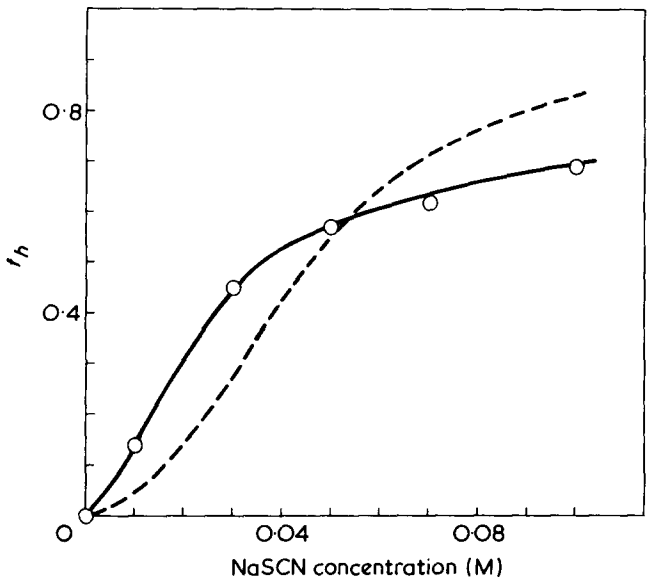

Figure 10 Helix content of PLHA in aqueous solutions of NaSCN. (– – –), Theoretical curve calculated by assuming $K_0^b = 1.0\ M^{-1}$

The free energy change, ΔG, of the transition from coil to α helix is defined as:

$$\Delta G = w_h RT(1-\gamma_h)^2 - w_c RT(1-\gamma_c)^2 + \Delta G_0 \qquad (15)$$

where ΔG_0 is the free energy change of the transition between the uncharged conformers. Since ΔG is zero at the midpoint of the transition, ΔG_0 can be estimated by use of γ_h and γ_c values at the midpoint, which are calculated from equation (8) and the values of K_0^b. By use of $K_0^b = 1.0 \pm 0.2\ M^{-1}$ for binding of ClO_4^- with the guanidino group, ΔG_0 is estimated to be -180 ± 20 and -170 ± 20 cal/monomol at 25.0°C for PLHA and PLA, respectively. The entropy change of the transition, ΔS_0, can also be calculated from ΔG_0 and ΔH_0 determined above. These results are listed in *Table 2.*

According to Grourke and Gibbs[8] and Fu *et al.*[30], the relatively great helical stability of PLL compared with PLO relates to the more positive entropy change of the transition from coil to helix, because the longer aliphatic side chain of PLL enhances the hydrophobic interaction between the side chains in the helical form. The values of ΔG_0 and ΔS_0 for PLL have been reported to be -110 ± 30 cal/monomol and -2.5 ± 1.3 e.u., respectively[9,30–33]. Therefore, the helical structures of PLHA and PLA are more stable than that of PLL, probably due to the more positive ΔS_0. Other studies have also indicated that the hydrophobicity of the side chain of PLA is not less than that of PLL[11,34]. The smaller difference in the helical stability between PLHA and PLA than that between PLL and PLO may relate to the smaller difference in ΔS_0 between PLHA and PLA than that between PLL and PLO. The thermodynamic parameters for PLAGB were not obtained, because this polymer precipitated at the midpoint of transition.

The anion series (1)–(5) is consistent with the so-called 'lyotropic series'. The water structure breaking anions can approach the guanidino groups in the side chains of PLHA and PLA and form ion-pairs which reduce the charge density on the polypeptides. The binding constant of SCN^- with PLHA is shown to be about four times as large as that with PLL. Because of such strong binding, which may be due to the great ability of the guanidino group for breaking the water structure around it, and the more negative ΔG_0, PLHA and PLA undergo the coil to helix transition at smaller concentrations of the anions than PLL. In *Figure 10* is shown the theoretical helical content of PLHA in the aqueous NaSCN solution, which was calculated with the binding constant obtained in this study. Over a range of C_s less than 0.07 M, agreements with the experimental results are very good.

ACKNOWLEDGEMENTS

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