The Effect of Added NaCl on the Binding of Sodium Perfluorooctanoate to Lysozyme in Aqueous Solutions

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The binding isotherms of sodium perfluorooctanoate (SPFO) to lysozyme have been investigated as a function of added NaCl by a potentiometric method utilizing a perfluorooctanoate ion selective electrode. The general feature of the binding of SPFO to lysozyme resembled that of sodium dodecyl sulfate. Although the lysozyme–SPFO system is different in detail from that of lysozyme–sodium dodecyl sulfate system, resulting from the mutual phobicity and the difference in hydrophobicity, the binding mechanism proposed for lysozyme–sodium dodecyl sulfate system can be true also for the binding of SPFO to lysozyme.

The investigations on the interaction of surfactants with proteins have so far been restricted to hydrocarbon surfactants. Fluorocarbon surfactants have been extensively investigated on their physicochemical properties in aqueous solutions,[1-6] and are interesting with respect to the nonideality in mixed micelles with hydrocarbon surfactants.[7-11] The surface activities of fluorocarbon surfactants are stronger than that of the hydrocarbon surfactant with corresponding carbon number. The binding of fluorocarbon surfactants to protein is expected to be the same as that of hydrocarbon surfactants. In the previous paper,[12] the effects of added NaCl on the initial binding ($\bar{\nu}<10$) and the subsequent binding ($\bar{\nu}\geq 10$) of sodium dodecyl sulfate (SDS) to lysozyme were discussed separately. It is interesting to know whether the binding mechanism proposed in the previous paper can be applied to the binding of a fluorocarbon surfactant to lysozyme.

In this work, the binding isotherms of SPFO to lysozyme were determined at different NaCl concentrations. The effect of added NaCl on the binding of SPFO was examined for comparison with that of SDS.

Experimental

Perfluorooctanoic acid (HPFO) was purchased from PCR Research Chemicals, Inc., Lot No. 4764. HPFO was recrystallized four times from distilled carbon tetrachloride. Solutions of the sodium salt (SPFO) were prepared by neutralization with sodium hydroxide. The purity of SPFO was checked by surface-tension measurements below and above the CMC. No minimum was observed. The solutions of hen egg-white lysozyme and other chemicals were the same as mentioned in previous papers.[12,13] The binding isotherms were made by means of potentiometry as described in the previous paper.[12] The carrier used in the SPFO-selective electrode was prepared by mixing equivalent amounts of SPFO and dimethyldioctadecylammonium chloride (Kaō Soap Co., Ltd.) in water. The resulting milky white precipitates (dimethyldioctadecylammonium perfluorooctanoate) were collected by centrifugation (3500 min^{-1} for 45 min) and resuspended in distilled water. Decantation was repeated twice and followed by recrystallization from acetone. The preparation of the poly(vinyl chloride) gel membrane is essentially the same as mentioned in the paper by Shirahama *et al.*[14]

All measurements were carried out at pH 5.8 and 25 °C. NaCl concentrations were 10, 20, 50, and 100×10^{-3} mol dm^{-3}. Every calibration curve of the electromotive force (emf) of SPFO in respective NaCl solutions showed good linearity in the concentration range 2×10^{-4} mol dm^{-3} to its CMC, and gave a slope close to the Nernstian response (56.2 ± 0.7 mV per decade change in perfluorooctanoate ion, PFO^-, at 25 °C).

In order to examine the accuracy of each emf value for the sample solution, emf's for several SPFO solutions of known concentration were also measured after measurments of the three sample solutions.

Results and Discussion

The equilibrium concentration of SPFO in the initial binding region ($\bar{\nu}<10$) increased with increasing NaCl concentration, as is shown in Fig. 1. On the other hand, the equilibrium SPFO concentration in the subsequent binding ($\bar{\nu}\geq 10$) decreased with increasing NaCl concentration. These tendencies were also seen in the binding of SDS in NaCl solutions. The flat region where $\bar{\nu}$ is kept at about 10 in Fig. 1 becomes narrower with increasing NaCl concentration as was seen in the case of SDS. It should be noted that the initial binding begins at a concentration higher than that of lysozyme–

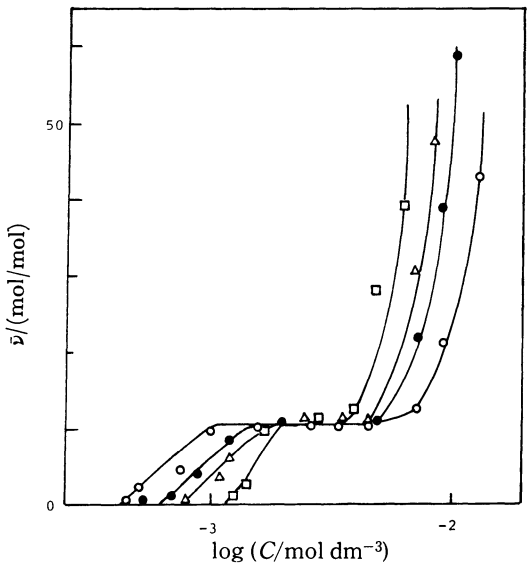

Fig. 1. Binding isotherms of lysozyme–SPFO system at 25 °C and pH 5.8.
$\bar{\nu}$: Binding number, C: equilibrium SPFO concentration, NaCl concentration; ○, 10, ● 20, △ 50, □100 mmol dm^{-3}.

SDS system. Hiramatsu *et al.* reported that the concentration of the initial binding depends on the hydrocarbon chain length of the surfactants for systems of bovine plasma albumin and cationic surfactants; the longer is the chain length, the lower the concentration of the initial binding.[15] Precipitation of lysozyme takes place when the binding begins, and it disappeares with further binding. The general feature of the binding of SPFO to lysozyme resembles that of SDS. So, the analysis of the binding isotherms can be made along a similar line to that discussed in the previous paper.[12]

The free energy difference of PFO^- ion between the states of the initial and final point of the flat region in Fig. 1, where $\bar{\nu}$ could be kept at 10, has been considered to be the free energy accumulation for the subsequent binding. This value (ΔG_{f-i}) is expressed as follows:

$$\Delta G_{f-i} = RT \ln \frac{a_f}{a_i} \simeq RT \ln \frac{C_f}{C_i}, \quad (1)$$

where a_i and a_f are the activities of the PFO^- ions in solution at the initial and final points of the region, respectively, and C_i and C_f are the respective equilibrium SPFO concentrations. The results obtained from Eq. 1 are shown in Fig. 2 together with the results obtained for lysozyme–SDS system. ΔG_{f-i}'s are larger than those of the lysozyme–SDS system in the concentration range of NaCl studied. It may be suggested that both the surface of the complex of lysozyme–PFO^- and singly dispersed PFO^- are less hydrophobic than that of the lysozyme–DS^- complex at a binding number just exceeding 10 and singly dispersed DS^- ion.

The plots of log $C_{\bar{\nu}}$ *vs.* log [Na^+] are given in Fig. 3. $C_{\bar{\nu}}$ and [Na^+] represent the equilibrium SPFO concentration where $\bar{\nu}$ ions of PFO^- are bound to a lysozyme molecule and total Na^+ ion concentration originating from NaCl and SPFO, respectively. The plot of log $C_{0.5}$ *vs.* log [Na^+] gives a straight line with positive slope o 0.3. At present, it is difficult to explain the meaning of the difference between 0.5 for SDS and 0.3 for SPFO. Nevertheless it is certain that the exchange between Cl^- and PFO^- ions and the binding of PFO^- ion to the positively charged sites take place simultaneously as in the case of SDS binding.[12]

According to the discussion in the previous paper,[12] the equilibrium surfactant concentration giving the binding number just exceeding 10 was considered to be the characteristic concentration of protein-ionic surfactant interaction. As is shown in Fig. 1, this concentration (C_{10}) seems to be independent of NaCl concentration. This result agrees with that of the lysozyme–SDS system discussed in the previous paper.[12] C_{10} for the lysozyme–SPFO system (4.8×10^{-3} mol dm^{-3}) is larger than that of the lysozyme–SDS system (2.9×10^{-4} mol dm^{-3}), corresponding to the large CMC in pure water (3.2×10^{-2} mol dm^{-3} at 25 °C) compared with that of SDS (8.2×10^{-3} mol dm^{-3} at 25 °C). Since the driving force for subsequent binding exceeding 10 is mainly hydrophobic interaction as described in the previous paper, C_{10} may be regarded as the concentration where SPFO molecule begins to form hemimicelles on the surface of the lysozyme. Thus there may exist a certain correlation between C_{10} and CMC. The differences between C_{10} and CMC of SPFO and of SDS are about 1 and 1.5 logarithmic units, respectively. The difference is a measure of the binding affinity, *i.e.*, the larger difference means easier binding to the complex compared with self-association (micelle formation) in bulk water. The smaller difference of SPFO means that the affinity between SPFO molecules and the lysozyme–PFO^- complex is smaller than that of the lysozyme–DS^- complex. PFO^- ions on the surface of lysozyme–PFO^- complex at C_{10} are more loosely interacting with each other and with hydrophobic part of lysozyme molecule than DS^- ions on the surface of lysozyme–DS^- complex at C_{10}, because of the shortness of hydrophobic part and mutual phobicity between fluorocarbon and hydrocarbon of lysozyme.[7-11] In other words, less hydrophobicity of lysozyme–PFO^- complex at C_{10} will be responsible for small difference between C_{10} and CMC, and large ΔG_{f-i}.

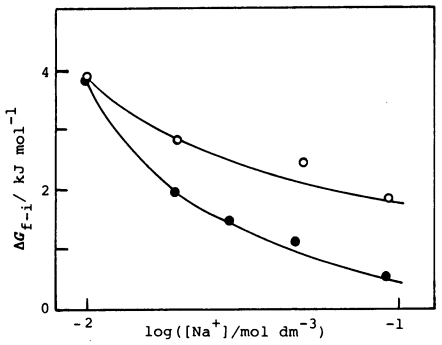

Fig. 2. ΔG_{f-i} *vs.* log [Na^+] plots evaluated from Eq. 1.
○: SPFO, ●: SDS.

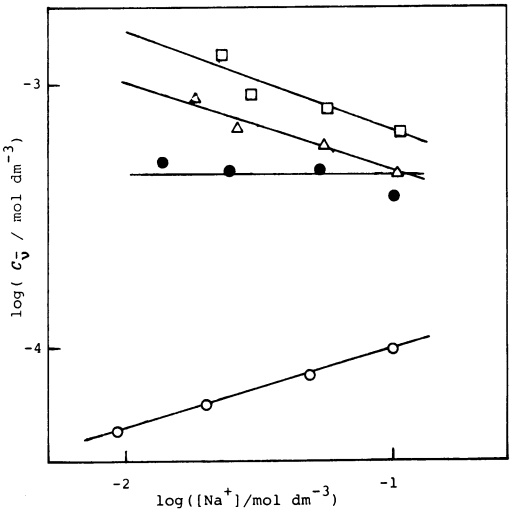

Fig. 3. log $C_{\bar{\nu}}$ *vs.* log [Na^+] plots.
$C_{\bar{\nu}}$: Eequilibrium SPFO concentration at binding number $\bar{\nu}$, $\bar{\nu}$: ○0.5, ● 10, △17, □ 40.

Plots of log C_{17} and C_{40} *vs.* log [Na^+] give straight lines with negative slopes, as is shown in Fig. 3. The standard free energy change of the micelle formation from the phase separation model is expessed as

$$\Delta G^\circ/RT = \ln X_{CMC} + \beta \ln X_{Na^+}, \quad (2)$$

where X_{f-i} and X_{Na^+} are the mole fractions of the

surfactant and Na^+ ion in the intermiceller solution at CMC, respectively, and β is the binding number of Na^+ ions per surfactant molecule in micelle.[16] As discussed in the previous paper,[12] the intercept and slope of the straight line in the plot of ln $X_{\bar{\nu}}$ *vs.* ln X_{Na^+} will give $\Delta G^\circ_{\bar{\nu}}$ and β, where $\Delta G^\circ_{\bar{\nu}}$ is the difference between the standard Gibbs free energy of a mole of monomeric hydrated PFO^- ion with β counterion and that of a mole of PFO^- ion with β counterion bound to the complex, and $X_{\bar{\nu}}$ is the mole fraction of DS^- ion in solution at the binding number $\bar{\nu}$. Equation 2 can be applied to the plots of log $C_{\bar{\nu}}$ *vs.* log [Na^+], if the common logarithmic concentration in mol dm^{-3} is converted into the natural logarithmic mole fraction. The results obtained by applying Eq. 2 to the plots in Fig. 3 are listed in Table 1 together with the data for the SPFO micelle itself. The β increases with increasing binding number and seems to approach the value of the micelle. $\Delta G^\circ_{\bar{\nu}}$'s are equal to ΔG° of SPFO micelle formation. These behaviors have also been seen in the lysozyme–SDS system. These suggest that the surface of lysozyme is also covered with PFO^- ions, likewise the lysozyme–DS^- complex, although the lysozyme–SPFO system is different in detail from that of lysozyme–SDS system, resulting from the mutual phobicity and the difference in hydrophobicity. The binding mechanism proposed for lysozyme–SDS system is also concluded to be true for the binding of SPFO to lysozyme.

Table 1. Thermodynamic parameters

$\bar{\nu}$	β	ΔG°/kJ mol^{-1}
17	0.35	−29
40	0.40	−29
Micelle[6]	0.55	−29

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