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Helix formation of poly (L-glutamic acid) in the presence of alkaline earth metal cations in aqueous alcohol solutions

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Abstract

Effects of divalent counterions (Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}) on the helix formation of poly(L-glutamic acid) are investigated in aqueous alcohol (MeOH, EtOH, 2PrOH, tBuOH) solutions. To observe helix formation inherent to the divalent counterions, the chlorides were added to a poly(L-glutamate) having an inert monovalent counterion, tetrabutylammonium cation. Marked counterion- and alcohol-specificities were observed for the dependence of helix content, θ, on the alcohol content. The θ values for most Mg^{2+} and Ca^{2+} systems decreased with increasing alcohol content in a higher alcohol region, while those for Sr^{2+} and Ba^{2+} systems were almost constant. A specific helix stabilization was observed for mixed divalent counterion systems. These results are self-consistently interpreted by taking into account contact ion-pair formation between the counterions and the polymer charges on the coil conformation.

Keywords: Poly (L-glutamic acid); Coil–helix transition; Contact ion-pair; Alkaline earth metal cations; Alcohols

1. Introduction

The coil–helix transition of charged polypeptides has been extensively studied both in aqueous and aqueous organic solvent systems [1–10]. The primary attention has been paid to effects of pH, temperature, inter- and intra-molecular hydrogen bonding on the helix conformation. However, studies on the effect of counterion binding [11–16], especially for fully charged polypeptides, are relatively less reported; α-helix conformation of Na poly (L-glutamate) was specifically induced by transition metal counterions such as Cu^{2+}, Cd^{2+}, but not by alkaline earth metals [17].

We have been studying coil-helix transition of poly(L-glutamic acid) [18,19] (PLG) and poly (L-lysine) [20,21] in aqueous alcohols and found that the helix content is markedly dependent on the counterion species. For example, sodium or lithium salt of PLG assumes α-helix in 80 vol% ethanol solution, while potassium or cesium salt remains random coil conformation [18]. The counterion specificity for helix formation, $Cs^+ \ll K^+ < Li^+ < Na^+$, which was confirmed for PLG in aqueous methanol (MeOH), ethanol (EtOH), 2-propanol (2PrOH) and t-butanol (tBuOH) solutions, has been interpreted as being caused by contact ion-pair formation between counterions and polymer carboxyl anions on the helix. Namely, reduction in electrostatic repulsive force between helix polymer charges due to the contact ion-pair formation should effectively stabilize the helix relatively to that in coil conformation. The

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counterion specificity has been explained by assuming that energy balance between desolvation and electrostatic energies involved with the contact ion-pairing is favorable to the smaller counterions, and/or polymer charge is not effectively neutralized by contact ion-pairing with the larger counterions [19].

Recently we have found a highly specific helix stabilization for PLG salts in mixed counterion systems [19]. For example, helix content is enhanced up to 50% for an equimolar mixing system of PLGLi and PLGK in 80 vol% MeOH + tBuOH (1 : 1) mixed solvent from ≈ 5% of the respective single counterion systems. It has been confirmed that the resultant helix content by counterion mixing is higher than simple average of respective single counterion systems for Li^+/K^+ or Cs^+ and $cNa^+ \rightarrow Na^+/K^+$ or Cs^+ combinations, but not for the Li^+/Na^+ system [22]. Similar effects have also been observed for monovalent/divalent and divalent/divalent counterion systems. We have tried to explain this unique effect by considering long-range electrostatic interaction between polymer charges as a component in free energy balance of contact ion-pair formation.

In the present study we report specific effects of alkaline earth metal counterions on coil–helix transition of tetrabutylammonium salt of poly(L-glutamic acid) (PLGTBA) in aqueous alcohols. The large monovalent counterion has been used to minimize the counterion mixing effect which prevents from observing a contribution of the divalent counterions to the helix stabilization. (A preliminary experiment for the PLGLi/PLGTBA system confirmed that any specific helix stabilization is not observed as a function of counterion composition.) The helix content in the presence of alkaline earth metal chlorides ($MgCl_2$, $CaCl_2$, $SrCl_2$, $BaCl_2$) has been measured by circular dichroism (CD) in aqueous solution of four kinds of alcohols (MeOH, EtOH, 2PrOH, tBuOH) as a function of alcohol composition, ratio of divalent to monovalent counterion concentration and counterion composition in mixed divalent counterion systems.

2. Materials and methods

Poly(L-glutamic acid) (PLG) ($Mw = 6.3 \times 10^4$) was obtained by precipitation in 2N HCl aq. of PLGNa which was prepared by saponification of poly(γ-methyl L-glutamate) (Seiko Kasei Co.). Tetrabutylammonium salt of PLG (PLGTBA) was then prepared by dissolving PLG in 1.90 N TBAOH (Sigma) and collected by freeze-drying after purification with ultrafiltration through Ultrafilter UP-20 (Toyo Roshi Co.). The alcohols and the divalent salts of analytical grade were purchased from Kanto Chem. Co. except for $CaCl_2$ from Merck (Suprapur).

CD measurements were carried out with a Jasco J-20A spectro-polarimeter at 25 ± 0.1°C. Helix content θ (%) was estimated by $\theta = [\theta]_{222}/400$, where $[\theta]_{222}$ is molar ellipticity at 222 nm [23]. Polymer solutions (PLGTBA concentration, $C_p = 6.8 \times 10^{-4}$ base mol/l) were prepared varying alcohol composition (20–80 vol%), ratio of divalent salt concentration, C_2, to C_p ($C_2/C_p = 0.1$, 0.3, 0.5, 0.7), and counterion composition in mixed divalent counterion system. Detailed experimental procedure is given elsewhere [18,19].

3. Results and discussion

3.1. Single divalent counterion systems

Fig. 1a–1d shows dependence of θ on alcohol composition at $C_2/C_p = 0.5$. (Results at $C_2/C_p = 0.1$ and 0.3 are not given here; no helix formation was observed at the former case and θ increased simply with alcohol composition, at most up to 25% at 80 vol% MeOH, at the latter one.) Two distinct features can be seen for the dependence. Namely, decrease in θ with increasing alcohol content at a higher alcohol region for Mg^{2+} and Ca^{2+} systems (except for Mg^{2+} in aq. tBuOH) and leveling of θ at ≈ 10% for Sr^{2+} and Ba^{2+} systems. Similar counterion specificity was also observed for PLG alkali metal salts/alkaline earth metal chlorides as reported in our preceding paper [19].

There we interpreted the decrease in θ and almost constant θ as follows: for the former we supposed that at higher alcohol contents contact ion-pair formation also occurs in the coil conformation to reduce the helix content through a relative stabilization of coil conformation. This assumption is based on a consideration that contact ion-pair formation on polyelectrolytes may be determined by the long-range electrostatic interaction between charged groups as well as by the short-range one between a counterion and a polymer charge and the desolvation energy. Namely, with a given increase in alcohol content at a lower alcohol content, contact ion-pair formation may take place only for helix conformation of a higher charge density, hence should have larger decrease in the long-range electrostatic energy upon the contact ion-pair formation. However, at a higher alcohol content (since contact ion-pair formation be-

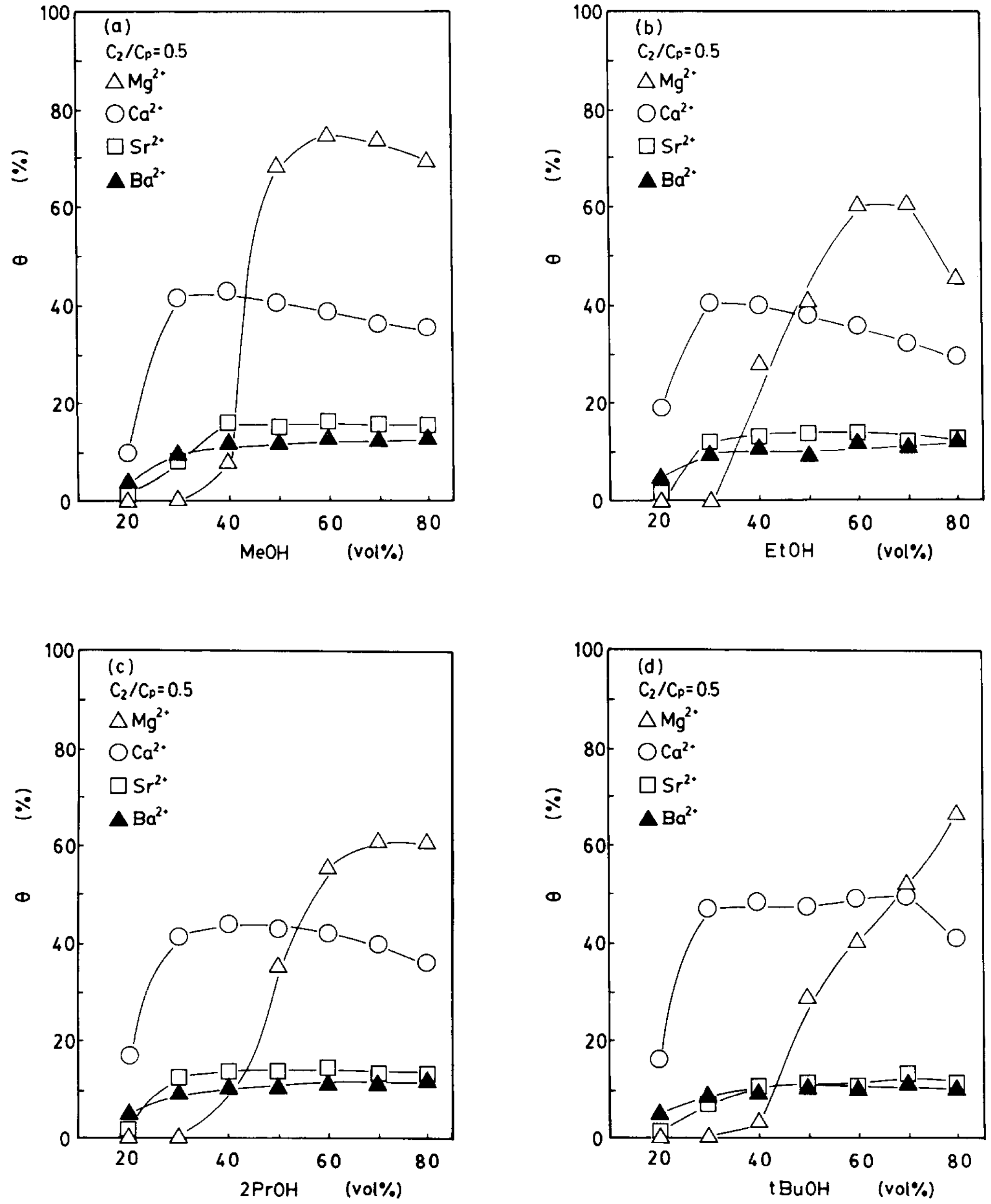

Fig. 1. Helix content of PLGTBA in aq. alcohol solution in the presence of alkaline earth metal ions. (a) MeOH; (b) EtOH; (c) 2PrOH; (d) tBuOH.

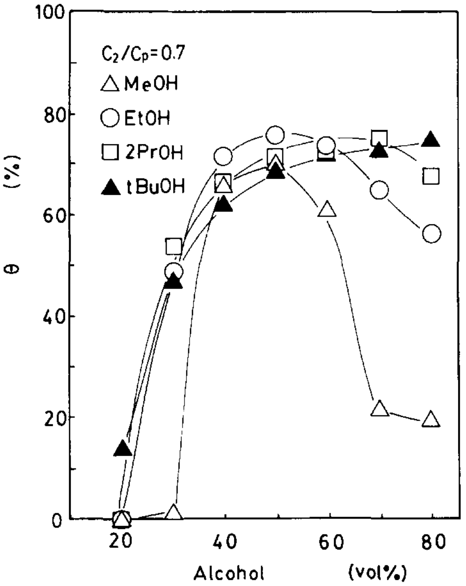

Fig. 2. Helix content of PLGTBA in aq. alcohol solution in the presence of Mg^{2+} ion. $C_2/C_p = 0.7$.

comes favorable with increase in alcohol content as shown later), such an ion-pairing may also occur for coil conformation of a lower charge density. In that case, the resultant decrease in the electrostatic free energy may be larger for the coil than that of the helix, probably because the degree of contact ion-pair formation on the helix tends to saturate at an alcohol content lower than in the case of coil.

For the latter, depressed θ values for Ba^{2+} and Sr^{2+} systems, it was ascribed to their large ion size; effective fractional charges left on the polymer charged groups after the contact ion-pair formation are likely to be still so substantial to prevent from the extension of the helix segments. These interpretations intact seem to be applicable to the present results.

A new finding in the present study is a fact that the extent of the decrease in θ (maximum θ value $-\theta$ value at 80 vol% alcohol) is significantly dependent on alcohol species. Namely, the decrement of θ is large in the order, tBuOH < 2PrOH < MeOH < EtOH, for Mg^{2+} and Ca^{2+} systems at $C_2/C_p = 0.5$. The specificity is more apparent for systems of $C_2/C_p = 0.7$ (Fig. 2), which has been measured only for Mg^{2+} system because of polymer solubility. In this case, the alcohol specificity is large in the order, tBuOH < 2PrOH < EtOH < MeOH. Here we invoke two solvent properties which have proved to be key factors for the contact ion-pair formation [18,19,21], i.e. dielectric constant, D, as a measure of counterion binding degree, and water activity, a_w, as a measure of preferential hydration and hence of desolvation energy. It is expected that contact ion-pair formation become favorable with decreasing the respective factors. Since a_w in mixed solvents with alcohols [24] is large in the order, MeOH < EtOH < tBuOH < 2PrOH, at the same vol% while D [25] is large in the order, tBuOH < 2PrOH < EtOH < MeOH, the specificity observed for $C_2/C_p = 0.7$ seems to be correlated with a_w rather than D. For example, in aq. MeOH which has low a_w values, ions are solvated by MeOH as well as water, while in aq. tBuOH of high a_w ions are mainly hydrated to become less favorable for forming contact ion-pair in respect of desolvation energy. In addition, the bulkiness of tBuOH may promote preferential hydration to the divalent counterions rather than the smaller 2PrOH. The monotonous increase in θ observed for tBuOH systems at $C_2/C_p = 0.5$ and 0.7 is thus explained by a reasoning that contact ion-pair formation takes place only for helix conformation because of the relatively high a_w and hence of high desolvation energy.

On the other hand, at $C_2/C_p = 0.5$ the decrease in θ is largest for aq. EtOH. This may be understood by taking into account electrostatic contribution to the contact ion-pair formation; the D values at the θ-decreasing region (60–80 vol% MeOH and EtOH) are 54.0 to 43.4 and 47.7 to 35.7, respectively. According to a counterion condensation theory [26], counterion binding degrees of divalent ones, λ_2, are estimated as follows; at $C_2/C_p = 0.5$, λ_2 on the coil conformation increases by $\approx 3\%$ for MeOH system and by $\approx 6\%$ for EtOH system in the θ decreasing region with the decrease in D. Although the estimated values should be different from the actual ones which contain contributions from contact ion-pair formation, they may be used as a measure of change in electrostatic contribution to the contact ion-pair formation. Thus, the larger increment in λ_2 seems to be responsible for the larger decrement in θ for EtOH system. As a matter of fact, at $C_2/C_p = 0.7$ the increment in λ_2 for EtOH system is estimated as $\approx 8\%$, which is compared to $\approx 7\%$ for MeOH system. Therefore aq. MeOH system seems to be most favorable for contact ion-pair formation also at $C_2/C_p = 0.5$, if compared under a same electrostatic condition, due to the low a_w.

3.2. Two divalent counterion systems

In our preceding paper [19], we investigated counterion mixing effect on the helix content mainly for two monovalent and monovalent/divalent systems; two divalent counterion systems were measured only for Mg^{2+} or Ca^{2+}/Ba^{2+} combination in 17.5 to 40 vol% EtOH. In the present study, all counterion combinations were tested, i.e. Mg^{2+}/Ca^{2+}, Mg^{2+}/Sr^{2+}, Mg^{2+}/Ba^{2+}, Ca^{2+}/Sr^{2+}, Ca^{2+}/Ba^{2+}, Sr^{2+}/Ba^{2+}, at total concentration ratio $C_2/C_p = 0.5$, for 30, 50 and 80 vol% aq. MeOH, EtOH, 2PrOH and tBuOH systems. For systems which do not contain Mg^{2+}, no appreciable helix stabilization was observed and the results were omitted here. Fig. 3 shows a typical helix stabilization with counterion mixing for Mg^{2+}/Mx^{2+} systems in 50 vol% EtOH. In the other alcohol systems too, similar results were obtained. The helix stabilization observed for mixed divalent counterion systems in the present study, as suggested in the preceding study, may also be ascribed to lower free energy in the long-range electrostatic interaction between polymer charges involved in the contact ion-pair formation for mixed counterion systems than simple average of the free energies of respective single counterion systems. The helix stabilization is most distinct for Mg^{2+}/Sr^{2+} rather than for Mg^{2+}/Ca^{2+}, if it is considered that the helix content of single Sr^{2+} system is much lower than that of single Ca^{2+} one. This result may be compared to a previous observation that counterion mixing effect is most distinct for Li^+/K^+ system.

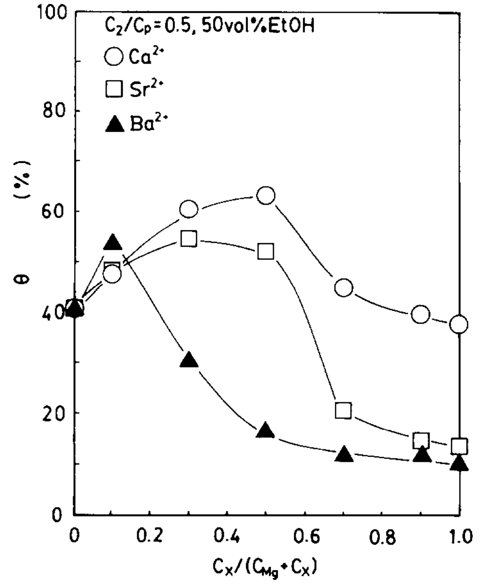

Fig. 3. Dependence of helix content on the divalent counterion mixing ratio for Ca^{2+}/Mg^{2+}, Sr^{2+}/Mg^{2+} and Ba^{2+}/Mg^{2+} systems. 50 vol% EtOH. $C_2/C_p = 0.5$.

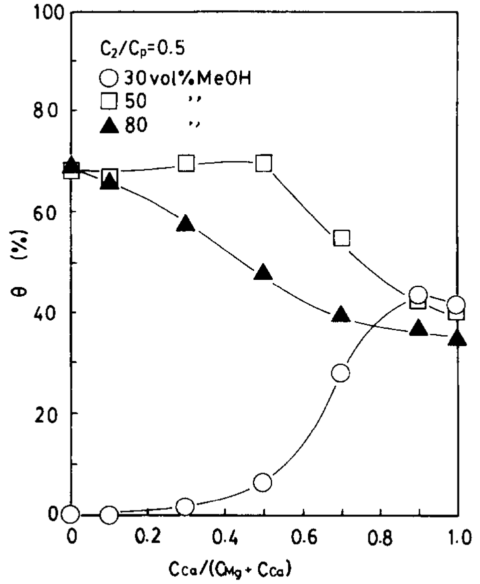

Fig. 4. Dependence of helix content on the divalent counterion mixing ratio for 30, 50 and 80 vol% MeOH systems. Ca^{2+}/Mg^{2+}. $C_2/C_p = 0.5$.

A new finding on the counterion mixing effect is the remarkable dependence on alcohol composition and species. In Fig. 4 and Fig. 5, results of Mg^{2+}/Ca^{2+} system are shown as typical ones for the dependence on MeOH composition and that on alcohol species at 80 vol%, respectively. As seen in Fig. 4, helix stabilization becomes more significant with increasing ethanol composition from 30 to 50 vol% while it is not effected by counterion mixing at 80 vol%. The same has also been observed for Mg^{2+}/Sr^2 and Mg^{2+}/Ba^{2+} systems. This dependence of counterion mixing effect on alcohol composition reminds us that of helix content in single divalent

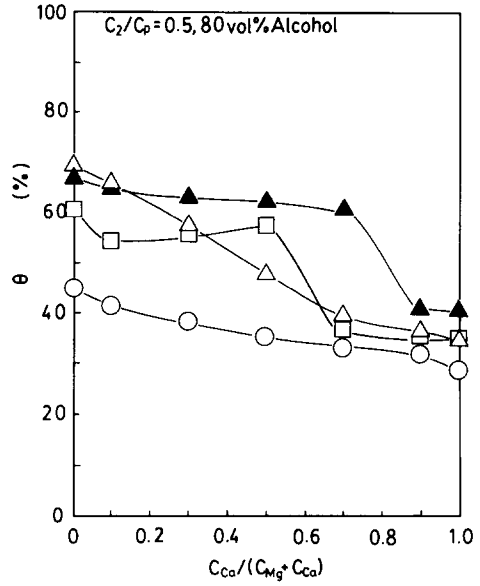

Fig. 5. Dependence of helix content on the divalent counterion mixing ratio for 80 vol% MeOH (△), EtOH (○), 2PrOH (□) and tBuOH (▲) systems. Ca^{2+}/Mg^{2+}. $C_2/C_p = 0.5$.

counterion systems, namely, decrease in θ of Mg^{2+} or Ca^{2+} system at higher alcohol compositions. As stated in the preceding section, the decrease in θ is ascribed to contact ion-pair formation on coil conformation. Since 80 vol% EtOH corresponds to the θ-decreasing region both for Mg^{2+} and Ca^{2+} systems, electrostatic interaction energy must decrease for coil conformation by counterion mixing as well as for helix. In other words, helix stabilization otherwise caused by the counterion mixing is canceled out by the stabilization of coil. The same explanation seems to be applied to the dependence on the alcohol species as shown in Fig. 5. Namely, the monotonous change in θ for 80 vol% MeOH and EtOH systems and the appreciable helix stabilization by counterion mixing for 2PrOH and tBuOH systems may be ascribed to 'ON' and 'OFF' of contact ion-pair formation on the coil state, respectively.

In conclusion, we have observed marked specificities for counterion and alcohol species in the helix formation of PLGTBA in the presence of the alkaline earth metal cations. Those specificities have been interpreted in terms of the same reasoning suggested in the previous studies on alkali metal salts of PLG. The self consistency in the present study with the previous ones, especially on the relation between the counterion mixing effect and contact ion-pair formation on coil conformation, seems to support the basic assumption in the series of studies, i.e. contact ion-pairing as the determining factor for helix formation of charged polypeptides in aqueous alcohol mixtures.

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