Solvent-induced and salt-induced coil–helix transition of poly(L-lysine) salts in water/alcohol mixtures

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Coil–helix transition has been studied for poly(L-lysine) salts, with counterion species Cl^- (PLLHCl), Br^- (PLLHBr) and NO_3^- ($PLLHNO_3$), in aqueous alcohol mixtures (water/MeOH, EtOH, 2PrOH, tBuOH) as a function of the alcohol composition and/or salt concentration. The dependence of the helix content on the solvent species and composition is interpreted in terms of the three solvent properties i.e. dielectric constant, Gutmann–Mayer acceptor number and water activity, which have been found to be critical for contact ion-pair formation in the study of poly(L-glutamic acid). The salt-induced transition is also analysed by taking account of the contact ion-pair formation. Comparisons are made with the results on the poly(L-glutamic acid) systems.

(Keywords: coil–helix transition; poly(L-lysine) salts; solvent properties)

INTRODUCTION

In the study of biological systems, it is important to elucidate the specificity of ions that are ubiquitous in such systems. The Na^+–K^+ pump, a basic supporter of cell activity, is considered to work in association with the conformational change of Na/K ATPase, which is sensitive to the presence of Na^+ or K^+ ions[1]. We have previously reported[2–4] that alkali and alkaline earth metal salts of poly(L-glutamic acid) (PLG) assume an α-helical conformation, depending on the counterion species as well as on solvent composition and solvent species. The counterion specificity found for alkali metal counterions, $Na^+ > Li^+ > K^+ \gg Cs^+$, was interpreted as resulting from contact ion-pair formation between the counterions and polymer charges in the helical conformation. (Here 'contact ion-pair' means an ion-pairing that has no intervening solvent molecules between the ions, thus involving desolvation in the formation process.) The solvent specificity was also correlated with the contact ion-pair formation through the three solvent parameters, i.e. dielectric constant (D), Gutmann–Mayer acceptor number (AN)[5] and water activity (a_w) of the mixed solvents, which control the electrostatic and desolvation energies upon the ion-pair formation[3].

In the present paper, we report the solvent- and the salt-induced coil–helix transition of poly(L-lysine) (PLL) salts[6] (PLLHCl, PLLHBr, $PLLHNO_3$) in water/alcohol mixtures. A comparison is made with the results on PLG systems, and the supposed mechanism for helix stabilization through the contact ion-pair formation is examined.

EXPERIMENTAL

PLLHBr ($M_w = 48\,000$) was purchased from Peptide Institute, Inc. Counterion exchange to PLLHCl and $PLLHNO_3$ was performed[3] by ultrafiltration of PLLHBr aqueous solution in the presence of excess NaCl or $NaNO_3$.

CD measurements were carried out at 25°C with a Jasco J 20A spectropolarimeter for the PLL salts ($C_p = 6.2 \times 10^{-4}$ basemol dm^{-3}) in water/MeOH, EtOH, 2PrOH and tBuOH (analytical grade from Kanto Chemical Co.) in the absence and in the presence of added salts, i.e. NaCl or NaBr (analytical grade from Nacali Tesque). $NaNO_3$ was not used for the salt-induced transition experiment because of its optical absorbance near the CD peak. The helix content, θ, was estimated from $\theta = -(\theta)_{222}/40\,000$, where $(\theta)_{222}$ is the molar ellipticity[7] at 222 nm. All the polymer sample solutions were prepared with deionized and distilled water.

RESULTS AND DISCUSSION

Solvent-induced coil–helix transition

Figures 1–4 show the dependence of θ (%) on the alcohol composition for PLL salts in water/MeOH, EtOH, 2PrOH and tBuOH, respectively. The alcohol compositions at which PLL salts start to assume helical conformation are lowest for $PLLHNO_3$, followed by PLLHBr and then PLLHCl. The θ values are also in the order, $PLLHCl < PLLHBr < PLLHNO_3$, except at higher alcohol compositions of 2PrOH and tBuOH. This counterion specificity is compared with that in PLG alkali metal salts, $Cs^+ \ll K^+ < Li^+ < Na^+$. The latter counterion specificity is much more marked than the former, since

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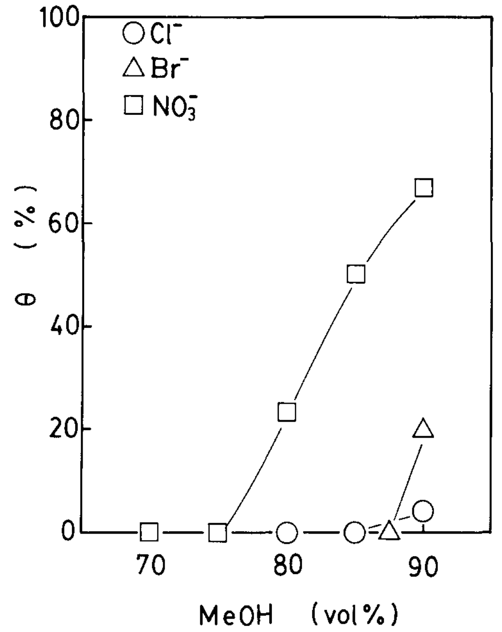

Figure 1 Solvent-induced coil–helix transition of PLLHCl, PLLHBr and $PLLHNO_3$ in aqueous MeOH

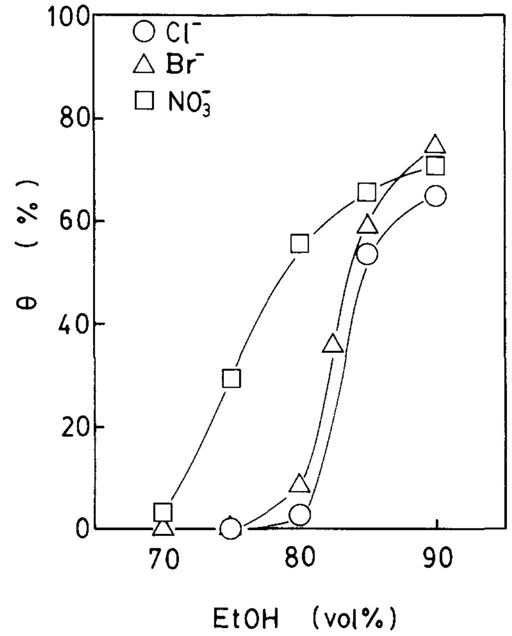

Figure 2 Solvent-induced coil–helix transition of PLLHCl, PLLHBr and $PLLHNO_3$ in aqueous EtOH

PLGK and PLGCs hardly assume helical conformation in the mixed solvents studied here. The counterion specificity for PLG is thought to be determined by a subtle energy balance in the contact ion-pair formation, namely between the desolvation energy and the electrostatic energy. This should be the case for the present counterion specificity for PLL salts. According to the order of the crystallographic ion diameters, the electrostatic interaction energy upon the contact ion-pair formation decreases as $Cl^- > NO_3^- > Br^-$, while the hydration energies, which we use as a measure of the desolvation energies involved with the ion-pair formation, are in the order[8] $Cl^- > Br^- > NO_3^-$. Thus the highest helix stability observed for the NO_3^- counterion is interpreted as a result of favourable contact ion-pair formation owing to the lowest desolvation energy. The position of Cl^- and Br^- in the ion specificity may also be explained using a similar rationale. On the other hand, the counterion specificity for PLG alkali metal salts is not primarily determined by the hydration energies of the counterions but by the electrostatic energy, since the helix is stabilized for Na^+ and Li^+ counterions, which should have higher desolvation energies and more negative electrostatic energies involved with the ion-pair formation.

Such a difference in the factors determining the counterion specificities between the two polypeptides may be qualitatively explained by invoking an empirical rule, that is 'ion-pair formations are favourable between

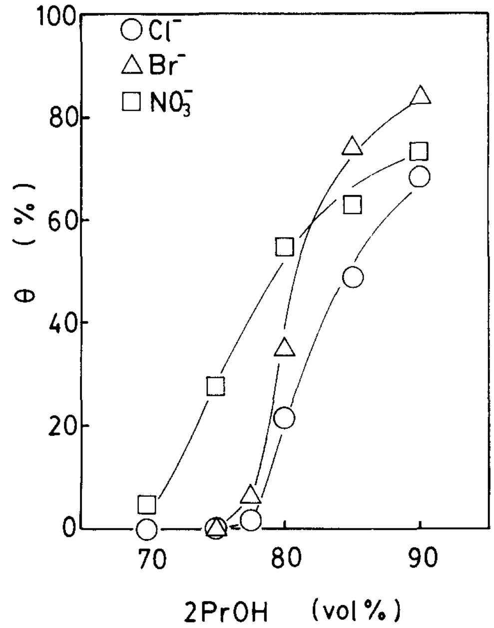

Figure 3 Solvent-induced coil–helix transition of PLLHCl, PLLHBr and $PLLHNO_3$ in aqueous 2PrOH

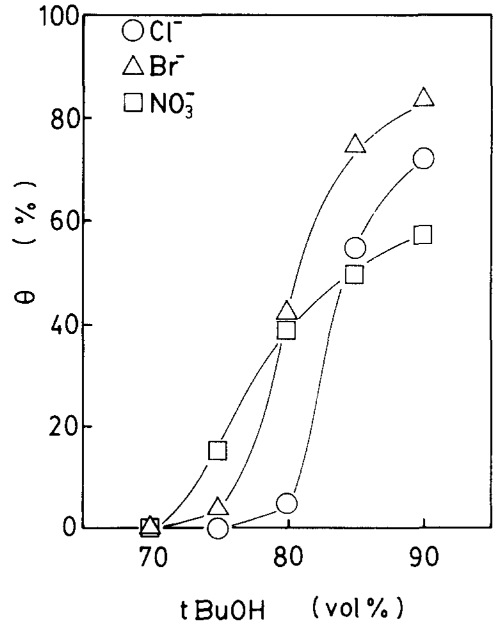

Figure 4 Solvent-induced coil–helix transition of PLLHCl, PLLHBr and $PLLHNO_3$ in aqueous tBuOH

Table 1 Dielectric constant (D), acceptor number (AN) and water activity (a_w) values of the solvents

Solvent	D^a	AN^b	$a_w{}^c$
H_2O	78.3	55	
MeOH	32.6	41	0.45
EtOH	24.6	37	0.70
2PrOH	19.9	34	0.88
tBuOH	12.5	–	0.90

[a] Ref. 15; values at 25°C
[b] Ref. 5
[c] Ref. 16; values for 80 vol% alcohol at 25°C

ions of like hydration structures or similar hydration energies'. This rule, which is based on Desnoyer *et al.*'s hydration sphere overlapping model[9], and is also apparently consistent with the HSAB principle[10], actually explains ion specificities observed for counterion binding in aqueous polyelectrolyte solutions. One example is given by the contrast in the counterion specificities between metal salts of polycarboxylates (e.g. poly(acrylic acid) and PLG), and those of polyelectrolytes having sulfate or sulfonate groups (e.g. dextran sulfate and poly(styrene sulfonate)); polyions with relatively small and hard carboxyl anions prefer Li^+ and Na^+ (Mg^{2+} and Ca^{2+}), and those with large and soft sulfate (sulfonate) groups prefer Cs^+ and K^+ (Ba^{2+})[11–14]. By comparing the hydration energies of the counterions ($-\Delta G_{hyd}$ (kJ mol^{-1}): Li^+, 481; Na^+, 375; K^+, 304; Cs^+, 274) with those of analogues to the polymer charged groups ($-\Delta G_{hyd}$ (kJ mol^{-1}): CH_3COO^-, ~400; HSO_4^-, ~270)[8], one can actually see the correlation of the counterion specificity with the hydration energy. In the present PLL systems also, the empirical rule explains the counterion specificity since NH_4^+, as an analogue for the side-chain ammonium group, has $-\Delta G_{hyd} = 292$ kJ mol^{-1}, which is close to the value for NO_3^- (306 kJ mol^{-1}) and much lower than the values for Cl^- and Br^- (347 and 321 kJ mol^{-1}, respectively). Thus an optimum position for the subtle balance between the desolvation and electrostatic interaction energies seems to exist at an ion-pair whose hydration energies are alike. In this context, the small difference (41 kJ mol^{-1}) between $-\Delta G_{hyd}$ values of Cl^- and NO_3^-, may explain the less marked counterion specificity for the helix formation than in the PLG systems, which have a much greater difference (207 kJ mol^{-1}) between the $-\Delta G_{hyd}$ values of the counterions.

The solvent specificity for the helix formation in *Figures 1–4*, 2PrOH ≧ tBuOH ≧ EtOH > MeOH, is also somewhat different from that for PLG alkali salts, EtOH ≧ 2PrOH > tBuOH > MeOH. The relatively unfavourable helix formation in MeOH(aq) and tBuOH(aq) for the latter case has been attributed to the high dielectric constant (D)[15] and acceptor number (AN)[5], and the high water activity (a_w)[16], respectively (*Table 1*). In other words, the lower these values, the more favourable the helix formation through the contact ion-pair formation. The same explanation, in principle, should be applied to the present solvent specificity. However, since the hydration energies for the counterions and the charged group in the PLL system are significantly lower than those of PLG, the relative contribution of preferential hydration on ions, which is measured by a_w, to the contact ion-pair formation energy must be lower than in the PLG systems. The relatively high helix stability in 2PrOH(aq) or tBuOH(aq) actually suggests that the high a_w values of those solvents are not as unfavourable for the contact ion-pair formation as in the PLG systems. In this context, one should note the apparently unexpected behaviour of $PLLHNO_3$, namely that θ increases less markedly in 2PrOH(aq) and tBuOH(aq) (*Figures 3* and *4*) than in MeOH(aq) and EtOH(aq). Since the former solvent systems are thought to be favourable for contact ion-pair formation in the PLL systems, the small increase in θ seems to be a result of a relative destabilization of the helix through a contact ion-pair formation on the coil conformation rather than a direct destabilization of the helix itself. It is considered that, with the increase in the alcohol composition, D, AN and a_w decrease, leading to a promotion of the contact ion-pair formation of NO_3^- on the coil conformation as well as on the helix.

Salt-induced coil–helix transition

Figures 5 and *6* show the dependence of θ on counterion concentration, C_c (mol dm^{-3}), for PLLHCl and PLLHBr. The qualitative behaviour of the salt-induced transition seems to be similar for these two counterion systems, except for that in tBuOH(aq). Such variation of θ with the counterion concentration has been analysed based upon counterion condensation theories[3,17], namely, a coil–helix transition of fully charged polypeptides is accompanied by extra counterion binding due to the higher charge density of the helical conformation. According to Record *et al.*[18], equation (1) is obtained:

$$COIL + Nu\Delta\lambda C_c \overset{K}{\rightleftharpoons} HELIX \qquad (1)$$

Here $COIL$ and $HELIX$ mean the respective cooperative units containing Nu charged groups, and $\Delta\lambda$ is the excess degree of counterion binding, i.e. $\lambda h - \lambda c$. K is the thermodynamic equilibrium constant for equation (1) and is given by:

$$\begin{aligned}\ln K &= \ln(\theta/1-\theta) + \ln(\gamma h/\gamma c) - Nu\Delta\lambda \ln C_c \\ &= \ln K_{obs} - Nu\Delta F_{el} - Nu\Delta\lambda \ln C_c \qquad (2)\end{aligned}$$

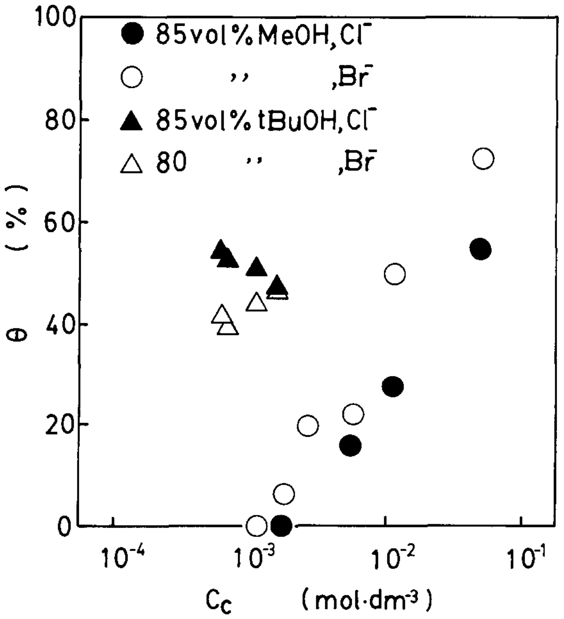

Figure 5 Salt-induced coil–helix transition of PLLHCl and PLLHBr in aqueous MeOH and tBuOH. C_c is the counterion concentration

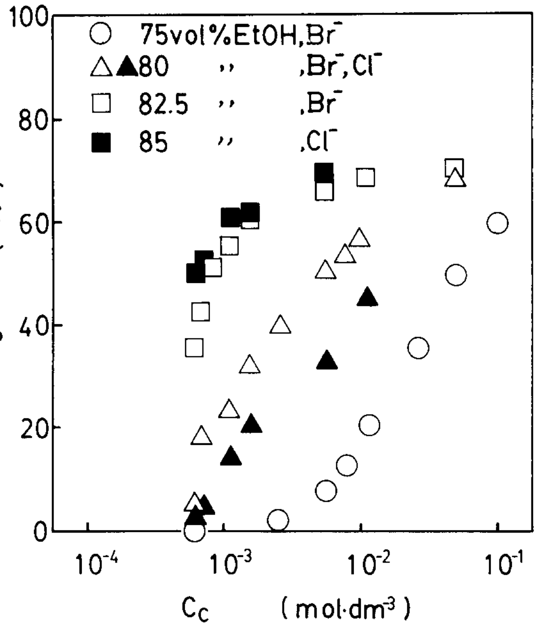

Figure 6 Salt-induced coil–helix transition of PLLHCl and PLLHBr in aqueous EtOH. C_c is the counterion concentration

Here C_c is used instead of the counterion activity as a first approximation. γh and γc are activity coefficients of the cooperative unit in helical and coil conformation, respectively. They are derived from excess energies of electrostatic interaction between polymer charges, F_h and F_c. ΔF_{el} is the difference in electrostatic energy between the coil and the helical conformations, i.e. $\Delta F_{el}=(F_c-F_h)/RT$.

It should be noted that equations (1) and (2) treat only the effects of electrostatic interaction and counterion binding on the conformational transition, and not the effects of polymer molecular weight, i.e. the equations implicitly assume that end effects are neglected. It is known that the coil–helix transition of short polypeptides becomes appreciably sharper with increase in the molecular weight[19]; therefore to represent dependence on molecular weight in the equations, *Nu* must be taken as a parameter accounting for the end effect as well as the charge number in a cooperative unit.

Equation (2) reveals that not only the difference in degree of counterion binding, but also the binding mode (e.g. contact ion-pair or atmospheric binding), influence the salt-induced transition through the ΔF_{el} term. We have shown that the sharpness parameter, Q, of the transition can be utilized as a measure for the contact ion-pair formation of PLG salts[3]:

$$\begin{aligned} Q &= \mathrm{d}\ln K_{obs}/\mathrm{d}\ln C_c \\ &= Nu(\Delta\lambda + \mathrm{d}\Delta F_{el}/\mathrm{d}\ln C_c) \qquad (3) \end{aligned}$$

Q values for the present PLL systems obtained at $\theta \approx 0.5$ are summarized in *Table 2*. These values are rather lower than those obtained for another PLLHCl sample in a preliminary study[20]; this may be attributed to the much higher molecular weight ($M_w=2.3\times10^5$), hence larger *Nu* values, of the latter sample. In the preliminary study, we discussed the variation of Q values in terms of $\Delta\lambda$ because it was impossible to estimate *Nu* and $\mathrm{d}\Delta F_{el}/\mathrm{d}\ln C_c$ terms with sufficient accuracy to explain the small changes in the Q values. On the other hand, in our study on PLG alkali metal salts, the last term of equation (3) proved essential for the marked dependence of Q on the solvent species, i.e. 27 in 80 vol% MeOH to 0.5 in 75 vol% 2PrOH for PLGNa[3]. Therefore in the present study, we take into account the *Nu* and $\mathrm{d}\Delta F_{el}/\mathrm{d}\ln C_c$ terms in a qualitative elucidation of the dependence of the Q values on the solvent species and composition.

The *Nu* value, when the end effects are neglected, is related to the cooperativity parameter, σ, of the conformational transition[21], as $Nu \approx \sigma^{-1/2}$. We assigned a value of 10^{-4} to σ (i.e. $Nu \approx 100$) for the PLG alkali metal salts with $M_w=7.1\times10^4$. In the present study, however, we assume that $\sigma \approx 10^{-3}$, thus $Nu \approx 10$ because of the lower molecular weight and the lower charge density. (Actual charge spacing of PLL should be larger than that of PLG, which has a shorter side chain than the former.) This is based on the fact that the higher the charge density of polypeptides, the higher the cooperativity of the coil–helix transition[22]. $\Delta\lambda$ values are estimated by our counterion condensation model[3,23] as 0.2 for $D=40$ of 85 vol% MeOH to 0.14 for $D=18$ of 85 vol% BuOH. This is because λc increases at a higher rate than λh with the decrease in D. Because $\mathrm{d}\Delta F_{el}/\mathrm{d}\ln C_c = -\Delta\lambda/2$, according to the condensation model[3,23,24], equation (3) gives Q in the order of unity for the present systems. This seems to be consistent with the Q values in solvent systems of higher D, while the Q values in 80 and 85 vol% tBuOH are significantly lower than estimated. This suggests that the salt-induced coil–helix transition cannot be fully explained by the counterion condensation concept alone. The same situation was found in the study of PLG salts, where contact ion-pair formation was introduced into the electrostatic stabilization factors of the helical charged groups. Thus, by making the same assumption as in the PLG systems[3], we obtain Q values varying between 1 and 0. For example, in the limit of 100% contact ion-pair formation on the helix, $F_h \rightarrow 0$, while F_c remains positive. Then, an increase in the ionic strength will reduce only F_c, resulting in such a negative value of $\mathrm{d}\Delta F_{el}/\mathrm{d}\ln C_c$ that the $\Delta\lambda$ value is cancelled.

In the present study, we have compared the helix formation of PLL salts in mixed solvent systems with that of PLG systems. The less marked specificities in the helix formation among the counterion species and the solvent species have been ascribed to the lower desolvation energies of the component ion or ionic groups and their smaller differences than those in the PLG systems. Since the counterion selectivity found for the helix formation of PLG alkali salts in aqueous solutions is much less apparent[25], polymer/solvent systems with high, as well as low, solvation energies seem inadequate to afford a distinct counterion specificity to the secondary

Table 2 Sharpness parameter, Q, of PLL salts in aqueous alcohols

Solvent	Concentration (vol%)	PLLHCl	PLLHBr
MeOH	85	0.81	0.73
EtOH	75	–	0.66
	80	0.65	0.38
	82.5	–	0.92
	85	0.36	–
2PrOH	80	0.71	0.39
tBuOH	80	–	0.27
	85	−0.25	–

transition of charged polypeptides. In this respect, the elaborate selectivity between Na^+ and K^+ in ATPase (if the counterion specificity found in the PLG systems is relevant to this) may also be dependent on its solvation environment.

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