Counterion-specific helix formation of poly(L-glutamic acid) in a crosslinked membrane

Yang-Il Huh, Tadahisa Inamura, Mitsuru Satoh and Jiro Komiyama
Department of Polymer Science, Tokyo Institute of Technology, Ookayama, Meguro-ku, Tokyo 152, Japan
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A poly(L-glutamic acid) (PLGA) membrane was prepared by crosslinking with poly(ether diisocyanate). The counterion specificity in helix stabilization was $Li^+ \geqslant Na^+ > K^+ \geqslant Cs^+$ when the membrane was immersed in aqueous alcohols and $Na^+ > Li^+ \geqslant K^+ \geqslant Cs^+$ in aqueous dioxane. These counterion specificities were interpreted as a result of contact ion-pair formation between the polymer charge and the bound counterion. The coil–helix transitions of PLGA in membrane/solvent mixture systems were compared with those in solution systems.

(Keywords: poly(L-glutamic acid); coil–helix transition; membrane; contact ion-pair; cooperativity)

INTRODUCTION

We have studied the coil–helix transition of charged polypeptides, namely poly(L-glutamic acid) (PLGA) and poly(L-lysine), in water/organic solvent mixtures and found that the helix content depends on the counterion species, the organic solvent species and composition, and the added salt concentration[1–3]. We have proposed that the counterion specificities of the helix formation should be attributed to the difference in the helix stabilities as caused by the contact ion-pairs formed between polymer charges and bound counterions. In the case of poly(L-glutamate) (PLG) alkali-metal salts, the highest stability of the helix conformation has been observed for PLGNa, which was ascribed to an optimum balance between the desolvation energy and the electrostatic interaction energy for contact ion-pair formation. We have suggested[3] three solvent properties as the determining factors for contact ion-pair formation, namely dielectric constant of the solvent mixture, the activity of water in the mixture and the Gutmann–Mayer acceptor number[4] of the organic component of the mixture. The first is a measure of the degree of counterion binding, *not necessarily in contact ion-pair form*. The second is used as a measure of the extent of selective hydration of the polymer charges and counterions and hence for the desolvation energies. The last is a measure of the desolvation energy of anions (in the case of PLG systems, its carboxyl anion groups). It is expected that contact ion-pair formation at the charged groups on the helix conformation becomes more favoured with the decrease in these three factors. Contact ion-pair formation as caused by the decrease in the three factors greatly reduces the effective polymer charge on the helix, which leads to a stabilization of the helix conformation. We have shown that the dependences of the conformational transition on the organic solvent species in the mixture and on the salt concentration are explained in terms of these three factors[3].

The counterion specificity found for the helix stability of PLG alkali-metal salts, namely $Na^+ > Li^+ > K^+ \geqslant Cs^+$, is noteworthy not only from a physicochemical point of view but also from a biological one; for the latter, one may suppose a basic relevance to cation selective phenomena in membrane proteins, such as the Na^+/K^+ pump, since conformational transitions of the constituent polypeptides are incorporated in the processes[5].

The permeability regulation of small molecules through synthetic polypeptide membranes, which utilizes the helix–coil transition, has been attempted by many workers[6–10]. In most studies, however, helix formation is induced by pH change in the medium, few studies being available on counterion-specific permeation control. In a previous paper[11], we reported that helix stabilization is higher for PLGNa than for PLGK in a blend membrane with poly(vinyl alcohol) immersed in water/2-propanol mixtures. The volume flux of the solvent through the membrane was responsive to the conformational transition. This blend membrane system was chosen to give an alcoholic environment to PLG, while it turned out that it was difficult to prevent the elution of PLG from the membrane in the long term over days.

In this study, we prepared a PLG membrane crosslinked with a poly(ether diisocyanate) to get a stable membrane in alcoholic solvent systems. The helix formation of PLG, depending on the counterion species, solvent species and composition, is discussed in comparison with those in the corresponding solution systems.

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EXPERIMENTAL

Poly(L-glutamic acid) (PLGA; $M_w = 44000$) was obtained by reprecipitation of PLGNa in 2 N HCl(aq.). PLGNa was prepared by saponification of poly(γ-methyl L-glutamate) from Seikou Kasei Co.

Synthesis of the crosslinking agent and membrane preparation were carried out according to Tanaka *et al.*[12]. Poly(ethylene glycol) (average molecular weight = 300, PEG300) from Kanto Chemical Co. was dried under vacuum at 70°C for 10 h. Hexamethylene diisocyanate (Tokyo Kasei Co.) was mixed with the PEG300 at 2:1 molar ratio under N_2 atmosphere at 80°C for 5.5 h to obtain a poly(ether diisocyanate) (PEGDI). The stoichiometric consumption of the isocyanate groups was confirmed by titration with dibutylamine. The casting solution was prepared by mixing 1.0 g of PLGA and 2.3 g of PEGDI in 30 ml of dimethylacetamide (DMA). The crosslinked PLG membrane was obtained by slow evaporation of DMA from the solution cast on a glass plate at 70°C for 24 h, followed by evacuation for 5 h. The degree of crosslinkage per PLG carboxyl group was estimated as ca. 1 mol% by pH titration. No phase separation over a 1 μm scale was observed by scanning electron microscopy.

Counterion species of PLG in the membranes were altered by immersing the dry membrane in the same aqueous organic solvents as used for the optical rotatory dispersion (o.r.d.) measurements for 24 h, namely 75–90 vol% aqueous methanol (MeOH), 70–90 vol% aqueous ethanol (EtOH) and aqueous 2-propanol (2PrOH), and 60–80 vol% aqueous dioxane, containing 0.01 N alkali hydroxide (LiOH, NaOH, KOH, or CsOH). For the sake of membrane stability or to minimize the elution of PLG, organic solvent compositions lower than the above values were not employed. After o.r.d. measurements, each membrane sample was immersed in otherwise the same solvent containing 0.02 N HCl to change the PLG salt to PLGA in the membrane. With these membranes containing PLGA, o.r.d. was measured again.

Optical rotation of the PLG membranes was measured with a Jasco 20A Spectropolarimeter at 25°C. The sample membrane of a salt-type PLG (PLGLi, -Na, -K or -Cs) or of an acid-type PLG (PLGA) was set inside a 1 cm cell filled with an appropriate mixed solvent containing 0.01 N alkali hydroxide or 0.02 N HCl, respectively. We used the optical rotation at 233 nm for the PLGA membrane as a reference and estimated the relative helix content of each membrane from the ratio of the optical rotation at 233 nm to that of the corresponding PLGA membrane. Namely the helix content θ (%) was estimated as $100\,(H/H_0)(R/R_0)$, where H means the peak height at 233 nm, R the swelling ratio of the membrane area and suffix 0 indicates the corresponding quantity for the PLGA-type membrane. With this convention, ambiguity due to fluctuations of the crosslink density and/or the polymer concentration among the membrane samples was eliminated in the evaluation of the relative helix content.

The volume swelling ratios (cm^3/cm^3) were estimated by measuring changes of the thickness and the area for membranes immersed in the same mixed solvents as used in the o.r.d. measurements. The elution of PLG from the membrane was evaluated by circular dichroism (c.d.) measurements at 222 nm by assuming 100% helix form for eluted and acidified PLGA.

RESULTS AND DISCUSSION

Figure 1 shows the volume swelling ratios for water/MeOH and water/dioxane systems. The swelling ratios tend to decrease with increase in the organic solvent composition in the range of 4 to 2. Essentially the same tendency was observed for water/EtOH and water/2PrOH systems. This is a natural consequence of the preference for water by the charged groups in the membrane. On the other hand, no systematic trends were observed for the dependences on counterion species, organic solvent species and helix content. It is considered that the scatter among the data for different counterions seen in *Figure 1* may be caused by non-uniformity of the crosslink density among the membrane samples. However, even if differences in the swelling behaviour have an influence on helix formation, it must be removed from our qualitative examination for the counterion specificity in helix stabilization because we employ the relative helix content as stated in the 'Experimental' section. Furthermore, since the elution of PLG due to the swelling was found to be less than 5 mol%, we can safely discuss the counterion specificity as below.

In *Figures 2–5*, we plotted the relative helix contents of the respective salt-type PLG against the composition of aqueous MeOH, EtOH, 2PrOH and dioxane, respectively. The figures also contain the results in the corresponding solution systems[1,3,13]. First, we notice that the coil–helix transition regions of PLG salts in the

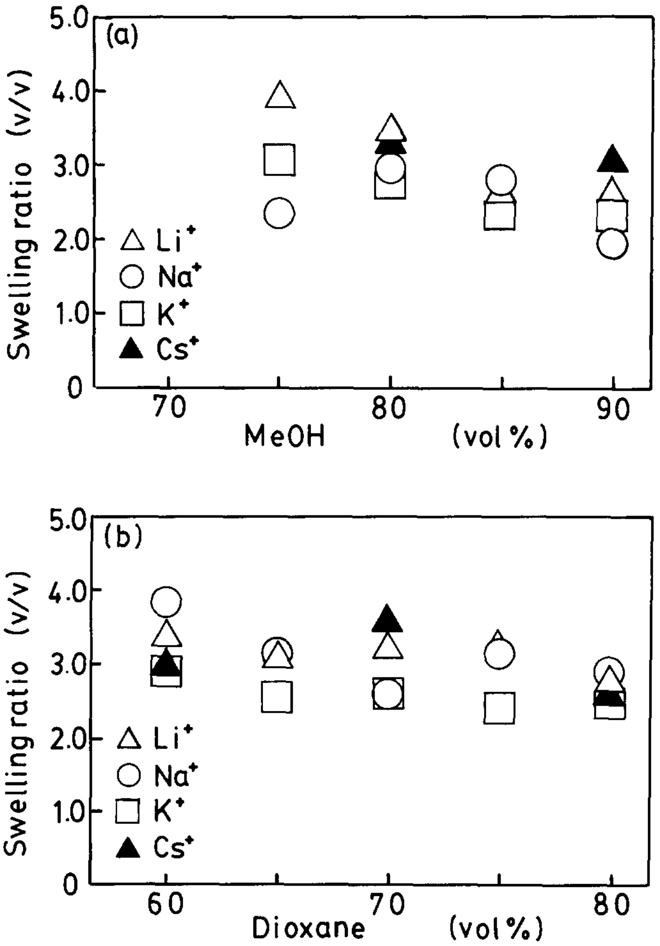

Figure 1 Volume swelling ratios of PLG membranes immersed in (a) water/MeOH and (b) water/dioxane mixed solvents

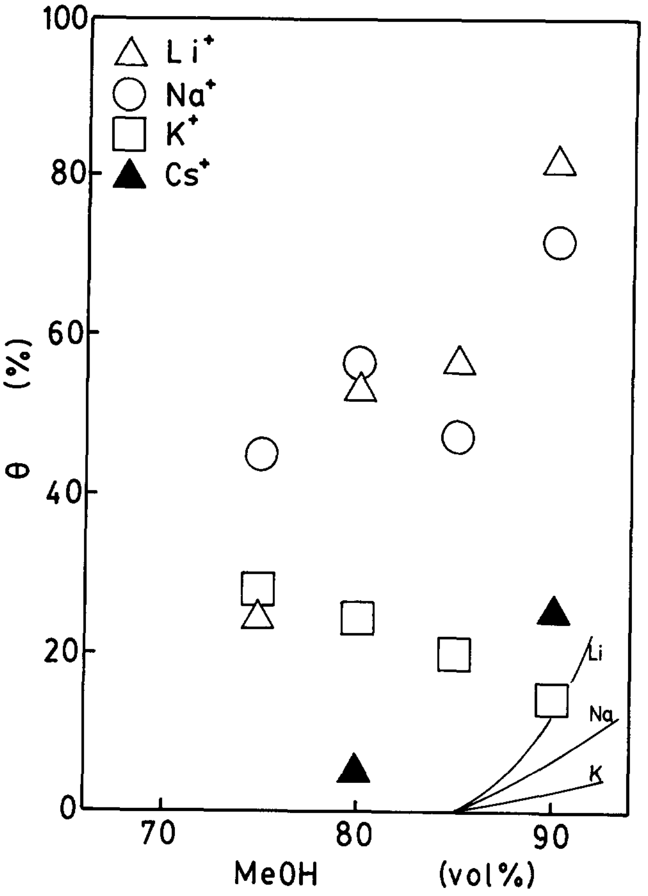

Figure 2 Helix content (θ) of PLG salts in membranes immersed in water/MeOH mixed solvent; full curves are results in solution systems[1]

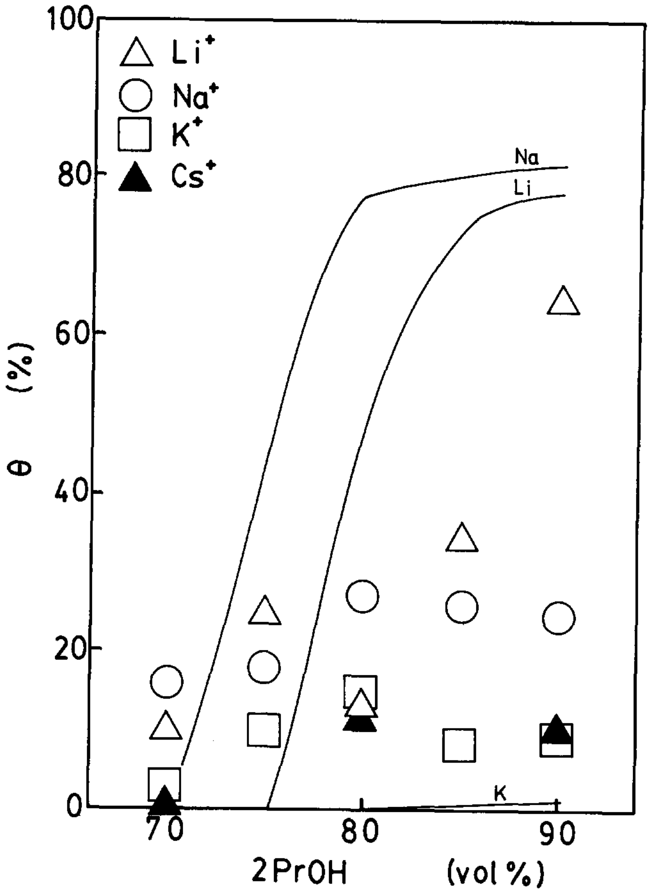

Figure 4 Helix content (θ) of PLG salts in membranes immersed in water/2PrOH mixed solvent; full curves are results in solution systems[3]

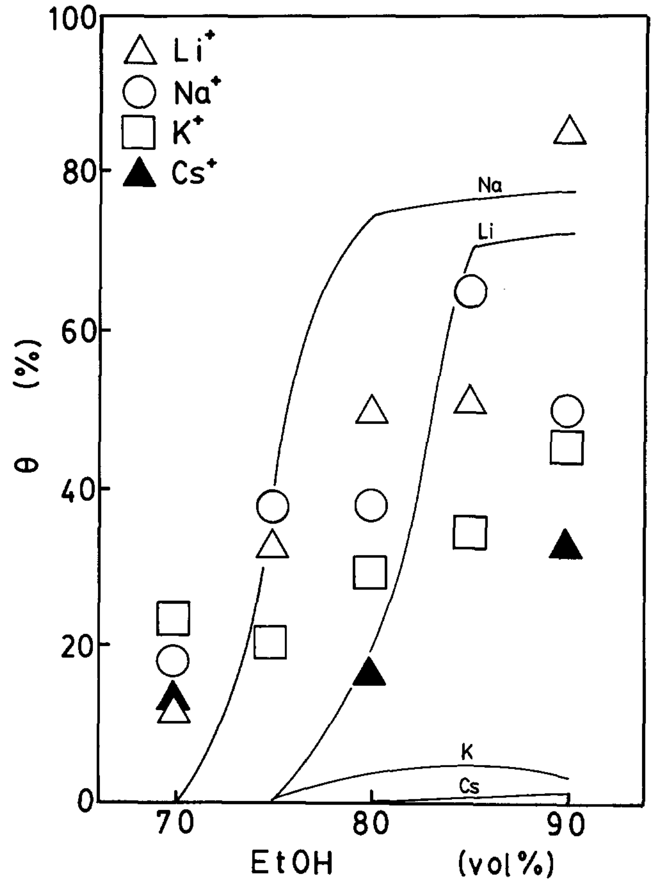

Figure 3 Helix content (θ) of PLG salts in membranes immersed in water/EtOH mixed solvent; full curves are results in solution systems[3]

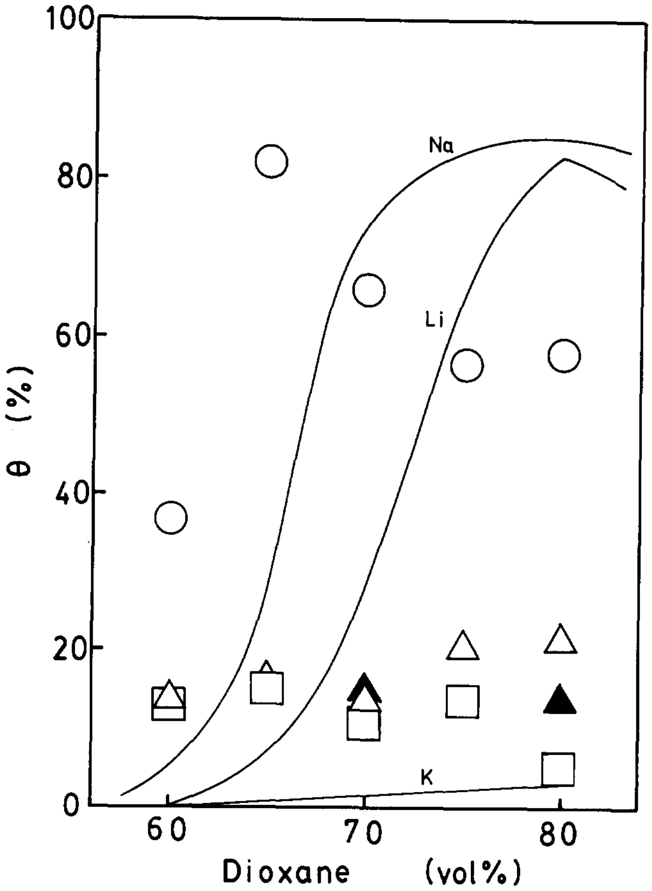

Figure 5 Helix content (θ) of PLG salts in membranes immersed in water/dioxane mixed solvent: (△) PLGLi, (○) PLGNa, (□) PLGK, (▲) PLGCs; full curves are results in solution systems[13]

membranes are rather wider than those in the respective solution systems. Further, in the MeOH system, PLG salts in the membrane show appreciable helix formation as opposed to the insignificant helix content even at 90 vol% MeOH in the solution systems. These two facts suggest that the cooperativity of the conformational transition in the membrane system is lower than that in the solution systems. It is expected that the nucleation of helix growth will be retarded by the crosslinking, although it is as low as 1 mol%. The experimental results that oppose this expectation may be explained either by the fact that PLGA in the helix conformation was crosslinked or by the uncharged nature of the crosslinking site. The latter interpretation is more likely since the nucleation parameter σ is known to increase with the decrease in the charge density of PLG[14].

As another feature in *Figures 2–4*, the counterion specificity in the helix content, $Li^+ \geqslant Na^+ > K^+ \geqslant Cs^+$, is noted. Contrary to the membrane systems, the helix content of PLGLi is lower than that of PLGNa in solution. As stated in the 'Introduction', the highest helix content of PLGNa in solution is ascribed to an optimum balance between the desolvation energy and the electrostatic interaction energy upon contact ion-pair formation. The lower helix content of PLGLi in solution is understood along the same lines; its high (de)solvation energy prevents contact ion-pair formation despite the favourable electrostatic interaction. Therefore, the counterion specificity observed in the present membrane systems immersed in aqueous alcohols, that is in the order of the ion size, suggests that the desolvation energy is lowered to become less significant for contact ion-pair formation. Here it seems appropriate to invoke three factors essential for helix and contact ion-pair formation, i.e. the dielectric constant D, water activity A_w and acceptor number AN. The latter two factors are concerned with the desolvation energy. Although one may hardly predict whether AN increases or decreases with the change from the solution system to the membrane, A_w should decrease in the membrane owing to the relative decrease in the volume concentration. Since the hydration energies of the relevant small ions are more negative than the solvation energies by the alcohols by $4\text{–}20\,\mathrm{kJ\,mol^{-1}}$ (ref. 15), and hence the dehydration energies more positive by the same amount, the decrease in A_w favours contact ion-pair formation, especially for Li^+, which has the highest solvation energy. Helix stabilization by the lowering of A_w should be effective for the other counterions. This is actually observed for PLGK and PLGCs systems, in which their helix contents are appreciably higher than in the solution systems. In the PLGNa systems, however, decreasing trends of the helix content are observed at higher organic solvent compositions (*Figures 3–5*). This helix destabilization may be attributed to contact ion-pair formation at polymer charged sites on the coil conformation as well as on the helix[16].

As seen in *Figure 5*, the counterion specificity for helix formation, $Na^+ > Li^+ \geqslant K^+ \geqslant Cs^+$, in the membrane/aqueous dioxane system is parallel to that observed in solution systems. This suggests that the cooperativity of the transition and the desolvation energy upon contact ion-pair formation in the membrane/aqueous dioxane system are not as different from those in the solution system. The D, A_w and AN values[3,4,17,18] of aqueous dioxane are 17.9 at 70 vol%, 0.84 at 70 vol% and 11 for the pure solvent, respectively. The D and AN values are significantly lower than, for example, 33.5 and 34 of 2PrOH, and A_w is somewhat lower than 0.89, respectively. It is known that lowering of D gives rise to an enhancement of the cooperativity of the transition through the dipole interaction of the peptide groups[19]. Further, it is expected that extremely low AN values result in enhanced selective hydrations on the anionic groups, leading to relatively high desolvation energies upon ion-pair formation.

In the present study, it has been confirmed that counterion-specific helix formations occur in the crosslinked membrane system of PLG alkali salts. The counterion specificity observed for the present membrane/aqueous organic solvent mixture system and its correlation with the solvent properties are essentially important for construction of ion-selective permeation systems. A study on such ion transport through the present membrane systems is now being undertaken.

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