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Exclusion of poly(ethylene glycol) from liposome surfaces

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The electrophoretic mobility of vesicles is measured for concentrations of poly(ethylene glycol) from 0 wt.% to 10 wt.%. Mixtures of phosphatidylcholines and phosphatidic acid are used. The zeta potential calculated from the electrophoretic mobility and the viscosity of the suspension becomes more negative for all vesicles studied. Binding of poly(ethylene glycol) to the phospholipid surface by addition of the poly(oxyethylene)-containing surfactant $C_{12}E_8$ has the opposite effect and a decrease of the zeta potential is observed. Independent measurements of the surface potential of the vesicles in the presence of PEG by use of a positively charged spin probe and ESR spectroscopy and a fluorescent pH indicator and fluorescence spectroscopy show that actually the surface potential is not changed. A theory of the electrophoretic behaviour of vesicles in the presence of PEG is given which explains the contradiction between the two methods. It is assumed that the polymer is excluded from the vesicle surface (depletion layer) and that the viscosity near the surface is lower than the viscosity in the bulk phase. The thickness of the depletion layer is calculated from the experiments. The decrease of the zeta potential in the presence of poly(oxyethylene) chains linked to the vesicle surface results from the friction increase.

Introduction

It has recently been established that PEG is excluded from the surface of water-soluble proteins [1]. It seems that there is a water layer around the proteins which is impermeable to PEG. The solvent composition is perturbed from that in the bulk in a region near the protein and the surface is preferentially hydrated [2]. This concept of PEG exclusion from the protein should also be applicable to membrane surfaces which consist of a protein and a phospholipid part of hydrophilic character. A partial or complete exclusion of PEG from the water layer between interacting bilayers in multilamellar systems has been described [3]. Evans and Needham [4] developed a depletion-based theory to explain the PEG-induced aggregation of giant phospholipid vesicles. Van Oss et al. [5] found from the quantitative discussions of the polar surface tension parameters that PEG and membranes immersed in water repel each other with a sizeable repulsion energy.

However, experiments performed to characterize the interaction of PEG with membranes have given contrary results. Using tritiated PEG an interaction with liposomes has been described [6]. Many other authors concluded from changes of the structural and dynamical properties of membrane components after addition of PEG that these polymer molecules interact directly with the membrane surface. However, in our experiments it was found that such influences of PEG on the membrane can also be explained by an indirect action of PEG on the membrane due to an alteration of the physicochemical properties of the aqueous phase [7–9]. Addition of PEG raises the osmotic pressure [10,11], decreases the polarity and the dielectric constant [7,8] of water and it has a high ability to bind water molecules [12]. The influence of PEG on the approach of bilayers in the lamellar phase [3], on phase transitions and phase separations [8,9,13], partition coefficients [14] and the organization of membrane proteins [15] can be explained by the influence of PEG on the properties of the water phase.

In this paper it is shown that the polymer exclusion phenomenon occurs for the individual phospholipid vesicles as well. Measurements of the electrophoretic mobility of charged liposomes in the presence of PEG support the idea that PEG has a lower concentration near the membrane surface than in the bulk phase. A first report about such measurements has been given very recently [16]. A similar behaviour of the electrophoretic mobility was observed for erythrocytes in the presence of dextran [17] and PEG [18]. The electrophoretic mobility of vesicles and erythrocytes decreases

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much less in the presence of PEG than expected from the change of the viscosity of the suspending medium, which would suggest an increase of the surface potential. In this paper such electrophoretic measurements are discussed for different phospholipid vesicles in the presence of PEG. The influence of PEG on the surface potential is determined by independent methods where a charged spin probe and a pH-sensitive fluorescence probe are used. These measurements do not show an influence of PEG on the surface potential. This contradiction can be overcome by introducing a distance dependent viscosity. This concept involves a dilution of the polymer near the vesicle surface.

It is possible to link PEG chains to phospholipid surfaces. It is known that poly(oxyethylene)-containing surfactants are incorporated into the bilayer system in such a manner that the alkyl part is imbedded in the phospholipid bilayer and the poly(oxyethylene) chains extend into the water phase. The opposite effect on the zeta potential is observed in such a system, i.e., the zeta potential is decreased. That indicates that these polymer chains, if fixed at the surface, increase the friction.

Materials and Methods

Dioleoylphosphatidylcholine (DOPC), dimyristoylphosphatidylcholine (DMPC) and dipalmitoylphosphatidylcholine (DPPC) from SERVA and dipalmitoylphosphatidic acid (DPPA) from FLUKA were used. Egg phosphatidic acid (egg PA) was prepared according to [19]. The phospholipids were used without further purification. All phospholipids show a single spot in thin-layer chromatography. Multilamellar vesicles (MLV) and large unilamellar vesicles (LUV) were prepared using the method of Bangham [20] and Düzgüneş et al. [21], respectively. Small unilamellar vesicles (SUV) were prepared by ultrasonication using a bath-type sonifier (800 kHz) or a KLN tip probe sonifier. Optically clear suspensions were obtained and used for measurements. For all experiments twice distilled water was used. PEG 6000 (FLUKA) was used without further purification. Measurements of the electric conductivity of PEG solutions showed that the concentrations of ionic impurities are negligible. Octaethyleneglycol mono(*n*-dodecyl) ether ($C_{12}E_8$) was purchased from Nikko Chemical Co. (Tokyo, Japan).

A PARMOQUANT-2 (Carl Zeiss Jena, G.D.R.) operating in the dark field mode was used for the measurement of the electrophoretic mobilities of MLV and LUV. In each case the device automatically calculated mean values and standard deviations of the electrophoretic mobility of a set of 100 particles. During the measurement the direction of the applied electric field was reversed. Particles drifting significantly faster in one direction than in the other were excluded from evaluation. The zeta potential was calculated according to Smolukhovski:

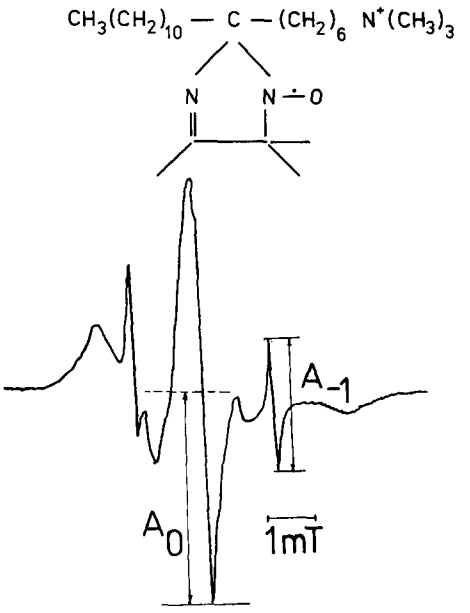

Fig. 1. ESR spectrum of the positively charged spin probe 7-imoxyloctadecane-trimethylammonium sulfonate (0.08 mM) in DOPC/DPPA (10:1) SUV (1.5 mg/ml total phospholipid). The spectrum is a superposition of two spectra which belong to unbound and membrane-bound spin probes. The parameters used for the calculation of partition coefficients are shown. The structural formula of the spin probe is also given.

$$\zeta = \frac{\eta \cdot u}{\varepsilon_r \cdot \varepsilon_0} \tag{1}$$

where u is the electrophoretic mobility, η is the viscosity, ε_r, the relative dielectric constant of the solution and ε_0, the electric field constant. The viscosity of the solution was measured with a Hoeppler viscosimeter.

For the determination of the surface potential by the electron spin resonance (ESR) method the positively charged spin probe 7-imoxyloctadecanetrimethylammonium methane sulfonate was used [22]. Its structure and spectrum are given in Fig. 1. For these measurements small unilamellar vesicles were used. This spin probe is only incorporated in the outer monolayer of the phospholipid membrane [14]. The spectrum is a superposition of two spectra which belong to spin probe molecules in the bulk phase and molecules incorporated into the outer phospholipid layer. From the change of the partition coefficient the change of the surface potential can be calculated by the following equation:

$$\Delta\psi = \frac{R \cdot T}{z \cdot F} \ln \lambda / \lambda_0 \tag{2}$$

The partition coefficient λ is calculated from the heights of the ESR signals A_0 and A_{-1} (Fig. 1). The influence of the polymer on the linewidth of the signal of the unbound spin probes was considered according to Refs.

14 and 23. The ESR spectra were measured on an ERS 231 spectrometer (Centre for Scientific Instrumentation, Berlin, G.D.R.). The ESR spectra were measured at a klystron power of 10 mW, a field modulation frequency of 100 kHz and a field sweep range of 100 G.

The fluorescent pH indicator 4-heptadecyl-7-hydroxycoumarin from Molecular Probes, U.S.A. was used for measurements of the surface potential of liposomes [24]. The probe molecules were added to the phospholipid/chloroform/ethanol solution at a molar ratio of 1:200. SUV and LUV were prepared as described before. Ionized and unionized forms of the probe can be distinguished by their different excitation and emission spectra. The degree of dissociation of the fluorophore is determined in the same way as described in Ref. 24. The surface potential was calculated from the shift in pK caused by changes in the surface potential relative to pK_0 for the uncharged surface:

$$\Delta\psi = 2.3\frac{R\cdot T}{F}(\mathrm{p}K_0 - \mathrm{p}K) \tag{3}$$

The value for pK_0 was obtained from measurements of unilamellar egg PC vesicles which are uncharged in the pH range used.

The concentration of the phospholipids was determined according to the method given by Stewart et al. [25].

Theory

For the calculation of the surface potential ψ_0 from the zeta potential ζ two assumptions are usually made: (i) the hydrodynamic plane of shear is located 0.2 nm from the phospholipid surface [26]. (ii) The potential profile in the aqueous phase can be described in terms of the classical double layer theory [27–29].

In the convential application of the Smolukhovski equation [1] to the determination of the zeta potential the viscosity of the suspension is used. This viscosity is different from that of the pure electrolyte because of the addition of the polymer. However, if an exclusion of the polymer from the surface of the membranes occurs the local viscosity near the surface is lower than the viscosity in the bulk phase. A position-dependent viscosity can be introduced by

$$\eta(x) = \eta_\infty\cdot\left[1+\left(\frac{\eta_\infty}{\eta_0}-1\right)\cdot h(x)\right]^{-1} \tag{4}$$

In this equation η_∞ is the bulk viscosity (increasing with the concentration of PEG), η_0 is the viscosity of the pure solvent (without PEG), x is the distance from the surface, x_S is the distance of the shear plane from the surface and $h(x)$ is a dimensionless function of the distance x from the membrane surface. The values of $h(x)$ decrease with increasing x monotonically from $h(x_S) = 1$ to $\lim_{x\to\infty} h(x) = 0$. The function $h(x)$ governs the viscosity profile $\eta(x)$. Eqn. 4 bears virtually no restriction to the concrete form of the viscosity profile.

Obviously η_∞ is the higher the higher the bulk concentration of PEG. The decrease of the local viscosity $\eta(x)$ from η_∞ in the bulk phase to η_0 near the shear plane is due to an assumed reduced local concentration of PEG in the vicinity of the membrane. $h(x)$ is assumed to be independent of η_∞.

The potential profile $\psi(x)$ follows from the Poisson-Boltzmann equation and is assumed to be not influenced by the presence of PEG. $\psi(x_S)$ is the electrostatic potential in the shear plane. In the classical theory of the double layer $\psi(x_S)$ is identical with the zeta potential. It is convenient to introduce a dimensionless potential ϕ by

$$\phi(x) = \psi(x)/\psi(x_S) \tag{5}$$

Because of the position-dependence of the viscosity the Helmholtz-Smolukhovski-equation has to be modified into

$$u = \varepsilon_r\varepsilon_0\psi(x_S)\cdot\int_0^1\frac{\mathrm{d}\phi}{\eta(x(\phi))} \tag{6}$$

where $x(\phi)$ is the inverse function of $\phi(x)$.

If Eqn. 1 is formally used to define an apparent zeta potential ζ_{app} and the viscosity profile is described in terms of $h(x)$ according to Eqn. 4, a linear dependence of the apparent zeta potential on the bulk viscosity is established:

$$\zeta_{app} = \psi(x_S)\cdot\left[1+\left(\frac{\eta_\infty}{\eta_0}-1\right)\cdot\int_0^1 h(x(\phi))\cdot\mathrm{d}\phi\right] \tag{7}$$

The last equation suggests that the apparent zeta potential ζ_{app} if calculated from the experimentally determined electrophoretic mobilities and the bulk viscosity η_∞ should depend linearly on the bulk viscosity. The potential $\psi(x_S)$ has the meaning of a real zeta potential because it is defined as the potential at the shear plane. On the assumption that the presence of PEG molecules does not alter the electric field in any way the real zeta potential $\psi(x_S)$ can be calculated from the electrophoretic mobility of liposomes in the pure solvent.

Provided that a plot of ζ_{app} vs. η_∞ resulting from electrophoretic measurements in fact gives a linear relation

$$\zeta_{app} = A + B\cdot\eta_\infty \tag{8}$$

the slope B (linear regression coefficient) is a measure for the formation of a depletion layer.

It would be desirable to come to more concrete conclusions.

If it can be assumed that the surface potential is so small that a linearization of the Poisson-Boltzmann equation is allowed, the potential profile is explicitly given by

$$\phi(x) = \exp(-\kappa(x - x_S)) \tag{9}$$

where κ is the Debye-Hueckel parameter. Then the integral in Eqn. 7 can be calculated analytically if a suitable choice for $h(x)$ is made, for instance according to Baeumler and Donath [17]

$$h(x) = \exp(-(x - x_S)/d) \tag{10}$$

d being an estimate of the width of the depletion layer. Then the apparent zeta potential is given by

$$\zeta_{app} = \psi(x_S) \cdot \left[1 + \left(\frac{\eta_\infty}{\eta_0} - 1\right) \cdot \left(1 + \frac{1}{\kappa d}\right)^{-1}\right] \tag{11}$$

and d can be calculated from the linear regression coefficient B according to

$$B = \frac{\psi(x_S)}{\eta_0} \cdot \frac{1}{1 + \frac{1}{\kappa d}} \tag{12}$$

Results

Electrophoretic measurements

The effect of PEG 6000 on the apparent zeta potential of different liposome systems is given in Fig. 2. The measured electrophoretic mobilities and the bulk viscosities determined in the corresponding PEG/water solutions are used for the calculations of the zeta potential from Eqn. 1. Because mixtures of neutral and negatively charged phospholipids are used negative zeta potentials are obtained. In all cases except the addition of sucrose the absolute value of the apparent zeta potential increases on addition of PEG and a linear dependence is observed as predicted by the theory given before. For MLV consisting of a mixture of DPPC and DPPA at a molar ratio of 1:1 a change of the apparent zeta potential from about −30 mV in the absence of PEG to −110 mV for 10 wt.% PEG 6000 is measured. For MLV consisting of mixtures of DMPC and egg PA at molar ratios of 10:1 and 1:1 the apparent zeta potential increases from −13 mV to −32 mV and from −42 mV to −155 mV, respectively. The same behaviour is observed for LUV. Results are given for a mixture of DMPC and egg PA at a molar ratio of 2:3 and an increase from −62 mV to −158 mV occurs. The other vesicles used have lower contents of negatively charged phospholipids with lower real zeta potentials. By use of Eqn. 12 the following characteristic lengths are calculated from the experiments (Table I).

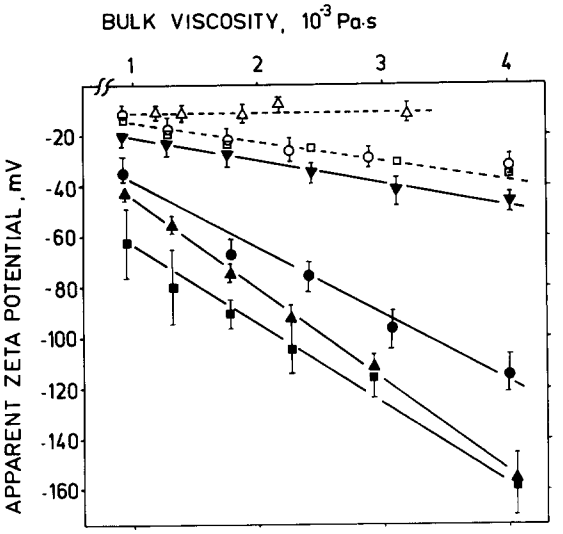

Fig. 2. Influence of PEG 6000 and sucrose on the apparent zeta potential of different vesicles. The vesicles were prepared from mixtures of DOPC, DMPC or DPPC with egg-PA or DPPA as indicated below. The zeta potential is plotted as a function of the viscosity of the suspension. The vesicles were prepared in 100 mM NaCl, 1 mM Tris, 1 mM EDTA (pH 7.4). The following MLV were used: DMPC/egg-PA (10:1)+sucrose (△); DOPC/DPPA (10:1) (□); DMPC/egg-PA (10:1) (○); DPPC/DPPA (1:1)+2 mM Ca^{2+} (▼); DPPC/DPPA (1:1) (●); DMPC/egg-PA (1:1) (▲). The following LUV were used: DMPC/egg-PA (2:3) (■).

As predicted by Eqn. 11 the slope of the $\zeta_{app} = \eta_\infty$ plot is influenced by the real zeta potential. This potential was changed in a broad range by changing the molar ratio of DMPC and egg PA in the DMPC/egg-PA MLV. As examples of these experiments the results are given in Fig. 2 for molar ratios of 10:1 and 1:1. In agreement with the theory the slope increases on increasing the concentration of negatively charged egg PA in the vesicles. The apparent zeta potential of the DMPC/egg-PA vesicle system was also measured as a function of temperature between 15°C and 35°C. The shift of the apparent zeta potential to higher values on addition of PEG is conserved over the whole temperature range (results are not given here).

TABLE I

Thickness of the depletion layer of PEG exclusion from vesicle surfaces

The characteristic length d was calculated from the change of the apparent zeta potential of liposomes on addition of PEG 6000 in a concentration range between 0 and 10 wt.% for different liposomes and in the presence of Ca^{2+}. The data presented in Fig. 2 and Eqn. 8 were used for the calculation of d. The vesicles were prepared in buffer solution (pH 7.4, 1 mM Tris/HCl, 100 mM NaCl, 1 mM EDTA, 25°C).

MLV	DMPC/ egg-PA 10:1	DMPC/ egg-PA 1:1	DOPC/ DPPA 10:1	DPPC/ DPPA 1:1	DPPC/ DPPA (1:1) +2 mM Ca^{2+}
d (nm)	0.61	0.66	0.40	1.15	0.62

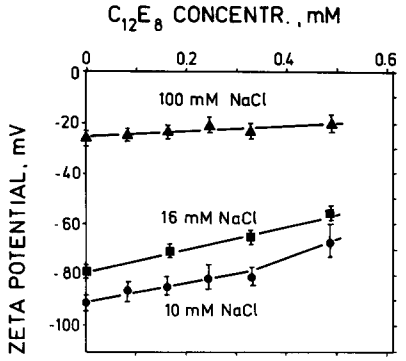

Fig. 3. Zeta potential of DMPC/egg-PA MLV vesicles as a function of the concentration of octaethyleneglycol mono(*n*-dodecyl) ether $C_{12}E_8$. Lipid concentrations of 1.8 mM DMPC and 1.0 mM egg-PA were used. The vesicles were prepared in 10 mM Hepes, 1 mM EDTA and pH 7.4.

The addition of Ca^{2+} to negatively charged vesicles results in the decrease of the zeta potential, mainly by direct binding to the phospholipid headgroups. As shown in Fig. 2 the zeta potential of about −35 mV of the DPPC/DPPA MLV is decreased to −20 mV by addition of 2 mM Ca^{2+}. Addition of PEG results in the increase of the zeta potential of this system as observed for the Ca^{2+}-free system (Fig. 2).

If instead of PEG sucrose is added to the DMPC/egg-PA MLV system the bulk viscosity is changed in a similar manner but the apparent zeta potential calculated in the same way is not changed. These data are also shown in Fig. 2.

The influence of the octaethyleneglycol mono(*n*-dodecyl) ether $C_{12}E_8$ on the electrophoretic mobility of DMPC/egg-PA MLV is given in Fig. 3. This surfactant consists of 8-oxyethylene units and a hydrocarbon chain of 12 C-atoms. At sublytic concentrations the surfactants are partly incorporated into the phospholipid bilayer and the hydrophilic oxyethylene part extends into the water phase, as demonstrated by NMR experiments [30]. At higher concentrations the turbidity rapidly decreases and a solubilization of the bilayers occurs [31]. In our experiments sublytic concentrations of the surfactant are used. As shown in Fig. 3 the zeta potential decreases in the presence of the surfactant.

The addition of the surfactant results in a lateral expansion of the bilayers and a dilution of the surface charge. However, the decrease of the zeta potential observed in our experiments cannot be explained by the reduction of the surface charge density alone. At the maximum concentration used the molar ratio of surfactant to phospholipid is about 1 : 6. Because a surfactant molecule occupies only half the area of a phospholipid molecule the surface charge density is reduced by 8% at this highest concentration. A reduction of the surface potential by about 5 mV results from calculations by use of the Gouy-Chapman theory. This change is smaller than the decrease of the zeta potential by more than 20 mV as measured for 10 mM and 16 mM NaCl.

Therefore, it can be concluded that the poly(oxyethylene) chains adsorbed on the bilayer surface exert an additional effect on the decrease of the zeta potential. The same behaviour was observed for the adsorption of these surfactants on silver iodide particles [32]. Mathai and Ottewill [32] explained this phenomenon by the shift of the position of the shear plane towards the outside of the original double layer so that the zeta potential decreases. In other words, the bound polymers increase the friction near the particle surface. This behaviour is completely different from the behaviour described above for the free polymers.

ESR and fluorescence measurements

In order to confirm the hypothesis that PEG does not actually alter the surface potential of vesicles use was made of the evaluation of ESR spectra of charged spin probes and of fluorescence spectra of a pH sensitive fluorescence probe using SUV and LUV consisting of the same mixture of DOPC/DPPA and DMPC/egg-PA as used for preparing the vesicles designed for electrophoresis.

To test the sensitivity of the method the influence of additional Ca^{2+} ions of varying concentrations both on the partition coefficient of the spin probe (ESR method) and on the surface potential (electrophoresis method) was investigated. From the values of the surface potential corresponding values of the partition coefficient were calculated. As Fig. 4b shows the two methods are in very good agreement (solid line: ESR partition coefficient; broken line; electrophoresis; values of surface potential indicated).

Obviously surface potential changes in an order of magnitude as observed in the apparent zeta potential should result in a drastic increase of the partition coefficient as given in Fig. 4a, upper curve. But, in fact, addition of PEG resulted in a slight decrease of the partition coefficient whether Ca^{2+} ions were present or not (Fig. 4a, middle and lower curve). The possibility that this decrease results from a change of the surface potential can be ruled out, because a similar decrease occurs for an uncharged probe of the same composition of the aliphatic chain. Previous studies of uncharged vesicles demonstrated a release of spin probes from the membrane resulting from a lowered polarity of the bulk phase in the presence of PEG [8,14].

In Fig. 5 the dissociation of the fluorescent probe 4-heptadecyl-7-hydroxycoumarin is plotted as a function of pH for DMPC SUV, DMPC/egg-PA (1 : 1) SUV and DMPC/egg-PA (2 : 3) LUV. The pK_0 value for DMPC vesicles is 8.7 whereas the pK was shifted to 9.7 and 10.3 for the DMPC/egg-PA (1 : 1) SUV and DMPC/egg-PA (2 : 3) LUV, respectively. According to Eqn. 3 the surface potential was increased from 0 mV

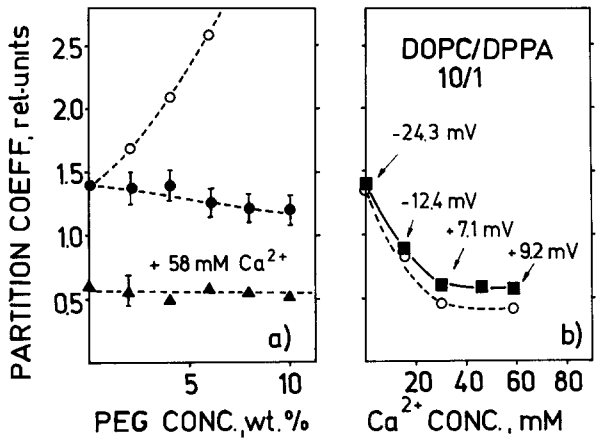

Fig. 4. Partition coefficients of the spin probe shown in Fig. 1 in DOPC/DPPA SUV as a function of added Ca^{2+} and PEG 6000. In (a) the partition coefficient is given as a function of the concentration of PEG in the presence (▲)and absence (●) of Ca^{2+}. The upper curve, (o------o) presents partition coefficients calculated according to Eqn. 2 from the electrophoretically mesured apparent zeta potentials and corresponding surface potentials of the DOPC/DPPA SUV. In (b) the partition coefficient is shown as a function of the Ca^{2+} concentration. The surface potentials are also given for each Ca^{2+} concentration as calculated from the electrophoresis measurement. Corresponding values of the partition coefficient are calculated from the surface potentials according to Eqn. 2 (broken curve).

for DMPC SUV (a surface potential of 0 mV is assumed for neutral phospholipids) to −57.5 mV and −90 mV for DMPC/egg-PA (1 : 1) SUV and DMPC/egg-PA (2 : 3) LUV, respectively. These values agree with the surface potential which can be calculated from electrophoresis measurements. As shown in Fig. 5 the addition of 5 and 10 wt.% PEG 6000 does not change the pK of the fluorescence probe in the DMPC/egg-PA vesicles which again demonstrates that PEG does not influence the surface potential of SUV as well as LUV.

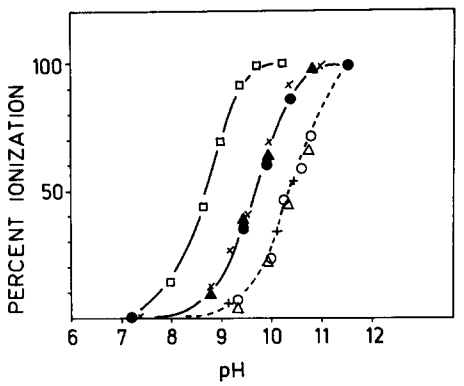

Fig. 5. pH dependence of the ionization of the fluorescence probe 4-heptadecyl-7-hydroxycoumarin incorporated into SUV and LUV at a molar ratio of 1 : 200. The vesicles were prepared in 100 mM NaCl, 4 mM Tes, 0.02 mM EDTA and pH 7.4. PEG 6000 was added after the preparation of the vesicles. DMPC SUV (□); DMPC/egg-PA (1 : 1) SUV at 0 wt.% (●), 5 wt.% (▲) and 10 wt.% PEG 6000 (×); DMPC/egg-PA (2 : 3) LUV at 0 wt.% (○), 5 wt.% (△) and 10 wt.% PEG 6000 (+).

The same behaviour was also found for other charged vesicles (Arnold, K. and Ohki, S., unpublished data).

So, after all, it is to be concluded that PEG does not affect the surface potential.

Discussion

The influence of PEG on the surface potential and zeta potential of liposomes of different phospholipid compositions was studied with different methods. The measurement of the electrophoretic mobility of the liposomes gives an increase of the apparent zeta potential on addition of PEG. The ESR measurements of the partition coefficients of a charged spin probe and the fluorescence measurements of the dissociation of a pH indicator indicate that the surface potential is not changed on addition of PEG. A theory is given which can explain the contradiction between the methods. It is assumed that the zeta potential is not actually changed. An apparent increase of the zeta potential on addition of PEG occurs because the increase of the viscosity in the bulk phase has a decreased influence on the motion of the particle due to a dilution of PEG near the liposome surface.

An opposite effect of PEG on the zeta potential was found when PEG was bound to the liposome surface via alkyl chains by use of a poly(oxyethylene) surfactant. The zeta potential decreased because the friction at the liposome surface was increased. It was shown by one of us that these surfactants lead to an increase of the repulsive force between bilayer membranes due to the presence of the PEG chains in the surface [33]. This demonstrates that free and bound PEG have different effects on the zeta potential and the interaction of bilayer membranes.

An increase of the zeta potential of erythrocytes in the presence of PEG was observed by Brooks and Seaman [34]. They concluded from these experiments that the surface potential is increased due to a dilution of monovalent ions near the membrane surface resulting in a reduced ionic screening. Such a dilution of ions was expected to arise from decreased solubilities in the presence of PEG. In the case of erythrocytes changes of the structure of the glycocalyx accompanied by the rearrangements of charged groups in the membrane surface could also occur.

For vesicles these alterations cannot play a role. Charges are arranged in a smooth surface and a drastic decrease of the ionic strength ought to occur in order to induce changes of the zeta potential from −60 mV to −160 mV as measured for the DMPC/egg-PA vesicles (Fig. 2). Calculations show that the salt concentration would have to decrease from 100 mM to less than 10 mM. The real influence of PEG on the solubility of ions is much smaller [34]. Pratsch and Donath showed that the increase of the zeta potential of erythrocytes can

also be explained by an exclusion of PEG from the erythrocyte surface [18].

On addition of PEG to lipid monolayers a decrease of the surface potential by several hundred millivolts was observed [35]. Since this change of the surface potential was not found for liposomes and erythrocytes we have to assume that the change of the surface potential observed for monolayers results mainly from changes of the orientation of molecular dipoles in the air/water interface.

The theory developed for the description of the influence of PEG exclusion on the electrophoretic behaviour provided the characteristic thickness of the depletion layer. The values obtained were of the order of 1 nm. This length is smaller than the hydrodynamic radius of a PEG 6000 molecule. It has to be taken into account that Eqn. 11 which is used for the calculation of the viscosity profile is a first approximation and that the values of the characteristic thickness have to be used in the context of this theory. The problems of the measurement of the viscosity profiles near surfaces are discussed in Ref. 36.

For the relatively small PEG molecules used in our experiments the steric contributions to the exclusion of the polymer from the surface should not play a role. The thermodynamically unfavoured interaction of PEG with the polar surface of the liposomes could be important as found for proteins [1]. Many of the conclusions about the PEG interaction with soluble proteins should be applicable to liposome surfaces as well, especially the extent of exclusion dependent on the hydrophobicity of the surface, pH, ionic strength and temperature [1].

The physical nature of the repulsive forces of the PEG–phospholipid interaction and the PEG–PEG interaction can be compared with the hydration repulsion observed between surfaces of phospholipid bilayers [5,37]. It is suggested that the depletion effect is significantly influenced by the hydration of the phospholipid surface as well as the hydration of the PEG molecules. Therefore, it is expected that the depletion is decreased if the phospholipid surface becomes more hydrophobic or the hydration of PEG is reduced. It is assumed that the hydration shell around PEG is disrupted for temperatures above the clouding temperature. Indeed, experimental evidence for a binding of PEG 20000 to didocecylphosphate vesicles was given [38]. This binding was explained by a decrease of the clouding temperature of PEG from about 103 °C to 40 °C due to the high concentration of phosphate groups in the vesicle/water interface of this system.

The finding about the exclusion of PEG from phospholipid surfaces is of basic importance for the interpretation of the molecular mechanisms of the PEG-induced aggregation of liposomes, cells and particles as virus and serum lipoproteins [39]. The application of this phenomenon to colloid dispersions was discussed in detail by Napper [40]. To summarize, the results strongly indicate that the PEG-induced aggregation of liposomes and cells is caused by the high osmotic pressure of PEG-water solutions together with a reduced concentration of PEG molecules in the water layer near the membrane surface.

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