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Kinetic Evidence for Proton Release and Uptake on Complexation between Two Polypeptides

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In kinetic experiments on complexation between poly(L-glutamic acid) and poly(L-lysine), slower proton uptake followed by fast proton release was found. Further, it was elucidated by the experiments using the polymers and small molecules having one or two ionizable groups that combination of the polymer and at least divalent ions was necessary for the proton release and uptake.

Polymer-polymer complexes, having new structure and function different from each component, have been investigated from viewpoint of not only physical chemistry but also biochemistry and polymer engineering. For a profound understanding of the properties of the polymer complex, elucidation of the complexation mechanism by kinetic experiments is necessary, but only a few kinetic studies[1-3] have been reported in contrast to a large amount of static information available. Then we carried out stopped flow measurements in order to reveal the mechanism of complexation between poly(L-glutamic acid) (PGA) and poly(L-lysine) (PLL). On the PGA-PLL complexation, release of proton from the glutamic residue has been observed statically,[4] and it was expected that the complexation could be followed by detection of the proton release. In contrast to our expectation, proton uptake process was observed and the release process was too fast to be followed. These proton release and uptake are of interest in connection with function of protein, since proton release and/or uptake have been observed in biochemical reaction, e.g., enzyme-substrate interaction,[5,6] proton pump,[7] etc. In the present paper, we discuss a fundamental view of mechanism of the proton release and uptake through the kinetic experiments.

Poly(L-glutamic acid) (mean degree of polymerization, $\overline{DP}$, 250, 60, 10), as the sodium salt was supplied from Ajinomoto Co., Inc., and poly(L-lysine) hydrobromide ($\overline{DP}$460) purchased from Sigma Chem. Co. The other chemicals used were of reagent grade. Two sample solutions of the same volume were mixed with each other, which were previously adjusted to the same pH of 5.5, where PGA is able to release protons sufficiently from undissociated carboxylic sites and PLL is mostly protonated. To facilitate the analysis of reaction mechanism, the experiments was carried out under the conditions where no precipitation is seen. The pH of complex solutions were measured immediately after mixing. Kinetic experiments were performed by the stopped flow method with three kinds of detections, i.e., conductance detection,[8] pH detection with pH sensor,[9] and optical detection with pH indicator, in which the second and last detection were utilized for the confirmation if the conductance change correctly reflects the pH change in solution. The dead times of conductance, pH, and optical detection apparatus are 15 ms, 3 ms, and 3 ms, respectively. All experiments were carried out at 25 °C without pH buffer.

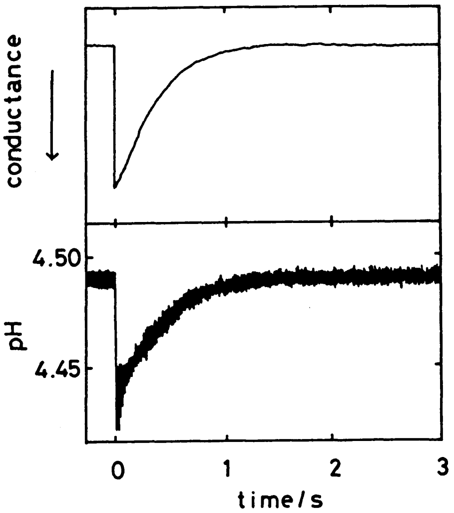
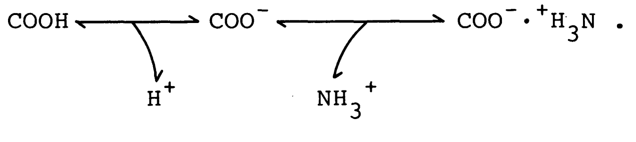


Fig.1. Kinetic evidence for proton uptake process on complexation between PGA($\overline{DP}$250) and PLL at 1×10^{-4} residue mol dm^{-3}. (a) Conductance detection; the downward arrow indicates increase in conductance. (b) pH detection; the curve is eliminated the effect from low ionic strength.[9)]

The complexation between PGA($\overline{DP}$250) and PLL was examined by the stopped flow method with conductance detection (in Fig.1(a)) and with pH detection (in Fig.2(a)). Only one reaction curve in a few seconds was observed in both measurements. Hence, the accordance in the experimental results in Fig.1 indicates that the change in conductance corresponds to that in proton concentration and the observed reaction is proton uptake. On the other hand, the process of proton release was found to be faster than 3 ms, since no reaction curve other than the proton uptake was observed. As a result, it is apparent that the complexation proceeds through at least such two steps.

Furthermore, kinetic experiments were carried out using PGA of various $\overline{DP}$s for examination of polymer length effect on the complexation. Then similar results were obtained even using PGA of low $\overline{DP}$ 10, which suggested that the proton release and uptake are not specific for the polymer-polymer complexation. Hence, the behavior of proton was examined for the systems including small molecules as well as the polymers. As shown in Table 1, the proton release and uptake were not observed in the systems of the polymer and monovalent compound. From these facts, the interaction between the polymer and monovalent compound was found to be weaker than that between the polymer and divalent compound. In general, monovalent ions have less tendency to form complex with other ions electrostatically than divalent ions. Additionally, the behavior of proton was not detected in the system of dicarboxylic acid and diamine. As a result, it was found that the combination of the polymer and at least divalent compound was necessary for the proton release and uptake.

Then the mechanism of the proton behavior would be proposed below. Firstly, in the proton release process, the binding of COO^- of acid to NH_3^+ of base causes the proton release from COOH of acid according to next equilibria,



$$COOH \underset{H^+}{\rightleftharpoons} COO^- \underset{NH_3^+}{\rightleftharpoons} COO^-\cdot{}^+H_3N\ .$$

In case of the polymer and monovalent ion, protons were hardly released owing to weak interaction.

Secondly, the subsequent proton uptake process may occur by protonation of COO^- owing to breaking of $COO^- \cdot {}^+H_3N$, that is, the above equilibria shift to left side. The breaking would result from irregular linkage and unstable structure formed by rapid complexation, as suggested by Tsuchida et al.[10] For such a linkage to the polymer, it is evident that not monovalency but at least divalency is necessary, as seen in the system of divalent metal ion and poly(acrylic acid), which make a linkage of the form COO-Metal-OOC.[11] The unstable complex in fast

Table 1. Observation of the kinetic process and pH change for several systems of compounds

Systems of compounds[a)]	Observation of the kinetic process (proton uptake)	pH change ΔpH[b)]
(polymer) - (polymer)		
PLL($\overline{DP}$460) - PGA($\overline{DP}$250)	observed	-1.01
- PGA($\overline{DP}$60)	observed	-1.03
- PGA($\overline{DP}$10)	observed	-0.89
(polymer) - (small molecule)		
PLL($\overline{DP}$460) - acetic acid	not observed	≃0
- propionic acid	not observed	≃0
- malonic acid	observed	-0.25
- glutaric acid	observed	-0.23
PGA($\overline{DP}$250) - methylamine	not observed	≃0
- ethylamine	not observed	≃0
- ethylenediamine	observed	-0.15
- 1,3-propanediamine	observed	-0.14
(small molecule) - (small molecule)		
malonic acid - ethylenediamine	not observed	≃0
(polymer) - (metallic ion)[c)]		
PGA($\overline{DP}$250) - Cu^{2+}	observed	-0.92
- Ca^{2+}	observed	-0.20
- Na^+	not observed	≃0

a) All the concentrations were 1×10^{-4} mol dm^{-3}, which was equivalent of acid and base except (polymer)-(metallic ion) systems. b) $\Delta pH = pH_{final} - pH\ 5.50$. The experimental error was within ±0.02 pH. c) PGA was 1×10^{-4} residue mol dm^{-3} and metallic ion 1×10^{-4} mol dm^{-3}.

step changes to stable one in structure, which is intra-complex process, and, at this time, some of linkages are broken. This breaking would be rate determining step of proton uptake process by considering the proton transfer reactions much faster than the observed process. In the system of weak and strong polyelectrolytes,[12)] the complexation has been reported to proceed through the two process, i.e., the fast binding process and relatively slow intra-complex process.[2,10)] In particular, Okubo et al., using the conductance stopped flow technique, observed two reaction curves of a rapid increase and a slow decrease in conductance. They did not, however, mention to the relationship between the conductance change and proton behavior. From the viewpoint of our results, it can be deduced that the conductance changes in their experiments correspond to the proton processes.

In conclusion, the proton release would occur by simple dissociation equilibrium and the proton uptake by intra-complex process on the complexation. In addition, these results suggest that the similar proton behavior may take place in biochemical systems accompanying complexation and proton movement, e.g., enzyme-substrate interaction,[5,6)] proton pump,[7)] etc.

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References

1) H.Ohno, H.Matsuda, and E.Tsuchida, Makromol. Chem., 182, 2267 (1981).
2) T.Okubo, K.Hongyo, and A.Enokida, J. Chem. Soc., Faraday Trans. 1, 80, 2087 (1984).
3) V.A.Izumrudov, T.K.Bronich, A.B.Zezin, and V.A.Kabanov, J. Polym. Sci. Polym. Lett. Ed., 23, 439 (1985).
4) A.Domard and M.Rinaudo, Macromolecules, 13, 898 (1980).
5) S.E.Halford, Biochem. J., 149, 411 (1975).
6) H.Nakatani, Y.Uehara, and K.Hiromi, J. Biochem., 78, 611 (1975).
7) Y.Kagawa, J. Biochem., 95, 295 (1984).
8) T.Ikeda, J.Nakahara, M.Sasaki, and T.Yasunaga, J. Colloid Interface Sci., 97, 278 (1984).
9) T.Nakatsuka, M.Nakamura, and T.Sano, Bull. Chem. Soc. Jpn., (in press).
10) E.Tsuchida, K.Abe, and M.Honma, Macromolecules, 9, 112 (1976).
11) F.Oosawa, "State of Binding of Counter Ions," in "Polyelectrolytes," Marcel Dekker, New York (1971), p.33.
12) The substances were sodium polyacrylate and Polybrene, polyethylenimine and sodium poly(ethylene sulfonate) in Ref. 2, and poly(methacrylic acid) and poly(N,N,N',N'-tetramethyl-N-p-xylylenealkylenediammonium dichloride) in Ref. 10.

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