Journal of Photoscience (2000), Vol. 7(2), pp. 63 – 66

Interactions of Lysozyme with Phospholipid Liposomes

Kyoung Ok Jung, Mi-Young Kim, In-Cheol Jeong, Andre Kim, Do-Hui Kim, Shin-Won Kang, and Jang-Su Park*
Department of Chemistry, College of Natural Science, Pusan National University, Pusan 609-735, Korea

A study of the interaction between lysozyme and phospholipid liposomes is essential for a systematic approach for understanding the action mechanism of lysozyme. So we want to clarify the interactions of lysozyme with phospholipid liposmes. We observed that lysozyme more interacts with negatively charged liposomes than with neutral liposome. More hydroxy groups of the phospholipid was very important on that interactions. We recognized the importance of electrostatic interactions in the process of fusion induced by lysozyme. As indicated by UV/Vis experiments, leakage and fusion are two uncoupled process.

key words: Lysozyme, Phospholipid, Liposome

INTRODUCTION

Lysozyme is a rather small protein (14.6 kDa), which was obtained from an egg albumen mainly. This protein whose size is 45 ×30 ×30 Å consists of 129 amino acid residues and is internally cross-linked by four disulfide bonds. Hydrophobic interaction of lysozyme is a unique factor for it's folding. It is well known that this protein can hydrolyze the peptidoglycan layer in the cell walls of some bacterias. A lot of studies have explained that phospholipid interaction is very important in protein activity [1], and many papers have released about liposomes and protein interactions [2,3,4,5]. However, the mechanism of lysozyme against biological membrane is not known.

A study of the interaction between lysozyme and phospholipid liposomes [6] is essential for a systematic approach for understanding the action mechanism of lysozyme. So we want to clarify the interactions of lysozyme with phospholipid liposmes.

MATERIALS AND METHODS

PG (phosphatidyl glycerol), PA (phosphatidic acid), PBut (phosphatidyl butanol) whose headgroup modified phospholipid analogues were synthesized by phospholipase D catalyzed transphospholylation from egg PC (phosphatidyl choline). Egg yolk phosphatidyl choline (EYPC) was isolated with silicagel column chromatography [7]. Egg yolk phosphatidyl glycerol (EYPG), phosphatidic acid (EYPA), and phosphatidyl butanol (EYPBut) were obtained by hydrolysis of EYPC with phospholipase D and were purified by silicagel column chromatography. The phospholipase D was partially purified from inner yellowish-white leaves of Savoy cabbage by heat treatment and acetone precipitation [8,9].

Small unilamella vesicles were prepared with two lipids consists of EYPC as a neutral vesicle, EYPC-EYPG, EYPC-EYPBut, and EYPC- EYPA as a negatively charged liposomes [6]. Phospholipid mixture was dissolved in small amount of diethyl ether and evaporated at 35℃, 60 rpm. The dried lipid complex was hydrated in 5 ml of Tris-buffer (5 mM, pH 7.4) and mixed by voltex mixer. The solution was centrifuged at 4 ℃, 15000 rpm for 40 min to eliminate multilamella vesicle and was diluted to 25 ml with the same buffer.

Unilamellar vesicles with CF (carboxy fluorescein) were prepared by similar method (We used Tris-HCl buffer (10 mM Tris, 100 mM NaCl, pH 8.0) with 100 mM carboxy fluorescein instead of Tris buffer). We monitored the fusion of liposome which is induced by lysozyme with UV/Vis spectrophotometer at 450 nm in every 15 sec for 5 min.

Fluorescein of liposome was measured by fluorescein exciting at 520 nm and at 450 nm. After injecting 50μl lysozyme into the liposome solution, we measured fluorescein in every 15 sec for 3 min [2,10,11,12]. We also observed blue-shift of tryptophan residue by following method. We dissolved lysozyme in Tris-buffer (5 mM Tris, 1 mM EDTA, 100 mM NaCl, pH 7.4) and monitored fluorescein peak of tryptophan residue in lysozyme. After that, we mixed lysozyme and liposomes (PC, PC/PG (3:1 w/w), PC/PA (3:1 w/w), PC/PBut (3:1 w/w) and monitored fluorescein peak of tryptophan residue in lysozyme.

Using Circular Dichroim, we monitored secondary structure change of lysozyme. We used lysozyme solution (0.1 mM lysozyme solution 300 μl, lysozyme solution 30 μl + liposome solution 270 μl) [3].

*To whom correspondence should be addressed.
E-mail : jaspark@hyowon.pusan.ac.kr
Received 30 April 2000; accepted 5 June 2000

RESULT AND DISCUSSION

Lysozyme is a basic protein which interact with neutral or acidic liposomes below pH 10. In this study, we modified liposomes variously, and monitored CF leakage to find fluctuation induced by interaction between lysozyme and liposomes. In other words, increase of fluorescein means more CF leakage induced by fluctuation.

Fig. 1 shows that lysozyme more interact with negatively charged liposomes (PC/other phospholipid) than neutral liposomes. This result shows that there are electrostatic interaction between liposomes and lysozyme, especially, number of the phospholipid's hydroxy groups is important for that interactions.

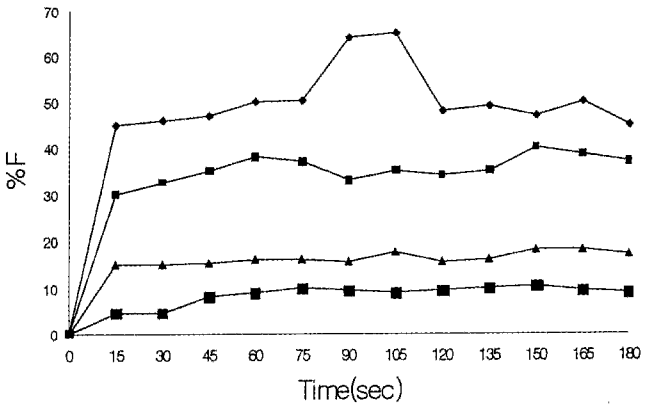

Figure 1. Percentage fluorescence in liposomes. Percentage fluorescence of PC/PG(4:1)(◆), PC/PA(4:1)( ), PC/PBut(4:1)(▲), PC neutral(4:1)(■) liposomes. The percentage of fluorescence (%F) was calculated according to the following: %F= $(F-F_0)/(F_t-F_0)$ × 100: where F0 and F are the donor fluorescence in the absence and in the presence of lysozyme, and F_t the fluorescence of Triton X-100

Aggregation was followed by measuring light scattering with the wavelength set up at 450 nm and 25℃ (Fig. 2) [13]. Here, absorbance change means size change of liposomes induced by fusion of liposomes. Fig. 2 shows that the absorbance change of PC/PG liposomes is the largest. It also means that the fusion of PC/PG liposomes occurred well. These results suggest that aggregation of liposomes induced by lysozyme is strongly dependent on the negative charges of liposomes rather than the neutral liposomes.

Tryptophan residue has a unique characteristic that the maximum of wavelength moves to lower wavelength when the residue moves into hydrophobic circumstance (blue-shift) [2]. This blue-shift is clear evidence for permeation of lysozyme. In this study, we could not find any blue shift (Fig. 3). These result shows that lysozyme does not permeate into liposomes but adsorb on liposomes. Circular Dichroism spectroscopy is a valuable technique for analyzing the secondary structure of proteins in solution. If one protein has a lot of α-

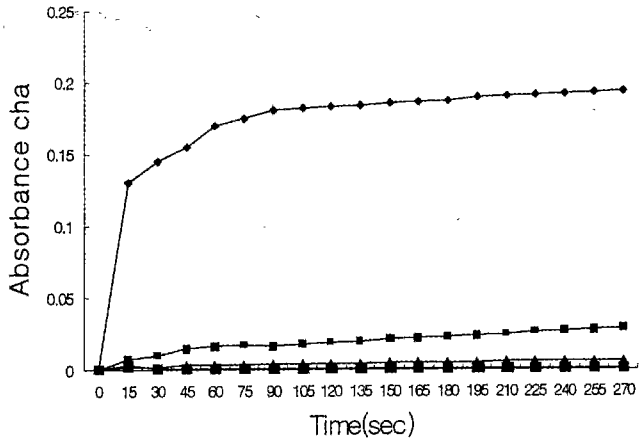

Figure 2. The light scattering of liposomes induced by aggregation of liposomes. Fusion of PC/PG(3:1)(◆), PC/PA(3:1)( ), PC/PBut(3:1)(▲), PC neutral(■) liposomes.

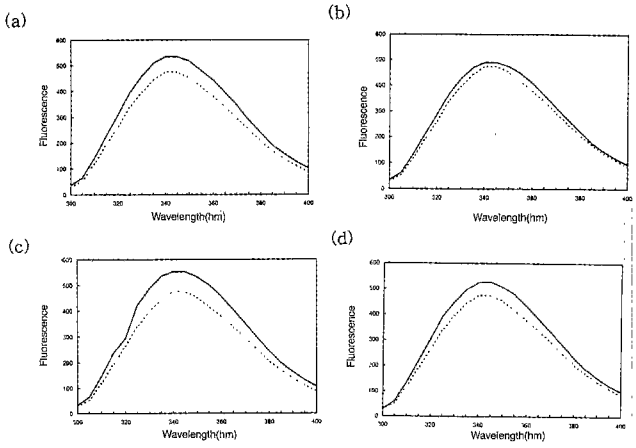

Figure 3. The measure of blue shift of tryptophan residue in lysozyme. Fluorescence spectrum of lysozyme in buffer solution(----), PC/PG(3:1)(a), PC/PA(3:1)(b), PC/PBut(3:1)(c), PC neutral(d) liposomes(———).

helix structure, we could find particullar peak at 208 nm and 222 nm. In Fig. 4 we could find few CD spectra change of lysozyme in negatively charged liposomes and neutral liposomes. It also means that lysozyme is not permeate into liposomes.

From our result, we suggest the different molecular interaction models of lysozyme between neutral and negatively charged liposomes (Fig. 5). Phospholipid unilamella vesicle bearing negative charges are so stable that they do not aggregate and fuse spontaneously, because of electrostatic repulsion (Fig. 5). However lysozyme is a basic protein, when interacts with negatively charged vesicles, both negative charges of phospholipid head group and positive charges of the protein would facilitate vesicle cross-linking and subsquently aggregation and fusion. Our results obtained with lysozyme are similar to those found with other basic proteins and peptides [8,14]. The results presented here do not

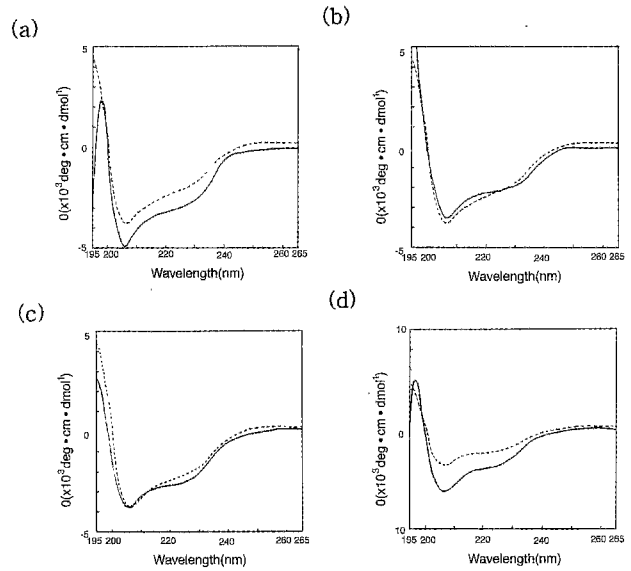

Figure 4. The CD spectra of lysozyme in buffer solution(----), PC/PG(3:1)(a), PC/PA(3:1)(b), PC/PBut(3:1)(c), PC neutral(d) liposomes (———). The concentration of lysozyme is 0.1 M.

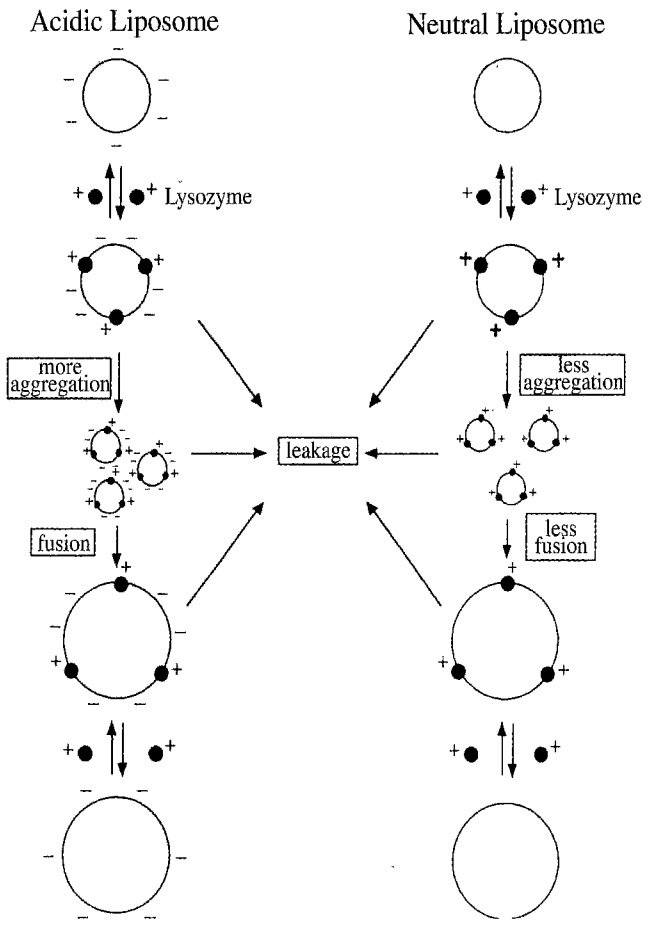

Figure 5. The molecular interaction of lysozyme with phospholipid liposomes. Relationships with fusion and leakage of aqueous content.

allow us to establish the molecular mechanism of fusion induced by lysozyme. Further studies will be needed.

REFERENCES

1. Kkyoko, S., Masanao, O., Tsutomu, H. and Kaori, W. (1994) Circular dichroism studies of the interaction between synthetic peptides corresponding to intracellular loops of β-adrenergic receptors and phospholipid vesicles. *J. Biochem.* **115**, 463-468.
2. Jesus, P. G., Cristina, C. and Derek, M. (1995) Interactions of hydrophobic lung surfactant proteins SP-B and SP-C with dipalmitoylphosphatidylcholine and dipalmitoylphosphatidylglycerol bilayers studied by electron spin resonance spectroscopy. *Biochemistry* **34**, 3964-3971.
3. Joanne, E. J. and Rosemary, B. C. (1994) Membrane-Binding Amphipathic α-helical peptide derived from CTP:Phosphocholine cytidyltransferase. *Biochemistry* **33**, 4327-4335.
4. Suchetana, M. and Wonhwa, C. (1996) Interactions of annexin V with phospholipid monolayer. *Biochim. Biophys. Acta.* **1279**, 58-62.
5. Bangham, A. D., Standish, M. M. and Watkins, J. C. (1965) Diffusion of univalent ions across the lamellae of swollen phospholipids. J. Mol. Biol. 13, 238-252.
6. Posse, E., De Arcuri, B. F. and Morero, R. D. (1994) Lysozyme interactions with phospholipid vesicle: relationships with fusion and release of aqueous content. *Biochim. Biophys. Acta.* **1193**, 101-106.
7. Morero, R. D., López Viñals, A. E., Bloj, B. and Farías, R. N. (1985) Fusion of phospholipid vesicles induced by muscle glyceraldehyde-3-phosphate dehydrogenase in the absence of calcium. *Biochemistry* **24**, 1904-1909.
8. Park, J. S., Jung, K. O., Kim, J. C., Kim, K. H., Park, N. G., Suh, H. S., and Kang, S. W. (1997) Interactions of Mastoparan B with phospholipid vesicles: relationships with fusion and leakage actions (**I**). *Bull. Korean Chem. Soc.* **18**, 258-261.
9. Davidson, F. M. and Long, C. (1985) Tetrahydrobiopterin analogues: Solution conformations of 6-methyltetrahydropterin, 7-methyltetra- hydropterin, and cis- and trans-6,7-dimethyltetrahydropterins as determined by proton nuclear magnetic resonance. *J. Biochem.* **69**, 458-466.
10. Shin, T. B., Leventis, R. and Silvius, J. R. (1991) Partitioning of fluorescent phospholipid probes between different bilayer environments. estimation of the free energy of interlipid hydrogen bonding. *Biochemistry* **30**, 7491-7497.
11. Wolf, D. E., Winiski, A. P., Ting, A. E., Bocian, K. M. and Pagano, R. E. (1992) Determination of the transbilayer distribution of fluorescent lipid analogues by nonradiative fluorescence energy transfer. *Biochemistry* **31**, 2865-2873.
12. John, P. N., Susan, F. M., Richard, G. P., and Roy, H. H.

(1995) Flow cytometric analysis of transmembrane phospholipid movement in bull sperm. *Biochemistry* **34**, 3907-3915.

13. Arag n, S. S. R. and Elwenspoek, M. (1982) Mie scattering from thin spherical bubbles. *J. Chem. Phys.* **77**(7), 3406-3414.
14. Surewiss, R. M., Epand, R. M., Vail, W. J. and Moscarelo, M. A. (1985) Aliphatic aldehydes promote myelin basic protein-induced fusion of phospholipid vesicles. *Biochim. Biophys. Acta.* **820**, 319-323.