Conformational Transition of Poly(L-glutamic acid) in Aqueous Decylammonium Chloride Solution

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(Received August 5, 1978)

The cooperative binding of decylammonium chloride to poly(L-glutamic acid) (PLG) was studied by the potentiometric measurement of the binding isotherm at pH 7.9. Circular dichroism spectra were also measured as a function of the degree of binding of surfactant ion (x). It was shown that the coil to α-helix transition of PLG-surfactant complex takes place with a change in x. PLG did not undergo an appreciable conformational change in the range of x below 0.55. But the helical content increased suddenly with further increase in x. An abrupt increase in the helical content of PLG-surfactant complex can be well interpreted in terms of the hydrophobic interaction among bound surfactant ions. On the basis of the theoretical analysis of the cooperative binding isotherm, it was concluded that the formation of a micelle-like cluster consisting of at least eight surfactant ions is required for the stabilization of a surfactant induced helical structure.

The conformational transition of polypeptide in surfactant solution is of special interest in connection with the effect of surfactant on protein structure. Although most studies so far reported have been confined to the conformational changes of poly(L-ornithine) (PLO) homologs with side chain R=$-(CH_2)_nNH_2$, some interesting features of such transition have been clarified.

PLO (n=3)[1,2] and its homologs (n=5—7)[3] adopt helical conformation in neutral solution of sodium dodecyl sulfate (SDS). The coil to helix transition of PLO is also noted in solution of sodium n-alkyl sulfate with 8 to 16 carbon atoms.[2] In contrast, however, poly(L-lysine) (PLL, n=4) assumes β-structure in neutral solutions of sodium decyl sulfate (SDeS) and its longer chain homologs,[2,4-8] while it assumes helical structure in sodium octyl sulfate solution.[2] Upon raising the pH of SDS solution, PLL undergoes a reversible β to helix transition.[7-9] These conformational changes occur even in the concentration of SDS much lower than the critical micelle concentration (CMC), suggesting a highly cooperative binding of surfactant ions to polypeptide.[2,7,8,10] An acidic solution of SDS causes poly(L-arginine) to undergo a coil to helix transition and poly(L-histidine) to change from a coil to β-structure.[11] In view of these observations, it is likely that the hydrophobic interaction between electrostatically bound surfactant ions plays a predominant role in the conformational change of charged polypeptide in surfactant solution. However, little is known about the factors which influence the type of a resulting conformation.

One might expect a similar conformational change of anionic polypeptide in cationic surfactant solution. The purpose of the present paper is to determine the binding isotherm of decylammonium ion to poly(L-glutamic acid) (PLG) and to find a correlation between the degree of binding and a resulting conformational change as inferred from circular dichroism (CD) spectra.

Experimental

Materials. PLG (Miles-Yeda Ltd., molecular weight 50000) was converted to sodium salt by dissolving it into dilute sodium hydroxide solution and then dializing against distilled water. The polypeptide concentration was determined by micro-Kjeldahl nitrogen analysis. Decylammonium chloride (DeAC) was prepared from 99% decylamine (Nakarai Chemicals Ltd.) according to a method described by Kolthoff and Strickes.[12] The pK_a of DeAC in 10^{-2} mol/dm^3 KCl solution was determined from pH titration to be 10.4 at 25 °C.

Binding Isotherm Measurement. Since PLG is a typical polyelectrolyte, the apparent dissociation constant (K) depends not only on the degree of dissociation (α), but on added salt concentration. For instance, in 5×10^{-3} mol/dm^3 NaCl, the pK value varies from 4.45 at α=0 to 6.0 at α≈0.9.[13] This means that about 10% of carboxylic acid groups remain still undissociated even at pH 7. In order to secure the complete dissociation of PLG, therefore, all experiments were performed with buffered solution at pH 7.9, which contains 4.32×10^{-3} mol/dm^3 sodium borate and 7.36×10^{-3} mol/dm^3 hydrochloric acid. The binding isotherm of DeAC to PLG was determined potentiometrically at 25 °C, using a poly(vinyl chloride) (PVC) plastic electrode which had been shown to respond to tetraalkylammonium ions.[14] The cell constructed was as in the following.

Reference electrode (Ag–AgCl)|1 mol/dm^3 NH_4NO_3 Agar bridge|Reference solution (DeAC, 5.238×10^{-4} mol/dm^3)|PVC membrane|Sample solution ($C_p=3.18\times10^{-4}$ residue mol/dm^3; DeAC, C_t; Borate buffer)|1 mol/dm^3 NH_4NO_3 Agar bridge| Reference electrode (Ag–AgCl),

where, C_p denotes the concentration of PLG in residue mol/dm^3 and C_t, the total concentration of DeAC, respectively. The PVC membrane was prepared by dissolving 0.6 g of PVC into mixed solution of 2.4 cm^3 of bis(2-ethylhexyl) phthalate and 2.4 cm^3 of 10^{-4} mol/dm^3 DeAC in tetrahydrofuran. The solution was then poured onto a covered glass dish and allowed tetrahydrofuran to evapolate slowly. A resulting film 0.30 mm thick was fixed to a PVC tube of 9 mm diameter by the use of a tetrahydrofuran solution of PVC. The electromotive force (EMF) (E) of the cell was measured with a Corning Digital 112 Research pH Meter with an accuracy of ±0.1 mV. Prior to the measurements on PLG solutions, the PVC membrane was calibrated with solutions of DeAC. Figure 1 shows the semilogarithmic plots of EMF *vs.* DeAC concentration. A linear relation with a slope of 57.9 mV suggests that the PVC membrane responds to surfactant ion exclusively in the concentration range studied. The concentration of PLG was

kept constant throughout a series of experiments.

Circular Dichroism. The CD spectra were obtained at 25 °C with a JASCO Automatic Recording Spectropolarimeter J-40A, using 1 cm path length cell equipped with water-circulating jacket. The spectra were recorded 3.5—4 h after mixing, because an appreciable time dependence was found during first 2—3 h.

Results and Discussion

In order to estimate the degree of binding (x) of decylammonium ion to PLG, it is necessary to assume that the polypeptide ions in an excess salt solution do not affect appreciably the activity coefficient of free surfactant ion.[10] On this assumption, which is compatible with a theoretical prediction,[15] x may be given as

$$x = C_b/C_p = (C_t - C_f)/C_p, \quad (1)$$

where, C_b represents the bound surfactant ion concentration and C_f, the free surfactant ion concentration which can be determined from Fig. 1.

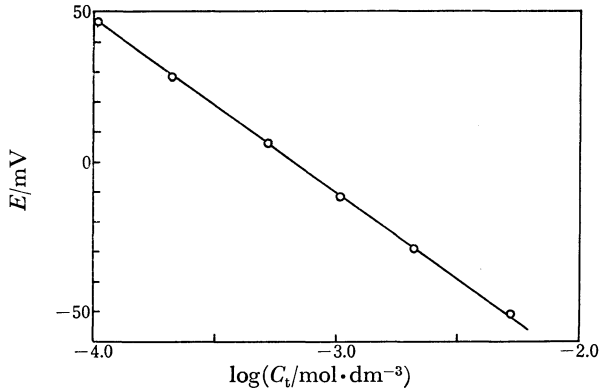

Fig. 1. Plots of EMF (E) of the cell *vs.* the logarithm of decylammonium chloride concentration in the absence of PLG at 25 °C.

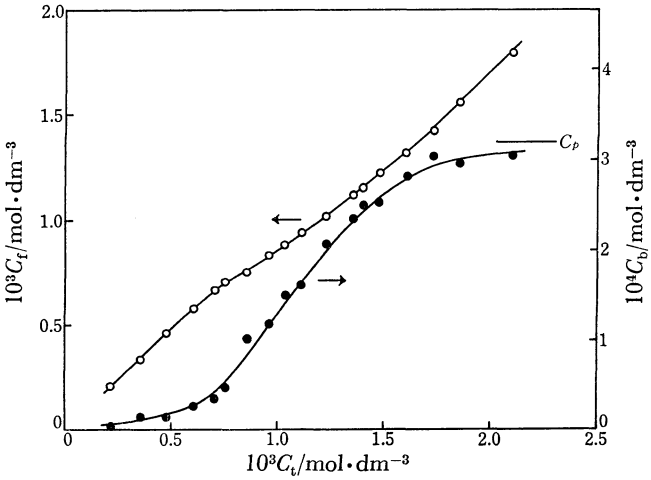

Fig. 2. Plots of C_f and C_b *vs.* C_t at 25 °C. C_p=3.18 $\times 10^{-4}$ residue mol/dm³. Arrows indicate the ordinate to be applied.

In Fig. 2, C_f and C_b observed with constant PLG concentration of 3.18×10^{-4} residue mol/dm³ are plotted against the total concentration of DeAC, C_t. Through a broad kink at about 7×10^{-4} mol/dm³, C_t first increases

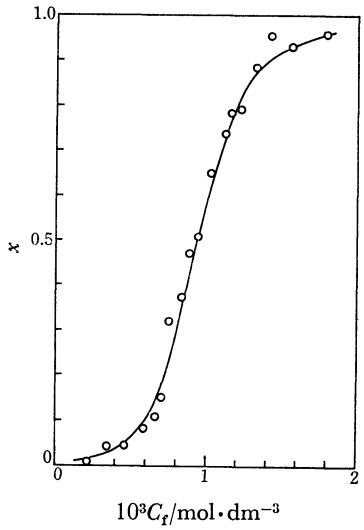

Fig. 3. The binding isotherm of decylammonium chloride to PLG at 25 °C.

almost linearly with increasing C_t and then increases gradually with further increase in C_t. In accordance with the change in C_f, C_b shows a sigmoidal increase with C_t and approaches eventually to PLG concentration, C_p. Figure 3 shows the binding isotherm of DeAC to PLG calculated from Fig. 2. Since the carboxylic acid groups of PLG are completely ionized at pH 7.9, decylammonium ion can interact electrostatically with carboxylate group, giving rise to an ion pair. As was pointed out by Satake and Yang,[10] however, the binding process of surfactant ion to polypeptide ion becomes cooperative because of an additional hydrophobic interaction among bound surfactant ions. In these circumstances, the bound surfactant ions will cluster side by side onto polypeptide chain even in small degree of binding and cause the binding isotherm to rise steeply in narrow range of C_f. This is indeed the case for the present result shown in Fig. 3, where x increases more rapidly than predicted by simple statistical binding model. In this connection, it is interesting to compare the present binding isotherm with those of sodium decyl sulfate to PLL and PLO.[10] In the case of PLL, the binding isotherm was found to rise almost vertically at a certain value of C_f, indicating a highly cooperative binding of decyl sulfate ion. This in turn results in a micellar clustering on the polypeptide chain similar to the micelle formation process of the surfactant alone above its CMC. On the other hand, the binding isotherm for PLO is not so sharp as for PLL,[10] but is still sharp as compared with the present result. These facts imply that the cooperativity in surfactant ion binding to polypeptide depends virtually on the total alkyl chain length of the complex species, *i.e.*, the sum of alkyl chain length of bound surfactant ion and polypeptide side group. The type of ionic group seems to play, if any, a minor role.

On the basis of Zimm-Bragg theory[16] for helix-coil transition, Satake and Yang[10] derived the following expression for the cooperative binding of small ions to polymer;

$$2x - 1 = (y-1)/[(1-y)^2 + 4yu^{-1}]^{1/2}, \quad (2)$$

where

$$y = C_f/(C_f)_{x=0.5}. \quad (3)$$

In Eqs. 2, and 3, u denotes a parameter, which is a measure of cooperativity, defined by

$$u = \exp[(2E_{01}-E_{11}-E_{00})/kT], \quad (4)$$

where, E_{ij} represents the interaction energy between neighboring i-j pairs, and suffix 0 and 1 refer to an unbound and a surfactant-bound polypeptide side chain respectively. The estimation of u can easily be attained by using a relation,

$$(dx/d\ln C_f)_{x=0.5} = u^{1/2}/4. \quad (5)$$

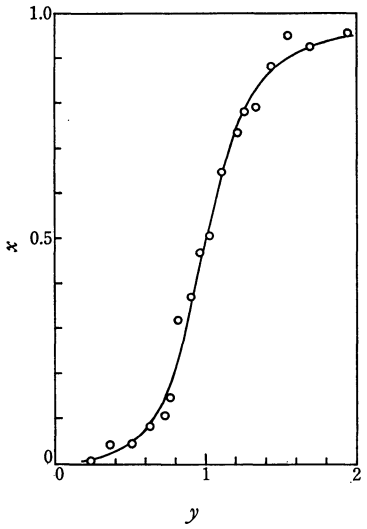

Fig. 4. Comparison of calculated and observed binding isotherm for PLG at 25 °C. ○, observed; solid line, calculated isotherm from Eq. (2) with $u=40$.

The most reliable value of u for the present system was found to be 40, reflecting well the cooperative nature of the binding process. The value of $u=40$ corresponds to an interchange energy $(2E_{01}-E_{11}-E_{00})$ of 9.12 kJ at 25 °C. Figure 4 shows the comparison of the calculated binding isotherm from Eq. 2 with $u=40$ with the observed one. The agreement is quite satisfactory over nearly whole range of x. As would be anticipated, the value of u obtained above is considerably small as compared with that of 77 for SDeS–PLO system.[10] This may reasonably be ascribed to a change in hydrophobic interaction between neighboring groups, arising from a difference in the alkyl chain length of polypeptide side group. According to a foregoing treatment,[10] the average cluster size ($\bar{m}$) of bound surfactant ions, *i.e.*, the average number of bound surfactant ions which constitute a one-dimensional micellar cluster on the polypeptide chain, is given as

$$\bar{m} = 2x(u-1)/[\{4x(1-x)(u-1)+1\}^{1/2}-1]. \quad (6)$$

In Table 1, the calculated values of $\bar{m}$ from Eq. 6 with $u=40$ are given as a function of x. It should be noted

Table 1. The calculated value of $\bar{m}$ as a function of x

x	0.1	0.2	0.3	0.4	0.5	0.55	0.6	0.7	0.8	0.9
$\bar{m}$	2.7	3.8	4.9	6.0	7.3	8.1	9.0	11.4	15.2	24.4

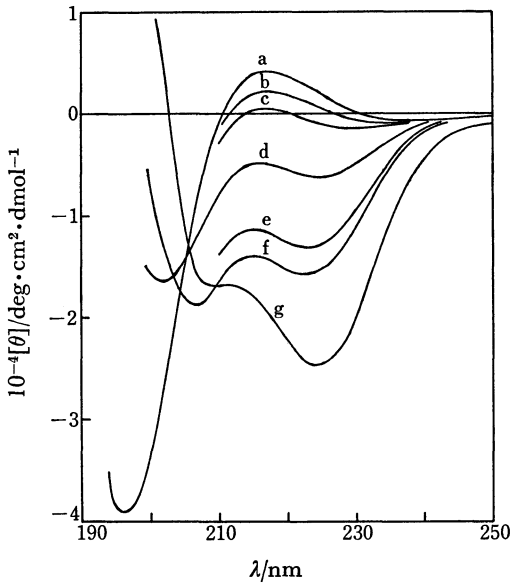

Fig. 5. The change in CD spectrum of PLG with respect to x at 25 °C.
a, 0; b, 0.35; c, 0.56; d, 0.64; e, 0.72; f, 0.80; g, 1 (C_t=0.1515 mol/dm³).

that the cluster size of bound surfactant ions increases rapidly with increasing x.

It is of great interest to find an interrelation between the degree of binding (x) or the average cluster size ($\bar{m}$) and the conformational change induced by surfactant ion binding. To this end, CD spectra were measured under the same experimental conditions as for binding isotherm measurement. Figure 5 shows the change in CD spectrum with respect to x. In the absence of surfactant, the CD spectrum of PLG is characterized by two extrema, a maximum centered at 217 nm with mean residue ellipticity, $[\theta]_{217}$, of 4300 and a deep minimum at 196 nm with $[\theta]_{196}$ of −39000. An additional shallow minimum is also observed at around 235 nm. These features agree well with the CD spectra observed for PLG[17] and PLL[18,19] in their random conformations. With increasing degree of binding, the CD spectrum of PLG tends to approach progressively to a typical spectrum which has a double minimum characteristic of α-helical conformation. A similar change in CD spectrum with surfactant concentration has been found for PLL in SDS solution[2,7] as well as for PLO in solutions of SDS[2] and SDeS.[10] At a concentration above CMC, where x may virtually be regarded as unity, the CD spectrum shows a deep minimum centered at 224 nm with $[\theta]_{224}$ of −25000 and a well defined shoulder located at 209—210 nm with $[\theta]_{209}$ of −17000. Hence, the ordered conformation of PLG in DeAC solution can reasonably be identified as an α-helical structure. It must be kept in mind, however, that the CD spectrum of surfactant induced helical conformation is somewhat different in shape and magnitude from those of helical PLG and PLL in water.[1,2,11] In the absence of surfactant, a double minimum of helical PLG or PLL has been found to locate at 221—222 nm with $[\theta]_{222}$ of −(32000—40000) and at 207—209 nm with $[\theta]_{207\text{-}209}$ of −(33000—39000).[17-20] Unfortunate-

ly, there is no conclusive evidence that accounts for the observed decrease in CD minima of helical polypeptide in surfactant solution. Grourke and Gibbs[1] have assumed that the environment produced by the bound surfactant ions affects the rotatory strengths of n-π^* and π-π^* transitions. Nevertheless, it seems equally probable that the surfactant induced helical structure contains small amounts of random conformation. It is true that the above situation makes it difficult to estimate the precise percentage of helix in PLG–DeAC complex, but $[\theta]_{222}$ is still available for a measure of helix content.

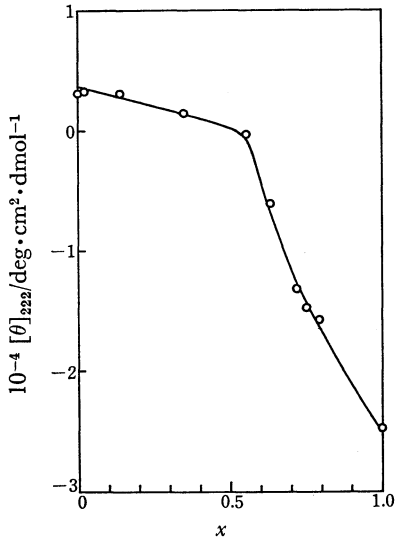

Fig. 6. Plots of $[\theta]_{222}$ *vs.* x at 25 °C. Value at $x=1$ is that in 0.1515 mol/dm³ decylammonium chloride solution.

In Fig. 6, $[\theta]_{222}$ is plotted as a function of x. A clear kink occurs at $x\approx0.55$, through which the magnitude of $[\theta]_{222}$ increases sharply with further increase in x. This implies that PLG does not undergo an appreciable coil to helix transition unless more than 50% of carboxyl groups are bound to decylammonium ions and that a helical structure of the complex become stable only when x exceeds this critical value. An abrupt increase in $[\theta]_{222}$ with x was also observed by Satake and Yang[10] for PLO in SDeS solution. In this case, however, the kink point locates at $x=0.3$—0.4, which corresponds to the average cluster size ($\bar{m}$) of bound decyl sulfate ions of 7—8.[10] This, in turn, corresponds to the number of residues included in two helical turns of α-helical structure. On the basis of these observations, they assumed that the hydrophobic interaction of each bound surfactant ion with another one locating one turn above or below is essential for the formation of a stable helical structure. If this is also valid for PLG–DeAC system, the kink point in $[\theta]_{222}$ *vs.* x plot should appear at x which corresponds to $\bar{m}=7$—8. In addition, the critical x value for the present system will be slightly large as compared with that for PLO–SDeS system, since the binding process is less cooperative in the former than in the latter. The estimation of $\bar{m}$ given in Table 1 supports well the above predictions. As would be expected, the critical value of $x=0.55$ corresponds to $\bar{m}\approx8$.

The authors wish to thank Prof. M. Tanaka and Prof. G. Sugihara, both of Fukuoka University and also to Prof. T. Inaba of Saga University for their helping in CD measurements.

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