FUSION OF PHOSPHOLIPID VESICLES INDUCED BY Zn^{2+}, Cd^{2+}, and Hg^{2+}

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Received February 19, 1985

The capacity of Zn^{2+}, Cd^{2+}, or Hg^{2+} to induce fusion of phospholipid vesicles composed of 50%/50%, 60%/40%, or 80%/20% dipalmitoyl phosphatidylcholine (DPPC)/bovine brain phosphatidylserine (PS) was investigated and compared to that of Ca^{2+} and Mg^{2+}. In vesicles composed of 50%/50% or 60%/40% DPPC/PS, Zn^{2+} and Cd^{2+} induced fusion at concentrations considerably lower than were required for Ca^{2+}-induced fusion. Only limited fusion of 80%/20% DPPC/PS vesicles occurred and Zn^{2+} was more effective than Ca^{2+} or Cd^{2+} in inducing fusion of these vesicles. Mg^{2+} and Hg^{2+} did not induce fusion in any of the vesicle systems.

Ca^{2+} ions induce fusion of phospholipid vesicles which contain negatively charged phospholipids (1-3), and they may be involved in regulation of cellular fusion processes in vivo (4-6). Studies of fusion induced by divalent cations other than Ca^{2+} have been limited to Mg^{2+}, Ba^{2+}, and Sr^{2+} (1,7).

We are investigating interactions of Zn^{2+}, Cd^{2+}, and Hg^{2+} with membranes to understand better their physiological effects. Concentrations of these divalent cations in vivo are generally rather low. However, Cd^{2+} and Hg^{2+} may bioaccumulate to concentrations which are toxic (8,9). In addition, because Zn^{2+} is a nutritionally important trace element, both low and high physiological levels of Zn^{2+} may produce adverse health effects (10). Some effects of Zn^{2+}, Cd^{2+}, and Hg^{2+} have been attributed to their interactions with membrane components, including phospholipids (11-13), so it is important to determine their capacities for inducing fusion.

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0006-291X/85 $1.50

We previously observed that Zn^{2+}, Cd^{2+}, Hg^{2+}, Ca^{2+}, and Mg^{2+} ions increase the phase transition temperature (Tc) of phospholipid vesicles composed of 60%/40% DPPC/PS, with the order of effects being $Zn^{2+}>Cd^{2+}>Hg^{2+}>Ca^{2+}>Mg^{2+}$ (14). In this report we compare fusogenic properties of Zn^{2+}, Cd^{2+}, and Hg^{2+} with those of Ca^{2+} and Mg^{2+}. Fusion was monitored using fluorescence energy transfer.

MATERIALS AND METHODS

Materials

DPPC and bovine brain PS were obtained from Sigma Chemical Co. and used without further purification. N-(7-nitro-2,1,3-benzoxadiazol-4-yl)phosphatidylethanolamine and N-(lissamine Rhodamine B sulfonyl)dioleoylphosphatidylethanolamine were obtained from Avanti Polar Lipids.

Methods

Preparation of Vesicles

Phospholipids were dissolved in chloroform to insure mixing and then the chloroform was evaporated. Buffer (10 mM HEPES, 100 mM NaCl, pH 7.4) was added and the dried phospholipids were hydrated at 45°C for 5-10 minutes. The sample was then sonicated to visual clarity. Small unilamellar vesicles (SUVs) were isolated by the procedure of Barenholz et al. (15). That is, following sonication, samples were centrifuged at 100,000 x g for 90 min and the upper portion of the supernatant fraction was recovered. When vesicles were labeled with NBD-PE and N-Rh-PE, 1 mol % of each was added to the chloroform solution of phospholipids.

Fusion Experiments

Fusion was monitored by the fluorescence energy transfer technique of Struck et al. (16). Vesicles labeled with fluorophores were mixed with unlabeled vesicles at a ratio of 1:4. Divalent cations were added as solutions of chloride salts and samples were incubated for 1 hr at 37°C. Following incubation, EDTA was added at a final concentration of 20 mM to eliminate aggregation of vesicles. Fluorescence emission spectra were recorded using an excitation wavelength of 470 nm. Triton X-100 was then added to a final concentration of 1% and spectra were recorded again. Fluorescence spectra were recorded on a Perkin-Elmer 650-40 spectrophotofluorometer with a 500 nm sharp cut filter in the emission path to reduce light scattering. Efficiency of resonance energy transfer was calculated from

$$E = 1 - F/Fo,$$

where F is the intensity of donor (NBD-PE) emission in the presence of N-Rh-PE (or absence of Triton X-100) and Fo is the donor emission in the presence of Triton X-100.

Results of fusion studies using resonance energy transfer were validated using gel filtration chromatography. Labeled and unlabeled vesicles were mixed at a ratio of 1:1. Samples were incubated for 1 hr at 37°C in the absence and presence of divalent cations after which 20 mM EDTA was added. Samples were chromatographed on a 1 x 50 cm Sepharose CL-4B column which had previously been "conditioned" with phospholipids by running several vesicle samples through it. Vesicles were eluted in 10 mM HEPES, 100 mM NaCl, pH 7.4

and were detected fluorometrically, exciting at 470 nm and monitoring the emission at 585 nm.

RESULTS

Shown in Fig. 1 is the effect of divalent cations on efficiency of energy transfer between NBD-PE and N-Rh-PE in vesicles composed of 50% DPPC/50% PS. Note that Ca^{2+} and Mg^{2+} are added at concentrations up to 20 mM while the highest concentration of Zn^{2+}, Cd^{2+}, and Hg^{2+} was 1 mM. Zn^{2+} and Cd^{2+} induced fusion at concentrations as low as 0.5 mM and Ca^{2+} induced fusion at concentrations of 15 mM and above. Mg^{2+} and Hg^{2+} did not induce fusion. Similar

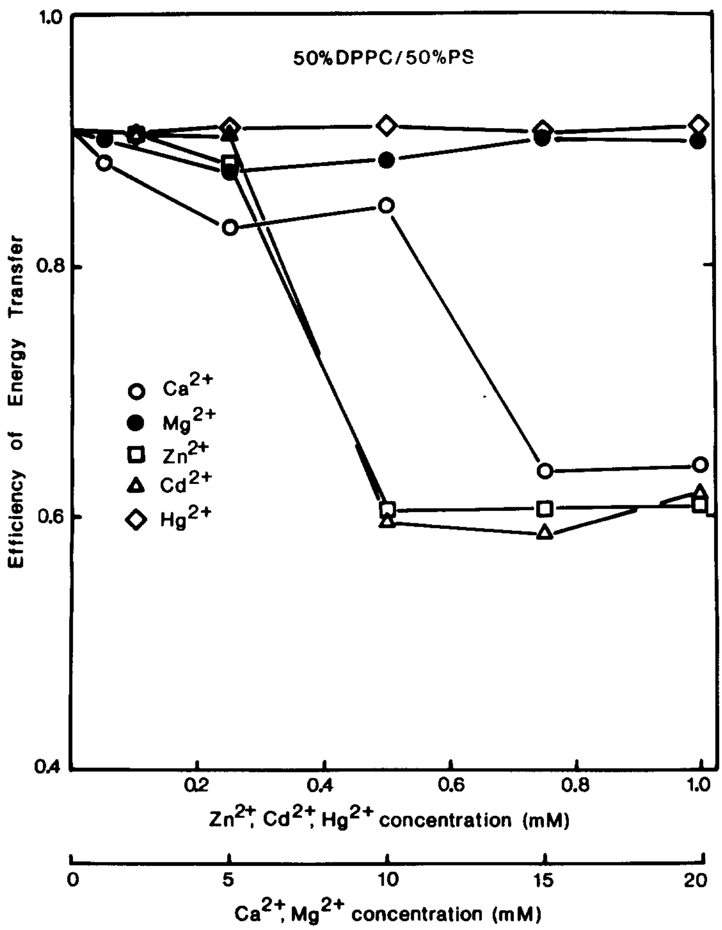

Fig. 1. Fusion of phospholipid vesicles composed of 50% DPPC/50% PS. Efficiency of energy transfer between NBD-PE and N-Rh-PE is plotted as a function of divalent metal ion concentration. Details of experiments are presented in MATERIALS AND METHODS.

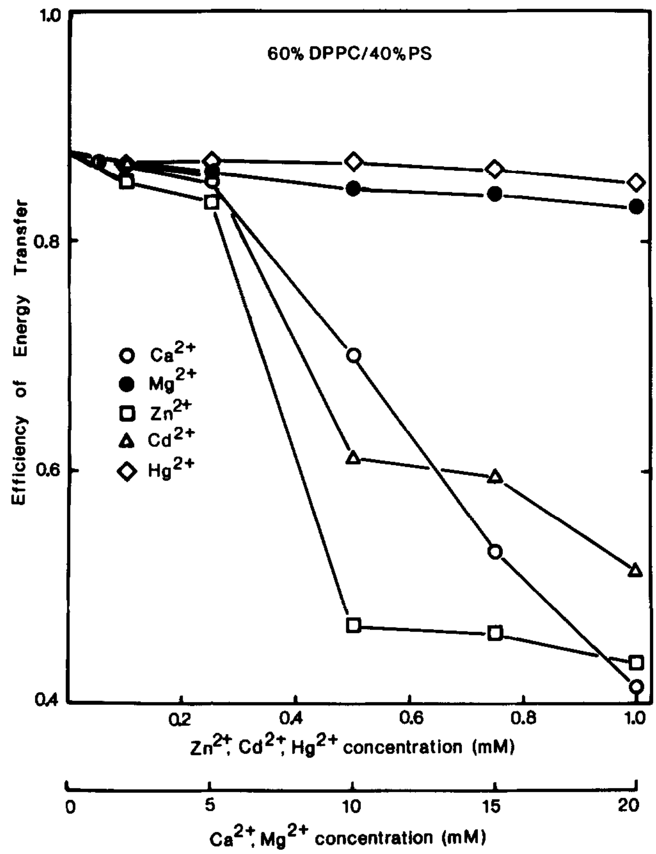

Fig. 2. Fusion of phospholipid vesicles composed of 60% DPPC/40% PS. Details of experiments are presented in MATERIALS AND METHODS.

trends were seen when fusion of vesicles composed of 60% DPPC/40% PS was investigated (Fig. 2). That is, Mg^{2+} and Hg^{2+} were not fusogenic, and 0.5 mM Zn^{2+} and Cd^{2+} induced fusion. Ca^{2+} was fusogenic at concentrations of 10 mM and above. That more fusion occurred at 10 mM Ca^{2+} with 60% DPPC/40% PS vesicles than with 50%/50% vesicles was unexpected. However, the general trends in fusion are the same with both types of vesicles, and in particular, much higher concentrations of Ca^{2+} as compared to Zn^{2+} and Cd^{2+} were required to induce fusion. In experiments using vesicles composed of 80% DPPC/20% PS, changes in efficiency of energy transfer were observed upon addition of Zn^{2+} and Ca^{2+} (Fig. 3). Changes were not as great as when vesicles containing higher concentrations of PS were used which probably indicates that fewer fusion events occurred with the 80%/20% vesicles. When 1 mM Zn^{2+} or 20 mM

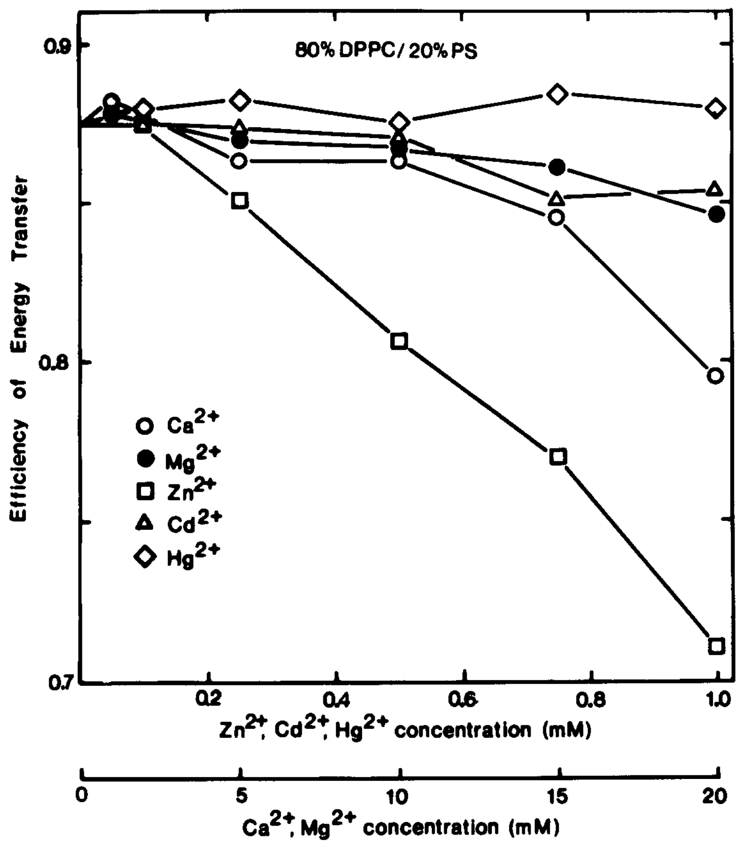

Fig. 3. Fusion of phospholipid vesicles composed of 80% DPPC/20% PS. Details of experiments are presented in MATERIALS AND METHODS.

Ca^{2+} was added to vesicles composed of 100% DPPC, changes were not observed in efficiency of energy transfer (data not shown).

Gel filtration chromatography was done to validate results from experiments using resonance energy transfer. Shown in Fig. 4 are elution profiles when vesicles composed of 50% DPPC/50% PS were chromatographed before and after addition of 1 mM Zn^{2+} or 5 mM Ca^{2+}. Recall that fluorescence studies indicated that fusion of 50%/50% vesicles occurred after addition of 1 mM Zn^{2+} but not after addition of 5 mM Ca^{2+}.

DISCUSSION

Induction of fusion by Ca^{2+} is consistent with observations of other investigators (1-3). In addition, Mg^{2+} is generally reported to be much less fusogenic than Ca^{2+}, though Mg^{2+} may induce a few rounds of fusion of SUVs

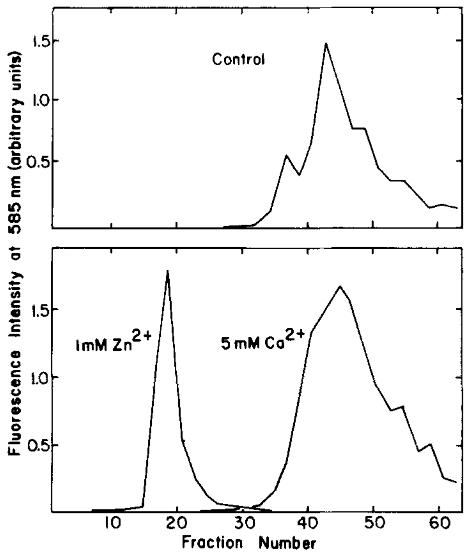

Fig. 4. Fusion of phospholipid vesicles composed of 50% DPPC/50% PS as detected by gel filtration chromatography. The chromatogram in the upper half of the Fig. is of a sample to which divalent cations were not added. Profiles shown in the lower half of the Fig. are for vesicles to which either 1 mM Zn^{2+} or 5 mM Ca^{2+} was added. Other details are presented in MATERIALS AND METHODS.

composed of 100% PS (17). We observed that Zn^{2+} and Cd^{2+} induce fusion of 50%/50% or 60%/40% DPPC/PS vesicles at concentrations between 0.5 and 1.0 mM. Interestingly, Hg^{2+} did not appear to induce fusion of phospholipid vesicles at concentrations up to 1.0 mM though previous experiments indicated that Hg^{2+} was almost as effective as Zn^{2+} and Cd^{2+} in increasing the Tc of 60% DPPC/40% PS vesicles (14).

Mechanisms for divalent cation-induced vesicle fusion have invoked changes in phase state (18), phase separation (19), and dehydration of polar head groups (20,21) to explain the process. However, recent studies have indicated that fusion is not dependent on changes in phase state (22) and that vesicle fusion occurs more rapidly than phase separation (23,24). Thus, divalent cations which induce fusion may be those which promote dehydration of polar head groups of phospholipids and thus allow contact between bilayers. A scheme depicting differences between Ca^{2+} and Mg^{2+} complexes with phospholipid vesicles has been proposed (20,21). Perhaps Zn^{2+} and Cd^{2+} behave similarly to Ca^{2+} in forming "trans" complexes in which the degree of hydration is greatly reduced and thus fusion occurs readily. Although Hg^{2+} behaves similarly to Zn^{2+} and Cd^{2+} in increasing Tc of DPPC/PS vesicles, it does not induce fusion. Thus, it will be interesting to apply these divalent cations to additional studies of the mechanism of vesicle fusion.

ACKNOWLEDGEMENTS

The authors thank Mrs. Sheila Early for her assistance in the preparation of this manuscript.

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