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THE INFLUENCE OF POLY(ETHYLENE GLYCOL) ON ION BINDING TO MEMBRANE SURFACES

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Abstract The binding of Pr^{3+} and Ca^{2+} ions to phospholipid bilayers was investigated in the presence of poly(ethylene glycol) (MW 400 and 6000). 30 wt.% PEG^{+} in water increased the Pr^{3+} concentration at the surface of unilamellar vesicles by a factor of about three. Qualitative similar results were obtained for the binding of Ca^{2+} ions to lipid phosphate groups in multilamellar liposomes. There is strong evidence to assume that increased ion binding is a general feature of action of PEG water solutions on membranes.

INTRODUCTION

PEG^{+} is a commonly used synthetic polymer for induction of cell fusion of different cell lines. From experiments on phospholipid model membranes we obtained evidence that the strong lowering of water activity after addition of PEG to solution together with reduced solubility of PEG molecules in the water layer near the membrane surface are

$^{+}$PEG - poly(ethylene glycol)

responsible for the PEG induced aggregation of cells.[1,2] After aggregation the bilayers of opposing cells have to get destabilized for fusion. The formation of nonlamellar lipid phases could be an indicator for bilayer destabilization processes. In some cases bilayer destabilization may be driven by membrane dehydration.[3] Membranolytic compounds as additives and impurities in commercial grade PEG's also destabilize bilayers.[4]

It is well known that increasing concentrations of di- and trivalent ions may induce nonbilayer phases in different lipid mixtures.[5] In the present paper we give experimental evidence for a third mechanism of membrane destabilization in the presence of PEG, the stronger binding of ions to membrane surfaces.

MATERIALS AND METHODS

A total egg phospholipid fraction was used in the experiments. The composition was checked by high resolution ^{31}P NMR and HPTLC plates (Merck). The molar ratio of phosphatidylcholine to phosphatidylethanolamine was 3.2 : 1. Further trace amounts of sphingomyelin, phosphatidylinositol, lysophosphatidylcholine, lysophosphatidylethanolamine and neutral lipids, altogether about 5 mol%, were detected.

^{31}P NMR investigations were performed on a Bruker HX-90 spectrometer at 36.4 MHz equipped with facilities for strong ^{1}H noise decoupling.

Unilamellar vesicles were prepared by ultrasonication of a lipid dispersion in heavy water,

containing 5 wt.% phospholipids. After sonication 10 µl of a 0.1 M $Pr(NO_3)_3$ solution were added to 1 ml of sonicated lipid dispersion. Liquid PEG 400 (Serva) was added under continous stirring of the dispersion.

Multilamellar liposomes were prepared by addition of 100 mg of a $^2H_2O/CaCl_2$ solution to 100 mg of dry phospholipid. Samples were homogenized by centrifugation back and foreward in sealed sample tubes filled with nitrogen to prevent lipid peroxidation. After a first ^{31}P NMR investigation the samples were opened and 1 ml of a 60 wt.% PEG 6000 (Serva)/^2H_2O solution was added.

All preparation procedures and measurements were performed at about 25°C.

EXPERIMENTAL RESULTS

Binding of Pr^{3+} ions

If the diameter of unilamellar vesicles is smaller than 100 nm the anisotropy of chemical shift and ^{31}P - ^1H dipole dipole interactions are averaged out. Slightly broadened isotropic ^{31}P NMR resonance lines are observed. After addition of Pr^{3+} ions to the dispersion the signal of lipid phosphate groups of the outer layer is shifted to lower field strength while the phosphate signal of the inner layer remains at the previous position if the vesicles are nonpermeable for Pr^{3+}. The difference of chemical shifts of phosphate signals of the inner and outer layers is directly proportional to the concentration of Pr^{3+} ions at the vesicle surface.[6] From the difference in are-

as of phosphate peaks an average vesicle diameter of 50 nm was calculated.

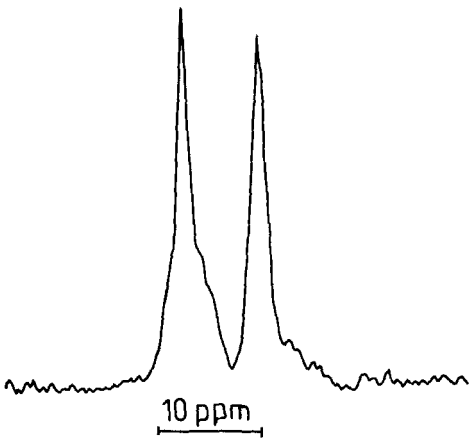

FIGURE 1. ^{31}P NMR spectrum of a dispersion of unilamellar egg phospholipid vesicles (5 wt.% in $^{2}H_2O$) after addition of 50 µl 0.1 M $Pr(NO_3)_3$ solution to 1 ml dispersion.

With increasing concentrations of PEG 400 in solution the difference of chemical shifts increased. At 30 wt.% PEG in water the Pr^{3+} concentration at the vesicle surface increased by a factor of about three. Around a concentration of 20 wt.% PEG massive vesicle fusion starts and the intensity of resonance signals decreased. At concentrations higher than 30 wt.% PEG most of the vesicles fused. There was no indication that bilayers became permeable for Pr^{3+} ions after addition of PEG.

Binding of Ca^{2+} ions

Ca^{2+} ion binding to lipid bilayers is of more

biological relevance than Pr^{3+} binding.

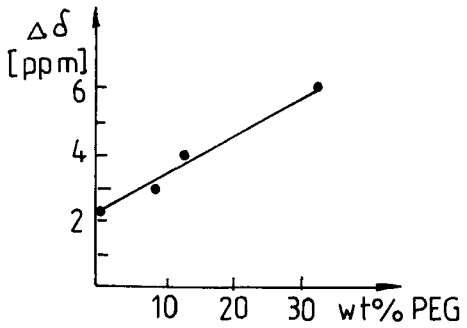

FIGURE 2. Difference of chemical shifts between phosphate NMR signals of lipids in the inner and outer layer in dependence on PEG concentration. Sample: 1 ml vesicle dispersion + 10 μl of a 0.1 M $Pr(NO_3)_3$ solution.

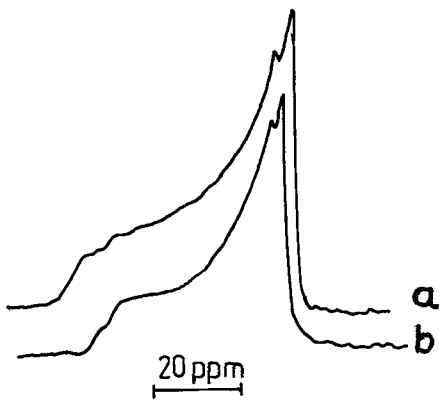

FIGURE 3. ^{31}P NMR spectra of multilamellar egg phospholipid dispersions with (a) and without (b) $CaCl_2$.

Unfortunately it is more difficult to detect Ca^{2+} binding, because Ca^{2+} ions influence the anisotropy of chemical shift of lipid phosphate groups only and not the isotropic chemical shift as in the case of Pr^{3+} ions. Therefore the experiments had to be performed with big multilamellar liposomes. The anisotropies of chemical shift are not averaged out for liposomes with diameters in the μm range.

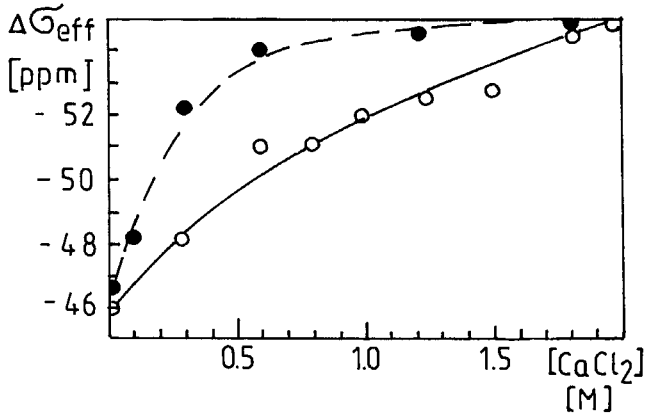

FIGURE 4. Anisotropy of chemical shift of phosphatidylcholine ^{31}P NMR signal in dependence on $CaCl_2$ concentration.
o without PEG
• 1 ml 60 wt.% PEG 6000 solution added

The ^{31}P NMR lineshape is a superposition of the spectrum of phosphatidylcholine ($\Delta\sigma_{eff}$ = -46 ppm) and of phosphatidylethanolamine ($\Delta\sigma_{eff}$ = -39 ppm).

$^{+}\Delta\sigma_{eff}$ - effective anisotropy of chemical shift

If the dispersions contain increasing $CaCl_2$ concentrations the anisotropies of chemical shift of both components increase. The reason for this increase is a structural change at the level of polar groups.[7]

In a previous paper[1] we showed that PEG alone has no significant influence on $\Delta\sigma_{eff}$. Changes in $\Delta\sigma_{eff}$ are related to the amount of Ca^{2+} ions bound to phosphate groups. In the concentration range between 0.1 M and 1.5 M $CaCl_2$ $\Delta\sigma_{eff}$ values in the presence of PEG correspond to higher $CaCl_2$ concentrations than without PEG.

DISCUSSION

The concentration of negatively charged phospholipids in the egg yolk phospholipid fraction is rather low. The electrophoretical mobility of multilamellar liposomes was comparable to that of egg yolk lecithin liposomes. Therefore the obtained results reflect the behaviour of a mixture of zwitterionic lipids. PEG in concentrations used to induce fusion of cells increases the surface concentration of Pr^{3+} and Ca^{2+} at least by a factor of two or three. Of course the ion concentration, especially the Ca^{2+} concentration in our experiments seems to be unrealistically high. These high concentrations were necessary to obtain a measurable influence of ions on phosphate NMR signals. It is well known that ion binding to membrane surface gets saturated with increasing ion concentration.[7] This behaviour is also reflected in Figure 4. We have therefore eviden-

ce that after addition of PEG the relative increase of bound ions at lower ion concentrations in solution is similar or even higher.

Increased ion binding could be a general feature of action of PEG water solutions on membranes. It could explain synergistic effects of PEG and Ca^{2+} ions in the fusion of phosphatidylserine containing vesicles.[8] Physical reasons for increased ion binding could be the decrease of dielectric constant of water after PEG addition[9] as well as a reduced solubility of ions in the water which has contact to PEG.

From our experiments we obtained no indication for a permeation of Pr^{3+} ions through bilayers in the presence of PEG. However this conclusion is valid for low PEG concentrations and unfused vesicles only. If the membranes would contain ion channels drastic increases in surface concentrations of ions could increase permeation rates of ions.

In any way, our finding has strong consequences for the mechanism of PEG induced membrane fusion. At higher Ca^{2+} concentrations several lipids form hexagonal phases.[5] Ion binding may induce instabilities of the lamellar phase state of lipids which could be related to further steps of membrane fusion after cell aggregation.

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