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SHORT COMMUNICATION

Dextran Enhances Calcium-Induced Aggregation of Phosphatidylserine Liposomes: Possible Implications for Exocytosis

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Summary

We have studied the calcium-induced aggregation of phosphatidylserine liposomes in the presence of various concentration of a high-molecular water-soluble polysaccharide dextran. It has been shown that threshold concentrations of calcium necessary to induce liposome aggregation in the presence of ~1 mM concentration of dextran is about one order lower than in the absence of dextran. Soluble intracellular polymers may thus play an important role in the process of exocytosis.

Key words

Exocytosis • Aggregation • Liposomes • Calcium • Dextran

Calcium is known to be an essential requirement in many biological fusion events. However, the mechanism of its action is not well understood, especially in the exocytosis which involves the fusion of a secretory organelle with the cell membrane (Masumoto *et al.* 1995, Edwardson and Marciniak 1995, Vogel *et al.* 1996, Constantin *et al.* 1996, Sen *et al.* 1997, Heidelberger 1998, Garcia *et al.* 1998).

It has been shown that negatively charged liposomes fuse upon introduction of certain polyvalent metal ions (Wilschut and Papahadjoupoulos 1979, Düzgünes and Ohki 1981, Ohki 1982).

In a recent study (Babincová and Machová 1997) we have shown that Ca^{2+} enhances the capability of the polysaccharide dextran to induce fusion of phosphatidylcholine liposomes. The aim of the present report was to show that an analogous effect also exists for the calcium-induced fusion of phosphatidylserine (PS) liposomes.

In order to gain insight into the mechanism of exocytosis and a deeper understanding of membrane aggregation and fusion, we have measured turbidimetrically Ca^{2+}-induced aggregation of PS liposomes in the presence of dextran.

PS from the bovine brain extracted and purified according to Rouser *et al.* (1972) was kindly supplied by Dr. P. Sourivong (North Dakota University, Grand Fork). Small unilamellar sonicated liposomes were prepared in

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tris-HCl buffer of pH 7.4 (Radelkis, Hungary) with 50 mM NaCl (Lachema, Brno). Preparations were made above the temperature of the phase transition of PS (68 °C).

The turbidity of PS liposomes suspension (total PS concentration 0.2 mM), quantified as a relative absorbance at 600 nm, was measured using spectrophotometer Specol 210 (Carl Zeiss, Jena). The absorbance was recorded 3 min after changing the Ca^{2+} concentration. These measurements were made in the presence of various amounts of dextran with molecular weight of 110 000 (produced by *Leuconostoc mesenteroides* Strain no. B-512, Sigma, St. Louis).

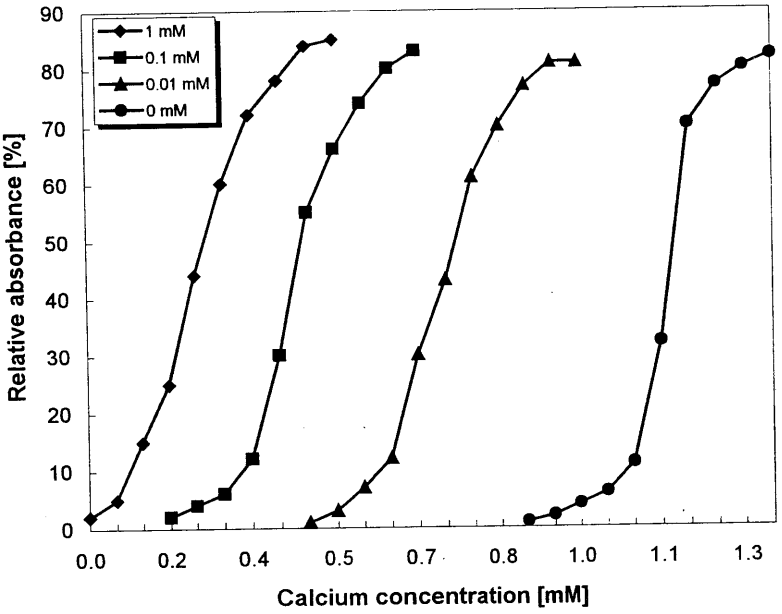

Fig. 1. *Relative absorbance of the small unilamellar phosphatidylserine liposomes as a function of Ca^{2+} concentration in the presence of various amounts of dextran.*

Figure 1 shows the changes of relative absorbance of the suspension of PS liposomes with respect to the Ca^{2+} concentration. The "threshold" concentrations of Ca^{2+} dications decrease with increasing amounts of dextran. It should be stressed that dextran itself is unable to induce fusion of PS liposomes. We have used dextran concentrations similar to those used in our previous study (Babincová and Machová 1997). As can be seen, the threshold concentration of calcium in the presence of ~ 1 mM dextran is about one order lower than in the absence of dextran, and with further increase of dextran is only slightly lowered.

The most important step in the induction of PS fusion is the formation of a transmembrane-Ca^{2+} complex as the site of direct interaction between two apposed membranes. As has been shown in recent theoretical studies (Nagarajan and Gahesh 1989, Raudino and Biancardi 1991) the Ca^{2+} binding to membranes is substantially enhanced by the presence of high-molecular uncharged polymers. This enhanced Ca^{2+} binding may cause local destabilization which rearranges, passing through intermediate intrabilayer micelles, eventually leading to merging of PS liposomes (fusion). In the cell (during exocytosis and endocytosis), the threshold Ca^{2+} concentration is about three order lower than in the fusion of liposomes (Plattner 1989). To explain this difference, special transmembrane proteins (e.g. synexin) have been suggested to be involved in this process. As has been shown in this study in explaining this discrepancy, the role of high-molecular intracellular polymers (e.g. nucleic acids, proteins and polysaccharides) should also be carefully analyzed as a possible source of the enhanced rate of exocytosis.

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