Communications to the Editor

[Chem. Pharm. Bull. 34(3)1391—1394(1986)]

NaCl-INDUCED AGGREGATION OF EGG PHOSPHATIDYLCHOLINE LIPOSOMES

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NaCl-induced aggregations of pure egg phosphatidylcholine liposomes were investigated as a function of NaCl concentration (0-1.5%) by following turbidity changes. The turbidity change seemed to have three stages with increasing salt concentration: Liposomes started aggregation at about 0.01% NaCl and continued mildly up to about 0.15%. At higher levels the turbidity increased sharply. Therefore, the threshold concentration of NaCl is thought to be 0.15%. The turbidity of liposomes swollen in 0.9% NaCl was 3.7 times that when swollen in distilled water. The aggregation phenomena were completely reversible on removing NaCl by dialysis, and the turbidity finally reached the level of the liposomes swollen in distilled water. These phenomena should be borne in mind in the use of neutral liposomes as drug carriers, especially in the reconstitution of lyophilized phospholipids-drug systems.

KEYWORDS —— egg phosphatidylcholine liposome; aggregation; sodium chloride; threshold concentration; reversible aggregation

The aggregation phenomena of liposomes are important in relation to the biological fate of liposomes when the vesicles are used as drug carrier systems, in addition to the initial step of membrane fusion.[1-3)]

Cation-induced aggregations of liposomes have been extensively studied in acidic vesicles where the aggregation is induced with various monovalent and divalent ions and with organic polycations.[4-7)]

Neutral liposomes are often selected as control vesicles relative to positively or negatively charged vesicles in the study of liposome disposition in vivo. The disposition is most probably affected by the size of vesicles irrespective of their charge.[8,9)] However, few studies have dealt with the aggregation of neutral liposomes.

The present study describes the effect of NaCl on the aggregation of pure egg phosphatidylcholine liposomes, following turbidity changes, as a function of the NaCl concentration. The results may provide useful information for liposome-drug preparations dispersed in distilled water or in NaCl solution, especially for the reconstitution of lyophilized phospholipids-drug systems.

MATERIALS AND METHODS

Phosphatidylcholine (PC) was extracted from egg yolk and purified by column chromatography on silicic acid (Mallinkrodt, St. Louis, MO).[10] Thin layer chromatography gave a single spot. All other chemicals were of reagent grade. Liposomes were prepared by the method of Bangham.[11] Briefly, a dried film of PC formed in a round bottom flask (50 ml) was dispersed in various NaCl solutions ranging from 0 to 1.5% (256 mM) on a vortex mixer at room temperature, so that vesicles were multilamellar. The PC concentration based on phosphorus was always maintained at 2 mM.[12]

The aggregation of liposomes was determined by measuring the turbidity of NaCl solutions in which the liposomes were suspended. The turbidity of the suspensions was measured at 660 nm in a 1-cm quartz cell, using a Shimadzu UV-260 spectrophotometer equipped with a constant-temperature cell holder and an agitator. The liposome samples were treated with NaCl in two ways: 1) by swelling them in NaCl solutions prepared in concentrations varying from 0 to 1.5%, and 2) by swelling them in distilled water to which varying amounts of NaCl were subsequently added to bring them to the same concentrations as those in the first part. Turbidity was measured immediately after the liposomes became swollen. Turbidity measurement was also carried out for the supernatants of samples allowed to stand 24 h for natural sedimentation and subjected to 1500 x g or 3000 x g centrifugation.

Liposomes swollen in 0.9% NaCl and ones swollen in distilled water to which 0.9% NaCl was added externally were dialyzed to distilled water at room temperature under atomospheric conditions, using a Visking cellulose tube (20/32). The distilled water was replaced each time the turbidity was measured.

RESULTS AND DISCUSSION

Figure 1 shows the relation of turbidity at various NaCl concentrations, when a dried film of PC was swollen in the NaCl solutions.

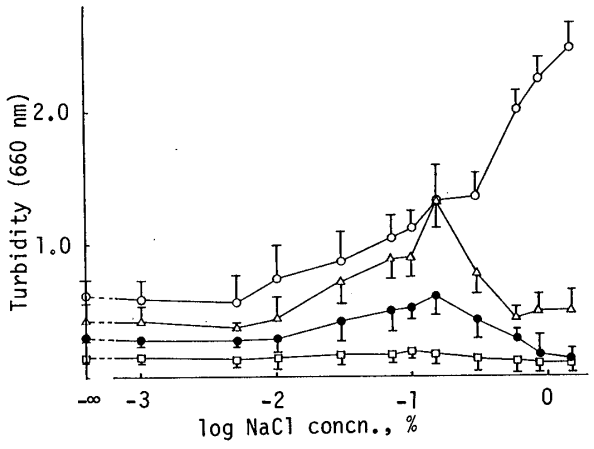

Fig.1. Effect of NaCl Concentration on the Turbidity of Liposomes Swollen in Various NaCl Solutions
Turbidity was measured immediately after swelling(○), supernatants after : standing 24 h(△), 1500 x g centrifugation(●) and 3000 x g centrifugation(□). Each point is the mean ± S.D.(n = 4). PC concn.: 2 mM.

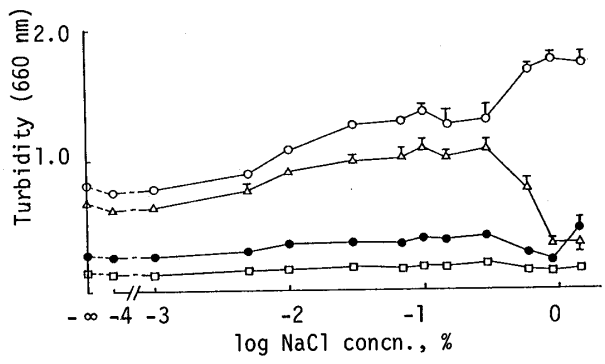

Fig.2. Effect of NaCl Concentration on the Turbidity of Liposomes Swollen in Distilled Water
Various NaCl solutions were added after swelling. Symbols are the same as in Fig.1. Each point is the mean ± S.D.(n = 3-4).
PC concn.: 2 mM.

The liposomes started aggregating at the NaCl concentration of about 0.01% (1.7 mM) and the turbidity gradually increased with increasing NaCl concentration. A sharp increase in the turbidity occurred starting from about 0.3 (51 mM) to 0.4% (68 mM), when very large flocculates were formed. The turbidity changes as a function of NaCl seem to have three stages.

The "threshold concentration" at which an additive induces vesicle aggregation is defined as the concentration corresponding to the maximum increase rate.[5] According to this definition, the threshold concentration of NaCl for egg phosphatidylcholine liposomes occurred at 0.4% (68 mM).

Natural sedimentation of the flocculates increased considerably as the NaCl increased beyond the threshold concentration, and the flocculates seemed to reach a balance at 0.15% NaCl (26 mM).

Centrifugation brought about more sedimentation of larger vesicles and flocculates. At 1500 x g a small peak of turbidity of the supernatants was observed at the same concentration of NaCl as in the natural sedimentation. Therefore it may be more reasonable to define the threshold concentration as the concentration at which the turbidity peak occurs, since this is a relatively narrower region; thus the threshold concentration of NaCl was thought to be 0.15% rather than the 0.4% defined previously. This is far below the 0.9% (154 mM, saline) often used as reconstitution aqueous media. At 3000 x g the flocculates were settled and the turbidity of the supernatants reached an almost constant level through the salt concentrations up to 1.5%.

Figure 2 shows the effect of added NaCl on the turbidity of liposomes swollen in distilled water. It is very likely here that the osmotic shrinkage of vesicles and their aggregations occur simultaneously. These processes affect turbidity changes in opposite directions. It is conceivable that the effect of the salt was not so large as the results shown in Fig. 1 due to the combination of these processes.

The reversibility of the vesicle aggregation was examined by dialysis of NaCl. Figures 3 and 4 show the turbidity changes of the aggregated liposomes swollen in 0.9% NaCl and those swollen in distilled water and aggregated by adding NaCl. The turbidity, i.e. the magnitude of aggregation, decreased with decreasing NaCl in the medium, where the decreasing rate reflects only the dialysis rate of the salt, and finally reached the initial level (Fig. 4). This indicates that the aggregation was completely reversed and there was little fusion of vesicles.

The role of NaCl in the aggregation reaction of egg phosphatidylcholine liposomes could not be characterized at this stage, but it seems unlikely that the results can be explained by electrostatic factors, such as surface potential, surface charge density or the direct binding of ions, that are generally postulated for the salt-induced aggregation of anionic vesicles.[13,14] However, the results are of practical importance in the reconstitution of lyophilized phospholipids-drug systems, because the morphologically different dispersed systems are produced by using either saline or distilled water.

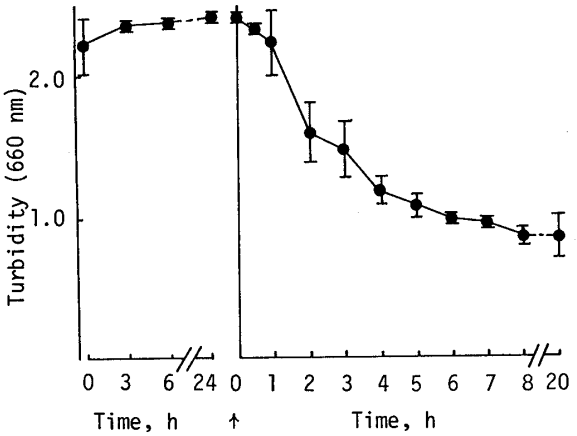

Fig. 3. Turbidity Changes of Aggregated Liposomes Swollen in 0.9% NaCl Solution
Dialysis started after the sample stood 24 h, i.e. at ↑. Each point indicates the mean ± S.D.
PC concn.: 2 mM.

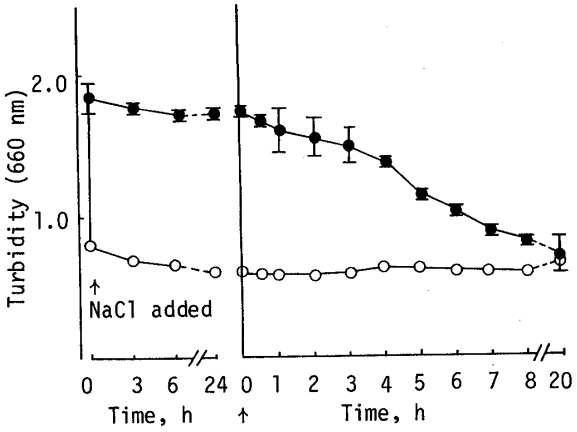

Fig. 4. Turbidity Changes of Aggregated Liposomes Swollen in Distilled Water
Liposome aggregation was induced by the addition of 0.9% NaCl and dialysis started after the sample stood 24 h, i.e. at ↑.
○: swollen in distilled water, control; ●: 0.9% NaCl, external.
Plots are the mean of 4 determinations. S.D. (open circles) ≦ 0.045,
PC concn.: 2 mM.

ACKNOWLEDGEMENTS This work was supported in part by Grants-in-Aid from the Ministry of Education, Science and Culture, Japan. The authors are grateful to Miss K. Kato for her technical assistance.

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(Received December 27, 1985)