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EFFECT OF PEG ON THE ELECTROPHORETIC MOBILITY OF LIPOSOMES

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INTRODUCTION

Poly(ethylene glycol) (PEG) is a widely used agent for the fusion of cells and it is used as a fractional precipitating agent for protein purification, isolation and crystallization[1].

It was demonstrated that some of the effects of PEG on membrane properties could be explained by an alteration of the physicochemical properties of the surrounding aqueous phase after addition of PEG[2]. PEG decreases the polarity and the dielectric constant of an aqueous phase[3]. Besides these effects PEG causes a high osmotic pressure and exerts an osmotic stress on the cell because high molecular PEG does not permeate cell membranes[4].

In this paper experimental results are given which support the hypothesis that PEG is excluded from the membrane surface. The electrophoretic mobility (EPM) of charged liposomes is measured as a function of the PEG concentration.

MATERIALS AND METHODS

Dipalmitoylphosphatidic acid (DPPA) and dipalmitoylphosphatidylcholine (DPPC) from FLUKA (West-Berlin) were used without further purification.

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The samples gave a single spot in thin-layer chromatography. Multilamellar vesicles (mixture DPPA/DPPC) were prepared by the method of DEAMER and BANGHAM[5]. The water used for the preparation of dispersions was twice distilled. The dispersions were buffered with tris. HCl (10^{-3} mol/l) from FLUKA. The aqueous dispersions were mixed with adequate volumes of aqueous solutions of PEG to get the appropriate final concentrations of the dispersions. PEG 6000 from FLUKA was used without further purification.

We measured the EPM of the liposomes for different concentrations of PEG by using a PARMOQUANT-2 (VEB Carl-Zeiss-Jena, GDR) in the dark field mode. 100 particles were measured in both directions for each experimental point.

In addition the viscosity of the suspension medium for different concentrations of PEG was measured by means of a Hoeppler viscosimeter.

RESULTS

Using the Helmholtz-Smoluchowski equation

$$\zeta = \frac{u \cdot \eta}{\varepsilon \cdot \varepsilon_0} \qquad (1)$$

where ζ : electrokinetic potential (zeta potential)

u : electrophoretic mobility

η : viscosity

ε : relative dielectric constant of the solution

ε_0: permittivity of the vacuum

we calculated values of the zeta potential from the EPM. Their absolute amounts grow with increasing PEG concentration.

Using eq.(1) means assuming tacitly that all premises under which it can be derived are fulfilled.

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For example the viscosity η of the suspending medium need not depend on the distance from the surface of the colloid particle, cell or liposome.

In order to explain electrophoretic measurements on erythrocytes performed in solutions of dextran BÄUMLER and DONATH[6] proposed a position dependent viscosity profile. If we adapt their strategy to systems with a smooth surface (liposomes) and (1) postulate a viscosity profile

$$\eta(x) = \frac{\eta_\infty}{1 + \left(\frac{\eta_\infty}{\eta_0} - 1\right) \cdot e^{-\frac{x - x_s}{d}}} \qquad (2)$$

η_∞: bulk viscosity (increasing with the concentration of PEG)

η_0 : viscosity of the pure solvent

x : distance from the surface

x_s : distance of the shear plane from the surface

d : characteristic length

and

(2) assume the value of the surface potential to be so small that linearization of the Poisson-Boltzmann equation is allowed,

we have to modify the Helmholtz-Smoluchowski equation as follows:

$$U = -\varepsilon\varepsilon_0 \int_{x_s}^{\infty} \frac{1}{\eta(x)} \cdot \frac{d\psi}{dx} \cdot dx \qquad (3)$$

$$= \frac{\varepsilon\varepsilon_0 \psi(x_s)}{\eta_\infty} \cdot \left[1 + \left(\frac{\eta_\infty}{\eta_0} - 1\right) \frac{1}{1 + \frac{1}{\varkappa \cdot d}}\right]$$

$\psi(x)$: electrostatic potential

Formal use of eq.(1) results in an apparent zeta potential

$$\zeta_{app.} = \psi(x_s) \cdot \left[1 + \left(\frac{\eta_\infty}{\eta_0} - 1\right) \cdot \frac{1}{1 + \frac{1}{\varkappa d}}\right] \qquad (4)$$

which turns out to be a linear function of the bulk viscosity.

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FIGURE 1 shows the apparent zeta potentials of the liposomes plotted vs. the bulk viscosity of the suspending medium using linear regression analysis. The characteristic length was estimated to be d = 1.8 nm.
Following this argument one can conclude that the polymer is excluded from the surface layer of the liposomes.

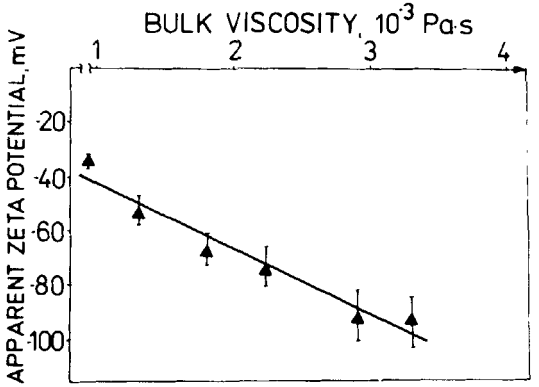

FIGURE 1. Dependence of the apparent zeta potential on the bulk viscosity.
DPPA/DPPC liposomes in 10^{-3} mol/l Tris, 10^{-1} mol/l NaCl, 10^{-3} mol/l EDTA, pH = 7.4, 25°C.

DISCUSSION

Different reasons have been given to explain the increase of the apparent EPM of erythrocytes in the presence of dextran, PEG and other polymers. An exclusion of counter ions from the surface and a decrease of the ionic strength accompanied by an increase of the zeta potential have been assumed to be the most probable cause[7]. BÄUMLER and DONATH[6] found that such changes of the surface potential do not sufficiently describe the behaviour of erythrocytes.

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Liposomes have the advantage that such a complex surface structure as the glycocalyx does not exist. The electrophoretic measurements are described by the assumption of a distance dependent viscosity. The characteristic length was calculated to be 1.8 nm. This value is in the order of the radius of an equivalent rigid sphere of PEG 6000 determined to be 2.6 nm from intrinsic viscosity measurements[8]. It is also consistent with the concept that PEG is effectively excluded from the hydration layer of the liposome surface. This result is of basic importance for the interpretation of the action of PEG on membranes and the fusogenic activities of PEG. Many of the effects of PEG on membranes have to be explained by an indirect action mediated by the changed properties of the water phase.

It has recently been established that PEG is excluded from the surface of water soluble proteins [9, 10]. It was concluded that there is a water layer around the protein which is impermeable to PEG. Such a behaviour is an expression of the difference between the interactions of water and PEG with the surface. These similarities in the behaviour of PEG at protein and liposome surfaces lead to effects comparable to those in the aggregation of proteins and liposomes in the presence of PEG.

A decrease of the surface potential of lipid monolayers by several hundred millivolts on addition of PEG was described by MAGGIO et al[11]. Such a decrease of the surface potential did not occur in our experiments for liposomes. Therefore, this change of the surface potential observed for monolayers should mainly result from orientation changes of molecular dipoles in the surface.

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