LETTERS TO THE EDITOR

Charge Asymmetry Does not Affect the Rate of Ca^{2+}-Induced Aggregation of Phospholipid Vesicles

Dear Sir:

Hall and Simon (1976) have proposed a model for Ca^{2+}-induced fusion of membranes that explains the phenomenon in terms of the induction of charge asymmetry. According to this model, Ca^{2+} binding and screening interactions reduce the electrostatic repulsions between negatively charged groups on the membrane surface, producing a tendency for contraction of the side of the membrane exposed to the ion. If both sides of the membrane are exposed, the two monolayers comprising the bilayer will contract to more or less the same extent, and minimal perturbation of the vesicle shape or stability will be made. However, if only the outside surface of the bilayer is exposed to Ca^{2+}, then the outside monolayer will tend to decrease in surface area while the inside monolayer will tend to retain its surface area. The model predicts that either bilayer strain or aberrations from spherical shape would result. These tendencies would represent the driving force for the fusion reaction.

The mechanism is supported by work done in model systems. Papahadjopoulos et al. (1977) have presented evidence that Ca^{2+} is less efficient in inducing fusion of phosphatidylserine vesicles when it is allowed to equilibrate across the membrane by means of an ionophore. We have studied the first partial reaction of the fusion process, membrane aggregation, in acidic phospholipids (Lansman and Haynes, 1975) and showed it to be quite similar to the fusion reaction in its dependencies on divalent cation concentration and membrane composition. It was proposed that aggregation depended upon and was rate-limited by the formation of complementary regions on the vesicle surface (Lansman and Haynes, 1975). The rate of vesicle aggregation is quite sensitive to the degree of binding of Ca^{2+} to the polar head groups of the acidic phospholipids and to the mole fraction of acidic phospholipids in the membrane. The extent of aggregation shows a great sensitivity to these parameters, which is accounted for by our statistical model (Lansman and Haynes, 1975). We would expect that the rate and extent of vesicle aggregation would be sensitive to bilayer strain and vesicle shape changes. Strain could affect the distribution of the acidic phospholipids in mixed lipid systems if the molecular volume or area of either of the component lipids varies with its mole fraction. Vesicle flattening would have the combined effects of increasing the surface area that can come into close apposition, and of producing "ends" having a low radius of curvature. Differences might arise in the composition and reactivity of the flattened and curved portions of the outside monolayer (Haynes and Simkowitz, 1977). In the expectation of such effects we have carried out experiments to determine whether charge asymmetry across phospholipid vesicles affects their rate and extent of aggregation.

Vesicles were made from phosphatidic acid (PA^-) derived from lecithin (PA^-, Na^+, salt; Koch-Light Laboratories Ltd., Colnbrook, Buckinghamshire, England) or PA^- and dimyristoyl-L-phosphatidylcholine (PC) by sonicating approximately 25 mg of lipid in 25 ml of medium (1 mM Kcl, 10 mM Tris-HCl, pH 7.4) until optical clarity was achieved. The ionophore X-537A (gift of Hoffman La Roche) was added to the vesicle suspension at the start of the experiment. The rates of Ca^{2+}-induced aggregation of PA^- and PC/PA^- vesicles were measured by observing the decrease in optical transmittance after rapid mixing in a stopped-flow apparatus. The rate of Ca^{2+}-induced vesicle dimerization was determined from the halftime for the first kinetic phase of transmittance decrease, as described in a previous study (Lansman and Haynes, 1975). Fig. 1 is a progress curve for an experiment in which PC/PA^- vesicles

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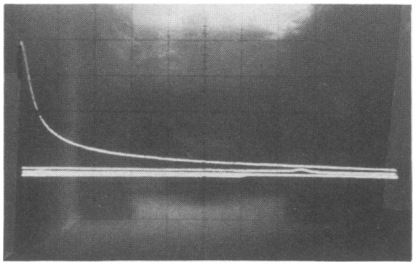

FIGURE 1 Oscilloscope trace of stopped-flow experiment. Syringe A contained PA^-/PC vesicles (3:1), 1.0 mg/ml in 10 mM KCl, and 10 mM tris buffer, pH 7.4, T = 25°C. Syringe B contained 10 mM Ca^{2+}. The experiment was performed with the Aminco-Morrow (4-8409) in the transmittance mode. The vertical axis is transmittance at 360 nm. The horizontal axis is time at 5 s per division. The oscilloscope was run in the repetitive sweep mode.

are rapidly mixed with Ca^{2+} to final concentrations of 0.5 mg/ml and 10 mM, respectively. The transmittance change obtained after the first sweep corresponds to a doubling of the sample optical density (OD) (turbidity), indicating that the vesicle dimerization reactions go to completion (Lansman and Haynes, 1975). Fig. 2 shows that the Ca^{2+}-induced aggregation can be fully reversed by addition of ethylenediaminetetraacetate (EDTA) 180 s after initiation of the reaction, as stated previously (Lansman and Haynes, 1975), proving that the vesicle fusion is not achieved during the lifetime of our experiments. Table I shows the lack of influence of the Ca^{2+} ionophore X-537A on the rates or extent of the aggregation reaction for PA^- and PC/PA^- vesicles for ionophore/lipid mole ratios up to 0.04. For vesicles composed of pure PA^-, the halftime for dimerization (~3 s) and the extent of reaction were not influenced substantially by rapid Ca^{2+} equilibration across the membrane. At the higher ionophore concentration (30 μM) the halftime for Ca^{2+} equilibration across phospholipid membranes is on the order of 50 ms (Chiu and Haynes, 1977, and work to be published). Our previous study (Haynes, 1974) has shown that the Ca^{2+} concentration is sufficient for binding to give full charge neutralization of the surfaces exposed. The halftime for binding is in the submillisecond range (Haynes, 1977).

Our findings suggest that the charge asymmetry effects on the fusion reaction are not due to changes in the degree of aggregation of the membranes for limiting size vesicles. The enhancement of the fusion

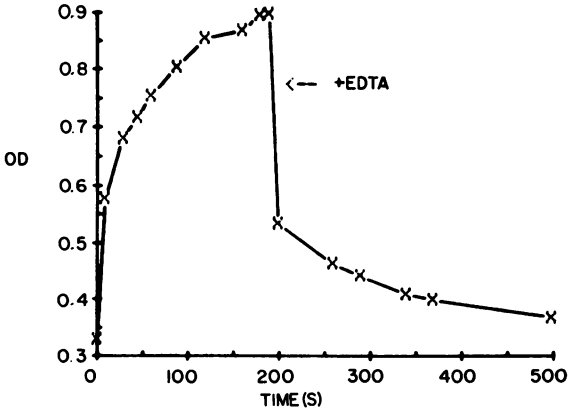

FIGURE 2 Vesicle disaggregation induced by EDTA. The OD increase of a 0.5 mg/ml sample of PA^- vesicles in 10mM KCl, 10 mM Tris buffer was measured at 350 nm in a 1-cm cell in a Zeiss spectrophotometer (Carl Zeiss, Inc., N.Y.). The reaction was initiated by addition of 10 mM Ca^{2+} at t = 0 and was reversed by addition of 15 mM EDTA at t = 180 s.

TABLE I
LACK OF EFFECT OF X-537A ON RATE AND EXTENT OF AGGREGATION

Lipid	[X537-A]	$t_{1/2}$	$\Delta OD/OD_0$
Phosphatidic acid	0	3.25 ± 0.35	1.0 ± 0.1
	3×10^{-6}	2.5 ± 0.3	0.9 ± 0.1
	3×10^{-5}	3.0 ± 0.3	1.2 ± 0.3
Phosphatidic acid/lecithin (3:1)	0	2.5 ± 0.2	0.61 ± 0.03
	3×10^{-6}	2.5 ± 0.5	0.5 ± 0.1
	1.5×10^{-5}	2.25 ± 0.35	0.57 ± 0.1
	3×10^{-5}	2.25 ± 0.35	0.57 ± 0.02

The experiments were carried out as in Fig. 1, except that syringe A also contained X-537A. The final concentration after mixing was as indicated. The final vesicle concentration after mixing was 0.5 mg/ml (ca. 6.8×10^{-4} M). $\Delta OD/OD_0$ is the fractional increase in sample OD. The values given are the average ± half difference of duplicate runs.

process must be attributable to bilayer destabilization or shape changes occurring in the aggregated state. Based on the absence of an effect on the dimerization reaction, we conclude that either shape changes do not occur in monomeric limiting-size vesicles, or they do occur but have no effect on the rate and extent of aggregation. We consider the former case to be more likely because flattening should increase the area available for interaction. Minimal shape changes would be expected for a limiting-size vesicle whose radius of curvature is determined primarily by the requirements of acyl chain packing.

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