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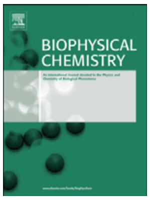


Double conformational transition of alkali metal poly(L-glutamate)s in aqueous ethanol

Counterion mixing effect revisited

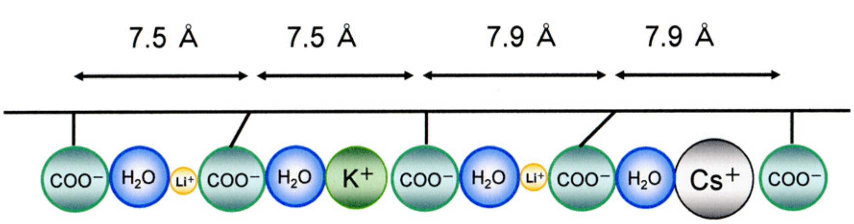
Masayasu Hasuike [a], Shigeki Kuroki [b], Mitsuru Satoh [a,*]

[a] Department of Chemistry and Materials Science, Tokyo Institute of Technology, Ookayama, Meguroku, Tokyo, 152-8550, Japan
[b] Department of Organic and Polymeric Materials, Tokyo Institute of Technology, Ookayama, Meguroku, Tokyo, 152-8552, Japan

HIGHLIGHTS

- ► Alkali metal poly(L-glutamate) shows "globule–helix" transition in aqueous ethanol.
- ► Mixing of smaller and larger counterions induces α-helix formation.
- ► Contact-ion pair formation is not necessary for α-helix formation.

GRAPHICAL ABSTRACT



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ABSTRACT

It was first found that alkali metal poly(L-glutamate)s show the coil–globule transition and the coil–helix transition sequentially in aqueous ethanol with increasing the solvent concentration. The counterion specificity for the former transition, i.e., $Na^+ > K^+$, $Rb^+ > Li^+$, Cs^+, proved to be somewhat different from that for the latter; $Na^+ > Li^+ > K^+ > Rb^+ > Cs^+$. Counterion mixing effects were also observed for both transitions; the most effective combinations to induce the transitions were Li^+/Cs^+ and Li^+/K^+, respectively. Solution viscometry, circular dichroism and alkali metal NMR line width measurements for the single-counterion systems revealed that the contact ion-pair formation hardly occurs for Li^+ even at the collapsed globule state and in the helix conformation. Unexpectedly, however, the specific binding of Li^+ was induced just by mixing with K^+ or Cs^+, when the helix content concomitantly increased. Mechanism for the counterion-specific "double transition" as well as the counterion mixing effect is discussed referring to the size-fitting model that has been proposed for the coil–globule transition of alkali metal poly(acrylate)s.

1. Introduction

Charged polypeptides such as poly(L-glutamic acid) (PLGA) and poly(L-lysine) (PLL) in aqueous solution transform their conformation from the random coil to the α-helix with neutralization of the charged group [1,2]. The coil–helix transition is induced due to decrement in the electrostatic repulsion among the charged groups and the resultant stabilization of the intra-molecular hydrogen bond. A similar transition also occurs in various kinds of aqueous organic solvent mixtures even at the fully charged state of polypeptides [3,4]. In our previous studies [5,6], the coil–helix transition of alkali metal poly(L-glutamate)s (PLGM) in aqueous systems was found to be induced by increasing organic solvent concentration, and interpreted in terms of two main factors; solvent effects on the intramolecular hydrogen bond and on the electrostatic repulsion among the charged groups. The latter was further divided into two contributions, i.e., nonspecific counterion binding such as what may be predicted by Manning theory [7] and regulated by the medium dielectric constant,

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[*] Corresponding author. Tel.: +81 3 5734 2133; fax: +81 3 5734 2888.
E-mail address: msatoh@polymer.titech.ac.jp (M. Satoh).

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and specific one such as contact ion-pair formation. Among others, with an analogy to the solvent-free system, the solvent-induced helix formation was ascribed to the polymer charge neutralization due to the contact ion-pair formation with polymer charges. Thus, the observed counterion specificity for the helix formation, i.e., $Na^+ > Li^+ > K^+ > Cs^+$, which proved to be common for other organic solvent systems [5], was also ascribed to the specific counterion binding.

In addition to the counterion-specific helix formation in the single-counterion systems, quite a specific helix stabilization was found in some mixed counterion systems [8]; the helix content of PLG in aqueous organic solvent mixtures was greatly enhanced just by mixing Li^+ and K^+ counterions, while a less significant stabilization was observed for Li^+/Cs^+ systems and it was negligible for all the other ones. As for the mechanism, an extra stabilization for the helix was tentatively explained in terms of the long-range electrostatic interaction between polymer charges that formed contact ion-pairs with Li^+ or K^+.

Another type of counterion-specific conformational transition has also been found for synthetic polyelectrolytes; namely, the coil–globule transition occurs for polyelectrolytes such as alkali metal poly(acrylate) s (PAAM) in aqueous organic solvent mixtures [9,10]. For example, in aqueous ethanol, the solution viscosity (or the reduced viscosity) significantly decreases with increasing the ethanol concentration to suggest the polymer chain collapse. Such a chain contraction was interpreted in terms of the nonspecific counterion binding that should be enhanced by the decrease in the dielectric constant with increasing the ethanol concentration, and the specific one that may be ascribed to the poorer solvency of the organic solvent to the ions than water. The collapse or the transition was easier in the order of $Na^+ > K^+ > Li^+$, Cs^+; PAANa chain almost collapsed at 50 vol.% ethanol, and PAAK at 60%, PAALi and PAACs at 70%, respectively. Although this counterion specificity was somewhat different from that for the coil–helix transition of PLGM, it had also been supposed that the contact ion-pair formation is essentially involved with the transition; the stronger the counterion binding, the more easily the transition occurs. However, this seemingly reasonable interpretation for the transition mechanism proved not always to be the case. According to NMR line width measurement for ^{23}Na, which was used as a measure of counterion binding strength, the supposed contact ion-pair formation did not always occur during the conformational transition [10]. On the basis of these experimental findings, Hayashi et al. proposed the “size-fitting model”, where the following four factors have been considered as essential determinants for the counterion specificity of the coil–globule transition of PAAM in aqueous organic solvent mixtures [9]:

1. Free energy gain from the electrostatic interaction upon the counterion binding, 2. Free energy loss due to desolvation of the polymer charge and/or counterion accompanying the binding, 3. Entropic loss of the polymer chain due to the ion-cluster formation (chain collapse), and 4. Poor solvency of the organic solvent to the counterion (and polymer charge). For PAA, the first factor should be more favorable for smaller counterions as $Cs^+ < K^+ < Na^+ < Li^+$, while the second one is, on the contrary, $Li^+ < Na^+ < K^+ < Cs^+$. As a result of this balance, the binding strength of these counterions to carboxyl anion should be determined. According to Collins [11,12], the order to form a contact ion pair formation is estimated to be $Na^+ > K^+ > Li^+ > Cs^+$, on the basis of the matching of Jones-Dole viscosity B coefficients of the cations and the anion in water. Although the order is apparently the same as that of the coil–globule transition of PAAM, the third and the fourth factors must also be taken into consideration because systems comprising of polyelectrolytes and organic solvents in addition to water are studied here. For the third one, the counterion dependency may vary with the polymer counterpart, because the entropy change for the ion-cluster (or “beads” of the pearl-necklace model [13,14]) formation should also depend on the stiffness of the main chain, length and bulkiness of the side chain, and hence the polymer charge array. As stated above, the coil–globule transition may occur without the contact ion-pair formation. Thus, the fourth one must be dominant for the chain collapse. Namely, addition of an organic solvent that is poorer to the charges than water should inevitably force the polymer take a conformation in which the charged group and the counterion are kept away from the poor solvent, e.g., as a globule state. Factors 1 to 3 may primarily determine the counterion specificity for the transition.

A counterion mixing effect was also found for the coil–globule transition of PAAM in aqueous organic solvent mixtures [10]. In this case, however, the most effective combination of two counterions was Li^+ and Cs^+, which is somewhat different from that for the coil–helix transition of PLGM system. A common feature for these two systems is that Na^+ is the optimum counterion for the respective conformational transitions. Thus, on the basis of the above four factors, the free energy balance must be optimum for Na^+ and the combination of a smaller Li^+ and a larger Cs^+ seems to cancel out or compensate each other the respective unfavorable energy balances. The same reasoning may be applicable to the Li^+/K^+ combination of PLG case.

Against the above backgrounds, we investigate the binding state of the alkali metal cations as counterions of PLG in aqueous ethanol via NMR line width measurement to see if the contact ion-pair formation is NOT a necessary condition for the coil–helix transition just as for the coil–globule one found for PAAM systems. Being different from the coil–globule transition in which charged groups must aggregate in a rather random fashion, the helix formation is accompanied by a regular array of the carboxyl groups around the helix axis. Thus, it may be expected that the third factor in the proposed size-fitting model, or actual size fitting of counterions to the polymer charge array, will be more essential to determine the counterion specificity. Furthermore we revisit the counterion mixing effect for PLGM system and consider the mechanism in comparison with that observed for the coil–globule transition of PAAM systems.

2. Experimental

2.1. Materials

Poly(L-glutamic acid) (PLGA) was obtained by hydrolysis of poly(γ-methyl L-glutamate) (Seiko Kasei, 10% dichloroethane solution). The molecular weight was estimated by viscometry as 5.0×10^4 [15]. Alkali metal poly(L-glutamate) (PLGM) samples (PLGLi, Na, K, Rb and Cs) were obtained by ultrafiltration of the respective alkaline solutions of PLGA through an ultrafilter Q2000 (ADVANTEC, fractionation molecular weight: 20,000) and the subsequent freeze-drying. As a reference polymer, poly(acrylic acid) (PAA) (Polyscience, $Mw = 5 \times 10^4$, 35% solution) was utilized. The respective alkali salts were prepared by neutralization by the corresponding alkali hydroxides (MOH). Ethanol (EtOH) and MOH were of analytical grade (Tokyo Kasei) and used as received. Deionized water was used for sample preparation.

2.2. Measurements

All the measurements were performed under the following conditions; polymer concentration: 20 mM, alkali concentration: 1 mM, EtOH concentration: 0–80 vol.%, temperature: 25 °C. The respective alkali hydroxides were added to ensure the full neutralization state of the polymers. In addition to the polymer concentration above, lower ones (5 mM for viscometry and 0.662 mM for circular dichroism (CD) measurements) were also used to see any effects from inter-polymer interactions. In the higher EtOH concentration region (≧70 vol.%), the sample solutions of 20 mM appeared cloud. The turbidity was most significant at 70 vol.% and for PLGCs and Rb, while those at 80 vol.% and for the other PLGM were only slight. Even with PLGCs (Rb) sample at 70 vol.% EtOH, however, the turbidity posed no hindrance for the measurements. On the other hand, samples of the lower concentrations remained clear.

The solution viscosity was measured with an oscillation viscometer (VM-10A, SEKONIC). Circular dichroism (CD) measurements were performed with J-720 circular dichroism spectropolarimeter (JASCO) and a 1 mm cell. The helix content (%) was estimated from the molar ellipticity (deg cm^2/dmol) at 222 nm ($[\theta]_{222}$) as $-[\theta]_{222}/40{,}000$ [16]. NMR line width measurements were carried out with DSX-300 (Bruker) NMR spectrometer at 116.63 MHz (^{7}Li), 79.38 MHz (^{23}Na), and 39.37 MHz (^{133}Cs). The parameters used for the measurement were as follows:

	^{7}Li	^{23}Na	^{133}Cs
The number of data points	32	128	256
The repetition time (s)	15	1	1
90° pulse width (μs)	21	18	18

The line width measurement for ^{87}Rb was abandoned because of the too large width, e.g., ca. 1300 Hz at 70 vol.% EtOH. Typical reproducibilities for the reduced viscosity, the molar ellipticity, and the line width were ±10%, ±5% and ±5%, respectively.

3. Results and discussion

3.1. Single counterion system

3.1.1. Viscometry and CD measurements

The reduced viscosity of PLGM (20 mM) in aqueous EtOH is shown as a function of the EtOH concentration as a relative value to that in water in Fig. 1. Essentially the same behavior was also observed for the diluted polymer samples (5 mM, data not shown) [10]. In the lower EtOH concentration region (0–60–70 vol.%), the reduced viscosity gradually and non-specifically lowered with EtOH concentration and then somewhat cooperatively and specifically decreased to an extent that suggests almost a complete collapse. The former gradual decrement in the viscosity may be attributed to the decrease in the dielectric constant of the mixed solvent and the resultant increase in the screening of the electrostatic repulsion among the charged groups due to the counterion condensation [7]. The latter counterion-specific collapse, on the other hand, may be or not be caused by the contact ion-pair formation. The apparent collapse points are 65 vol.% for PLGNa, 70 vol.% for PLGK and Rb, and 80 vol.% for PLGLi and Cs. The marked decrement in the viscosity observed for PLGM is comparable to the coil–globule transition observed for PAAM system in aqueous organic solvent mixtures. The counterion specificity for PLGM, Na$^+$ > K$^+$ (Rb$^+$) > Li$^+$, Cs$^+$ is also comparable with that of PAAM system, Na$^+$ > K$^+$ > Li$^+$, Cs$^+$. This coincidence suggests that the counterion specificity for the coil–globule transition is not so sensitive to polymer charge array when the polymer charged group is the same. It should also be noted here that the behavior of Rb$^+$ is similar to that of K$^+$ rather than to Cs$^+$. In order to establish the counterion specificity, however, experiments in other solvent systems should be necessary.

On the other hand, the coil–helix transition also occurred with increasing EtOH concentration: > 60 vol.% for PLGNa, and > 70 vol.% for PLGLi, K, Rb although the helix formation of PLGK and Rb was much less significant (Fig. 2). Essentially the same results were obtained for the much diluted polymer samples (0.662 mM, dashed lines in Fig. 2) except for the slight displacement of the starting point for helix formation of PLGNa and PLGLi systems. Thus, it may be considered that the turbidness observed for systems of higher polymer (20 mM) and EtOH concentrations (≧70 vol.%) does not affect the conformational transitions. Here it should be noted that the coil–helix transition for PLGNa started nearly the same time when the chain collapse due to the coil–globule transition was completed, while the two transition regions for PLGLi overlapped (70–80 vol.%). Namely, the helix formation seems to occur via globule state. Thus, PLGM in aqueous EtOH may be called “a double transition system”, in which coil–globule and globule-helix transitions continuously occur. This finding may be rather unexpected, because it has been believed that the α-helix formation of polypeptides is inevitably accompanied by transformation to a stiff rod like conformation. However, this is not the case in the present system as clearly shown by Figs. 1–3. The polymer chain almost completely collapsed while the conformation is in α-helix as exemplified by Fig. 3 where the typical double-peak around 208 and 222 nm [16] emerges with increasing EtOH concentration. These results strongly suggest that the α-helix polymers exist as collapsed train of short helical segments.

One may note that the counterion specificity, Na$^+$ > Li$^+$ > K$^+$ > Rb$^+$ > Cs$^+$, is different from that for the coil–globule transition; Li$^+$ shifted to the second-favorable position. As stated in Introduction, the counterion specificity of the coil–globule transition of PAAM was interpreted mainly by factors 1–3, and it also may be the case for PLGM system. Then, how are those counterion specificities for the coil–globule and the globule–helix transitions not the same? What causes the difference? Among the factors 1–3, the third one seems to be responsible because the other factors are common for the

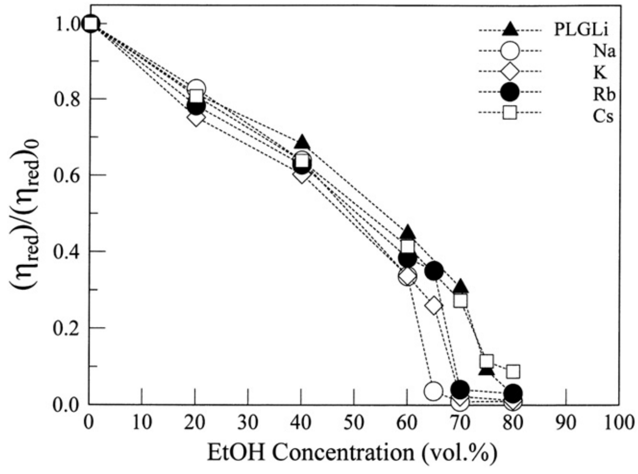

Fig. 1. Counterion specific chain collapse of PLGM in aqueous EtOH as detected by the reduced viscosity. (η_{red}): reduced viscosity and $(\eta_{red})_0$: reduced viscosity for water system. (The experimental values of $(\eta_{red})_0$ for the respective counterion systems (PLGLi, Na, K, Rb and Cs) were 70.8, 60.1, 80.3, 74.7, and 77.0 L/mol.)

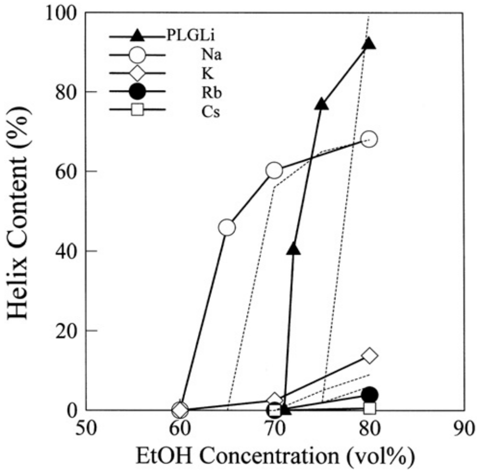

Fig. 2. Counterion specific helix formation of PLGM in aqueous EtOH. The dashed lines are respective data obtained for much diluted samples (0.662 mM). Each line corresponds to the near-by plots.

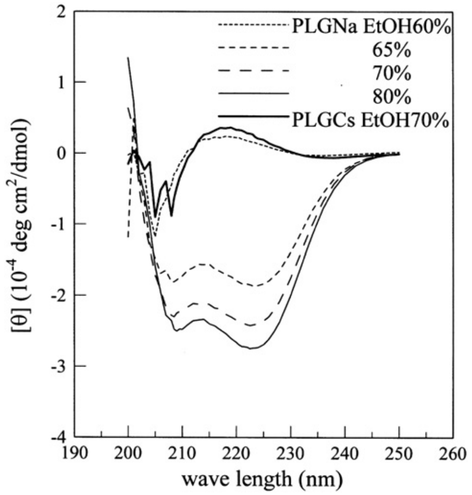

Fig. 3. CD chart for PLGNa and PLGCs in aqueous EtOH (polymer concentration: 20 mM). Due to the turbidness of PLGCs/70 vol.% EtOH sample, the CD spectrum in the shorter wavelength region below 210 nm showed some disarray, while in the longer wavelength region, a typical spectrum for the random coil conformation was observed.

transitions. In the globule or collapsed state, polymer charges aggregate to form a kind of “ion-cluster”, which may be comparable to that formed in ionomers [17]. In this case, polymer charges are able to accommodate the counterions within the cluster in a most favorable way. Since the polymer chain is free to rotate, ion clusters of various sizes and charge arrangements would be formed with a distribution of free energy change. Here we note an experimental fact that PLGLi and PLGCs have almost the same coil–globule transition region. This suggests that the average of the free energy change distribution involved with the ion-cluster formation is almost the same for these counterion systems. On the other hand, in the case of helix formation, the charge array is fixed to that of α-helix. Thus, the degree of freedom for the counterion binding may be rather restricted. The observed counterion specificity of the helix formation suggests that the free energy change involved with Li^+ counterion binding to the fixed charge array is more favorable than that of Cs^+. Further discussion on this point will be made again in the next section on the NMR line width measurements of counterions.

3.1.2. Line width of ^{7}Li, ^{23}Na and ^{133}Cs counterions

Since the nuclear spin of alkali metals is larger than 1/2, they have a nuclear quadrupole moment. Thus, the line width of the ions should be broadened if the symmetry around the pertinent nuclear became worse, e.g., upon the contact ion-pair formation where the counterion may be surrounded by the polymer charged groups as well as solvents [18]. Fig. 4a shows the relative values of NMR line width of ^{7}Li, ^{23}Na and ^{133}Cs counterions to those in water as a function of EtOH concentration. In Fig. 4b, those for PAAM are given as a reference, showing a similar increasing tendency for both polymer systems. Comparing the enhancement in the line width at 80 vol.% EtOH, however, the increment for Na^+ is more significant for PAA of globule state than PLG of mainly helix conformation, while those for Li^+ and Cs^+ show no essential difference between the two polymer systems. In the case of PLGNa system, one may note that the increasing tendency appreciably enhanced, especially at 50 vol.% and 65 vol.%. Since the latter solvent concentration is roughly coincident with that where the helix formation starts for PLGNa, the helix formation may be helped by charge neutralization due to the contact ion-pair formation of Na^+ with the polymer charges. Fig. 5a shows a model for the counterion binding of Na^+ to the carboxyl anions in the α-helical charge array as a schematic illustration. Here we postulated that one of the tightly bound two water molecules to the carboxyl anion [19] can be dehydrated upon the counterion binding in the presence of concentrated ethanol. In the sketch, the shortest charge spacing (0.75 nm) and the second one (0.79 nm) were selected from 1.01, 1.31, 0.79, 0.75, 1.43, 1.44 (nm) in sequence as the polymer charge array of PLGNa in α-helix conformation [20]. In fact, those values were estimated by assuming that the side chains are extended straight out along the radii of the helix and the charges are located at rotations of 100° apart with an axial translation of 0.15 nm per residue. Although the charge spacing should never be “fixed” and actually fluctuate at the end of the side chain, here we assume them to be constant. It may not be unreasonable if compared with the much higher degree of freedom of the charged groups in the globule state. For the drawing of the illustration, the following values (nm) were also employed for ion diameters, water diameter, and hydrogen bond length in the hydrogen bonding hydration to the carboxyl anion; Na^+: 0.19, $-COO^-$: ca. 0.30 [21], H_2O: 0.28 [22], $O_w-H…O^-$: ca. 0.26 [23]. Then, the total length for the binding mode is estimated as ca. 0.74 nm, which is almost conforming to the shortest charge spacing of the α-helical charge array. The counterion binding model as Fig. 5a, in fact, is consistent with an MD simulation for PLGNa in the α-helix conformation [24]; Na^+ counterions seem to be bound in between neighbor carboxyl groups as well as from outside of the carboxyl group layer around the polymer.

On the other hand, the relative line width value of Li^+ showed no significant increment at 80 vol.% EtOH despite of the nearly full helix formation. Although the slight increase in the line width above

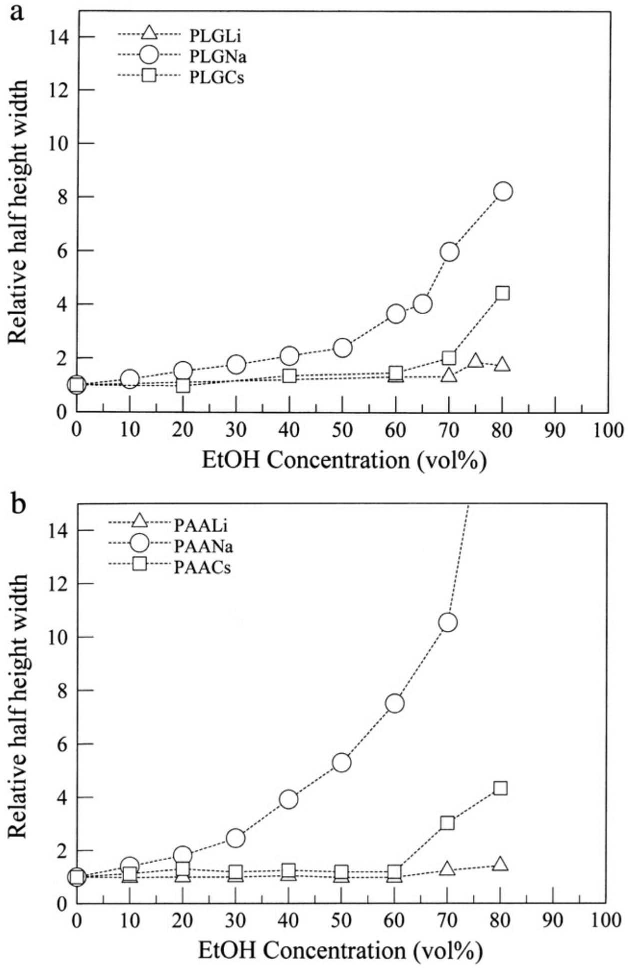

Fig. 4. Line widths of ^{7}Li, ^{23}Na and ^{133}Cs as counterions of polycarboxylates in aqueous EtOH. a. Relative values for PLGM (the respective values in water were 16.1, 23.9 and 7.0 (Hz) for PLGLi, Na and Cs) and b. relative values for PAAM systems that were calculated from the data in Fig. 6 of Ref. [10].

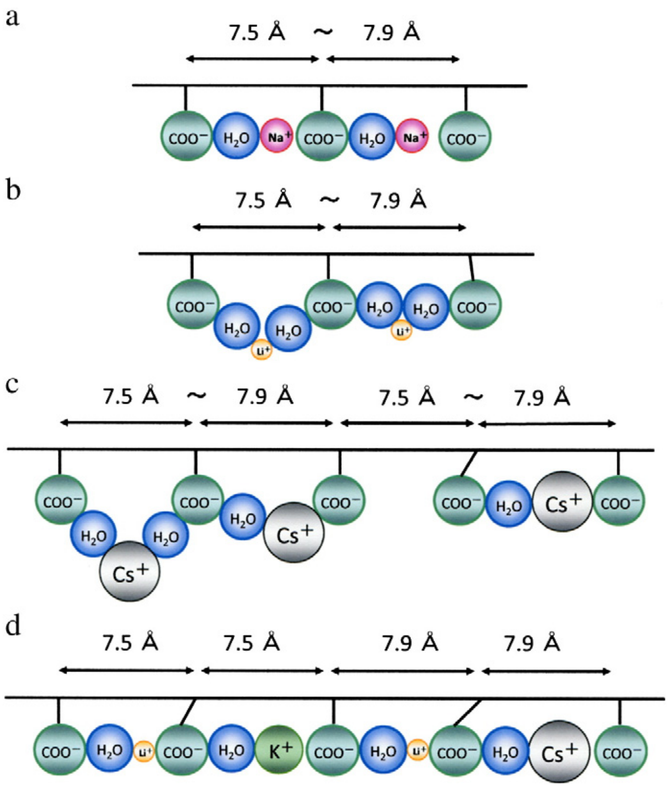

Fig. 5. Schematic illustrations for counterion binding models of PLGM in α-helix conformation. 7.5 and 7.9 Å are the nearest and the second nearest charge spacing in the helix charge array. a. PLGNa, almost just fitting to the nearest charge spacing. b. PLGLi, the counterion binding into the second nearest charge spacing seems to be more effective for the charge neutralization. c. PLGCs, the linear CIP–SSIP mode shown on the right seems to be ineffective due to the inevitable enhancement in the charge density around the binding site. d. Linear CIP–SSIP binding models for mixed counterion systems; Li^+/K^+ combination for 7.5 Å spacing and Li^+/Cs^+ for 7.9 Å.

70 vol.% EtOH may suggest that a contact ion-pair formation occurred, it must be only a minor component. Since PLGLi shows a typical coil–helix transition, the contact ion-pair formation is not a necessary condition for the helix formation. This situation may be depicted by Fig. 5b, where Li^+ ions are bound to the polymer charges as solvent(water)-shared ion-pair (SSIP). Although the polymer charge repulsion may not be effectively screened with the binding model for the shortest charge spacing shown left, it must be largely reduced for the second one shown right, where only a slight displacement of the charge spacing (ca. 0.1 nm) is necessary. In this type of counterion binding, Li^+ may keep the first hydration shell even in the bound state. Since the suppressed line width increment of Li^+ ion suggests that the surrounding of the ion is not largely different from that in water, the counterion-binding model is consistent with the experimental result.

In the case of PLGCs, an appreciable increment was observed above 60 vol.% EtOH. This means that the contact ion-pair formation is included to some extent in the counterion binding of Cs^+ in the globule state. On the other hand, since no helix formation occurred in the relevant solvent concentration region, the contact ion-pair formation is not a sufficient condition for the helix formation of PLGM in aqueous EtOH.

Now we return to the discussion on the counterion specificity of the "globule–helix transition". As stated in the previous section, the free energy change involved with Li^+ counterion binding to the helix charge array, as illustrated in Fig. 5b, must be more favorable than that of Cs^+. Then, what kind of binding mode may be assumed for the "hypothetical" helix charge array for PLGCs? Fig. 5c shows three likely binding models. In the left one, one Cs^+ ion forms two solvent-shared ion-pairs (SSIP) and in the middle, Cs^+ forms one contact ion-pair and one solvent-shared ion-pair (CIP–SSIP) with two nearest or second nearest charges. In the right one, CIP–SSIP mode is somewhat modified to accommodate the Cs^+ in between the two polymer charges (linear CIP–SSIP). As for the SSIP mode, the binding energy must be unfavorable than even the left one in Fig. 5b for Li^+ due to the larger ion size. In the case of CIP–SSIP, the charge neutralization might be comparable to the right one in Fig. 5b. However, since no helix formation was actually observed for PLGCs, the CIP–SSIP type counterion binding must also be unfavorable. One reason may come from the large difference in the hydration energies of the ions, i.e., $-COO^-$ and Cs^+; the hydration enthalpy of the former (CH_3COO^-) is −423 kJ/mol, while that of the latter is only −274 kJ/mol [20]. This means that a replacement of the hydration water of the carboxyl anion with Cs^+ is enthalpically unfavorable and it may not be fully compensated by the enthalpy gain from the attenuation of polymer charge repulsion with the CIP–SSIP mode. From a point of view of the charge screening, the linear CIP–SSIP model shown right in Fig. 5c may be near to an optimum state just as the case of PLGNa (Fig. 5a). However, it would inevitably enhance the charge repulsion around the site, which must be totally unfavorable for the helix formation. In other words, PLGCs could assume α-helix if the enhanced charge repulsion accompanying the CIP formation was somehow compensated. This was in fact realized by addition of Li^+ counterion as shown below.

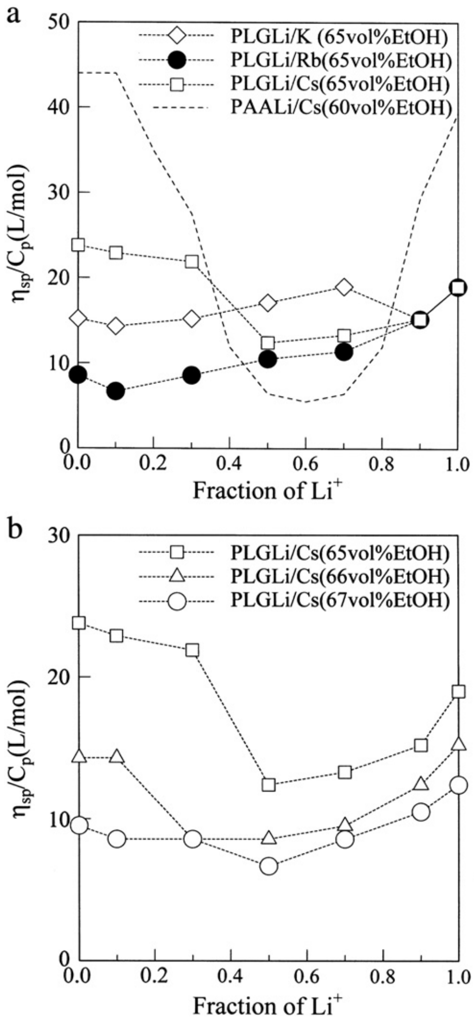

Fig. 6. Counterion mixing effect for PLG coil–globule transition in aqueous EtOH as detected by the viscometry. a. Comparisons among the counterion combinations and with PAA system. b. Effect of EtOH concentration for PLGLi/Cs system.

3.2. Mixed counterion system

3.2.1. Viscometry

According to the experimental results for PAA systems [8], the counterion mixing effect appeared for a combination of Li^+ and Cs^+, the sizes of which are smaller and larger than the optimum one of Na^+. Further, it became more significant at a solvent concentration that was a little lower than the collapse point. In the present case, the full collapse of PLGLi occurred at 80 vol.%. Thus, counterion mixing effects, if any, were tested around at 65 vol.% EtOH. As shown in Fig. 6a, PLG systems showed a counterion mixing effect for the combination of Li^+ and Cs^+, while the extent was just modest compared with that for PAA. With a slight increase in the EtOH concentration (65–67 vol.%), the effect became still less significant (Fig. 6b), which is contrastive to PAA systems where the counterion mixing effect was greatly enhanced by increasing organic solvent concentration [8]. These discreet counterion mixing effects suggest that some hindrance factor to the mixing effect exists in PLGM system. As a candidate, one may consider the charge spacing; the carboxyl group of PLG is away from the main chain by two methylene group and two adjacent side chains are separated by one amide group, which is looser than the charge arrangement for PAA. According to our "size-fitting model" to explain the counterion-specificity observed for the coil–globule transition of PAAM systems, fitting of the counterion size to the polymer charge array is one of the main factors to determine the ion-specificity. Since such a size-fitting should become less effective or result in lower selectivity when the charge array is flexible, the less significant counterion-mixing effect for PLG system may be rather natural.

3.2.2. CD measurements

In Fig. 7 compared are the counterion mixing effects for the helix formation at 70 vol.% EtOH. The combination of Li^+ and K^+ was the most effective to induce the coil–helix transition. Those for Li^+/Rb^+ and Li^+/Cs^+ combinations were also appreciable. Although the counterion specificity in the single counterion system showed that PLGCs forms no helix at least up to 80 vol.% EtOH, Cs^+ proved to be able to induce the transition upon mixing with Li^+. Thus, the apparently most unfavorable counterion size of Cs^+ for promoting the coil–helix transition seems to become a favorable one with the coexistence of Li^+ counterion. This rather striking result strongly suggests that the counterion-size-fitting to the polymer charge array largely contribute to the specific counterion binding when the polymer charges are arranged in the helix conformation. This point will be considered again in a later section.

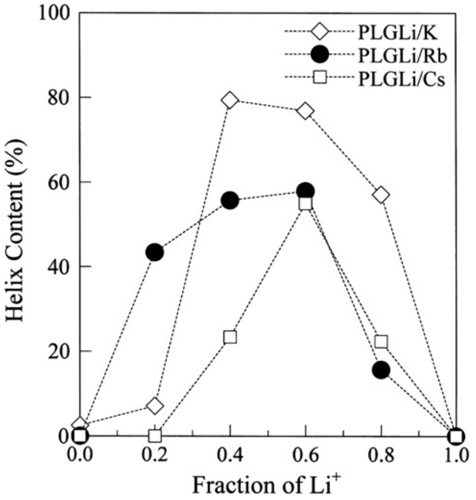

Fig. 7. Counterion mixing effect for PLG helix formation in aqueous EtOH (70 vol.%).

3.2.3. Line width measurements

In the previous study on PAA mixed counterion systems, a significant increment in the line width was observed for Cs^+ but not for Li^+. Namely, the induced coil–globule transition was only accompanied by the contact ion-pair formation of Cs^+. In the present PLGM system, however, the helix formation induced by the counterion mixing proved to be accompanied by the contact ion-pair formation of both cations, Li^+ and Cs^+, with the polymer charges as indicated by the significant increment in their line widths (Fig. 8). For example, the line width for ^7Li increased by ca. 60% from ca. 21 Hz in the single counterion system at 70 vol.% EtOH to ca. 34 Hz in the presence of 60 mol% of Cs^+. This broadening of the line width of ^7Li was more significant when mixed with K^+ (37 Hz in the presence of 40 mol% of K^+). The line width of ^{133}Cs when mixed with Li^+ was, on the other hand, more markedly enhanced; the maximum line width of Cs^+ at 70 vol.% EtOH was ca. 88 Hz in the presence of 40 mol% of Li^+, which is larger more than 8 times of ca. 10 Hz in the single counterion system. This marked enhancement is rather distinguished if compared with the broadening in the single counterion system with increasing EtOH concentration; 4.5 times from 7 Hz in water to 31 Hz in 80 vol.% EtOH. These results indicate that the counterion mixing is critically effective for the specific counterion binding when the charge array is in the α-helix conformation.

Comparing the two counterion mixing effects shown in Figs. 7 and 8, they seem to be coupled each other. However, a scrutiny confirms that there exists some contradiction. For example, at the mixing ratio of $Li^+:Cs^+ = 0.2:0.8$, the line width, especially of Li^+ was somewhat enlarged whereas the helix content was 0%. At the ratio of 0.8:0.2, no enhancement in the line width was observed both for Li^+ and Cs^+, while the helix was induced to some extent. Although the cause for the apparent inconsistency may not be identified at the present stage, the turbidness of the polymer samples might be related; as stated in Experimental section, PLGCs sample solution at 70 vol.% EtOH was turbid. In the counterion mixing systems, only those at $Li^+:Cs^+ = 0.2:0.8$ and 0.8:0.2 appeared cloudy. Thus, although the reason why the turbidity was restricted to those mixing ratios is unknown, the inconsistency between the CD and the line width results may be just apparent.

3.2.4. Counterion mixing effect

Finally we consider why and how the counterion mixing is so effective for the coil–helix transition of PLG. The related questions are "how could the simple mixing of counterions with different sizes

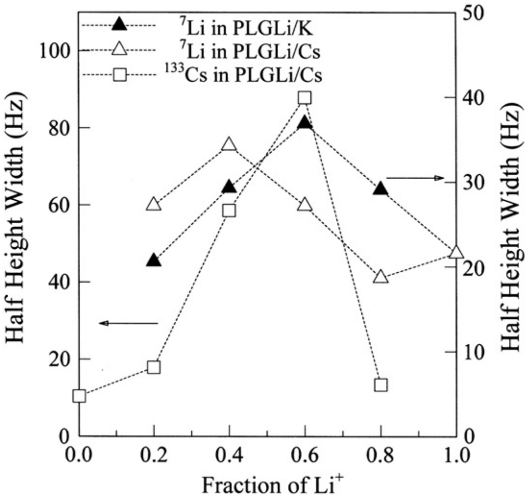

Fig. 8. Counterion mixing effect for the line width of ^7Li and ^{133}Cs in PLGLi/K or PLGLi/Cs systems.

(Li^+ and a larger one) induce the significant specific counterion binding (line width increment) and the concomitant helix formation, in spite of the fact that the transition to helix conformation of PLGLi occurred by increasing the EtOH concentration (~80 vol.%) without accompanying any significant line width broadening." The latter experimental fact means that in the single counterion system Li^+ ion size does not fit to the charge array of helical PLG; too small for the charge spacing if the linear CIP–SSIP type counterion binding should occur. The same may be the case for Cs^+; too large for the charge spacing (Fig. 5c). On the other hand, however, the mixing of Li^+ and Cs^+ (or K^+, Rb^+) counterions, or coexistence of the smaller and the larger counterions than the optimum one, Na^+, seems to compensate the "unfittingness" in the respective single counterion systems, and effect an equivalent behavior of a hypothetical single counterion system, the ion size of which is the mean value of the two counterions. For example the mean values are ca. 0.097, 0.104, and 0.115 nm for Li^+/K^+, Li^+/Rb^+, and Li^+/Cs^+, respectively [21], while the ion radius of Na^+ is 0.095 nm. Since the mixing effect for inducing helix formation is most significant for Li^+/K^+ system, this coincidence seems to support the "size-fitting mechanism" for the counterion mixing effect. A schematic is shown in Fig. 5d to show the reasonable fitting of the respective mixed counterion systems to the charge array, the degrees of which are in fact consistent with the counterion mixing effects observed in the CD and the line width experiments.

Finally, it may be appropriate to note the larger helix content in the Li^+/K^+ system (ca. 80% at 40 mol% Li^+) than that in PLGNa (ca. 60%) at 70 vol.% EtOH. A similar inversion of the helix content was also observed between PLGNa and PLGLi in the higher EtOH concentration region. To explain this apparent contradiction, one should remember that equilibrium between the coil (or globule) and the helix is treated here. Namely, any stabilization of the coil state occurred, it should lead to a decrease in the helix content. Since the significant increase in the line width of PLGNa system suggests that the globule state is largely stabilized with the specific counterion binding, one may explain why the increasing trend of the helix content of PLGNa became less significant in the higher EtOH concentration region and finally overtaken by PLGLi. A similar behavior has also been found for alkaline earth metal poly(L-glutamate) [25] and poly(L-lysine) [6] systems. In the former, the order of the helix content was $Ca^{2+} > Ba^{2+} > Mg^{2+}$ at 30 vol.% EtOH, while it was $Mg^{2+} > Ca^{2+} > Ba^{2+}$ at 60 vol.% EtOH. In the latter, it was $NO_3^- > Br^- > Cl^-$ at 80 vol.% EtOH and $Br^- > NO_3^- > Cl^-$ at 90 vol.% EtOH. These examples of the inversion in the helix content have been interpreted as being caused by stabilization of the coil conformation; namely, counterions that are favorably bound to the polymer charged group ($-COO^-$ or $-NH_3^+$), e.g., Ca^{2+} and NO_3^-, may stabilize not only the helix but also the coil conformation, especially in the higher organic solvent concentration region. Thus, the same mechanism seems to be applicable to the inversion of the helix content of PLGNa and PLGLi/K systems.

4. Concluding remarks

In the present study, we found that PLGM (M:Na or Li) changes the conformation from the random coil to the α-helix via the so-called globule state with increasing EtOH concentration in the aqueous mixed solvent. On the basis of our studies on polyelectrolytes such as PLG and PAA including the previous ones [5,9], the observed coil–globule–helix transition may be described as follows. In the lower EtOH region up to ca. 60 vol.%, the polymer chain in random coil conformation shrinks due to the screening of the electrostatic interaction among the charged groups, which may be mainly caused by the so-called counterion condensation that should be enhanced by a decrement in the dielectric constant of the medium. As a matter of fact, since the line width for ^{23}Na is already appreciably enhanced in this solvent region, the contact ion-pair formation should partly contribute to the gradual shrinking of PLGNa coil. The more steep decrement in the reduced viscosity or the chain collapse that appeared with further addition of EtOH, may be ascribed to two main causes, i.e., poor solvency of the mixed solvent to the polymer charged group including the counterion, and the contact ion-pair formation (for PLGNa and PLGCs). The observed counterion specificity for the collapse, $Na^+ > K^+ > Li^+$, Cs^+, may be interpreted in terms of the four factors as described in the Introduction. In such a collapsed state as the globule, the charged groups must be highly concentrated. Thus, the interaction between the counterion and the polymer charge may be depicted by the ion-cluster model where each counterion interacts with more than two charged groups rather than one-to-one type binding. Above the globule region, or in the higher EtOH concentration region (>60 vol.%), PLGNa and PLGLi assumed α-helix conformation. By increasing EtOH concentration for PLGM in the globule state, the polymer chain would suffer a further desolvation, both on the peptide group and the charged group. The former would lead to the α-helix conformation, which may be helped by the latter via the specific counterion binding. Since the concentrated state of the charged group in the globule state should be further enhanced in the transition to the helix conformation, some effective charge neutralization mechanism, such as the size-fitting model as depicted in Fig. 5, must be necessary for promoting the transition. Such a conformational transition behavior as above may be common for aqueous organic solvent mixture systems, because the coil–globule transition is ubiquitous among polyelectrolytes in poor solvents [26] and the coil–helix transition of PLGM has been observed in many kinds of such mixed solvent systems [5].

The counterion specificity, especially of the helix formation, has been interpreted in terms of the "size-fitting effect". Although the binding scheme as shown in Fig. 5 is just a hypothetical model at the present stage, the simulation results by Fedorov et al. for PLGNa in aqueous solution [24] seems to, at least partly, support the schema. The experimental fact that the counterion specificity is different between the coil–globule transition and the helix formation also suggest that the charge spacing is a key factor. Among others, the significant counterion mixing effect observed for the helix formation would never be explained without referring to the size-fitting model. In order to reach an ultimate elucidation of the counterion specific conformational transitions of polyelectrolytes, a number of studies are now being undertaken by us, including an MD simulation for helical PLGM in organic solvents and experimental studies for polyelectrolytes of different charge spacing and polymer charges, e.g. sulfonate and quaternary ammonium groups.

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