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Effect of trehalose in low concentration on the binding and transport of porphyrins in liposome-human serum albumin system

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Abstract

The influence of trehalose on the interaction of liposomes with porphyrins and with human serum albumin (HSA) was studied. Small unilamellar liposomes were prepared from 1,2-dimyristoyl-*sn*-glycero-3-phosphatidylcholine (DMPC) and from DMPC/1,2-dimyristoyl-*sn*-glycero-3-phosphatidylglycerol (DMPG) 19:1 w/w% and incorporated with mesoporphyrin IX (MP) or magnesium mesoporphyrin (MgMP). The fluorescence intensity and anisotropy of porphyrins were measured within the temperature range of 15–33°C, in the presence and in the absence of 3×10^{-2} M trehalose, to study the location of the porphyrins inside the liposomes and their partition between the liposomes and HSA. Based on the presented data and our earlier results (I. Bárdos-Nagy, R. Galántai, A.D. Kaposi, J. Fidy, Int. J. Pharm. 175 (1998) 255–267) we conclude that trehalose – even at this relatively low concentration – interacts with the head groups of the liposomes and that the presence of DMPG enhances the effect. This effect seems to hinder the binding of HSA to the liposome surface and influences the location of MgMP within the liposomes. In the case of MP, the porphyrin partition between the liposomes and HSA was affected by trehalose, while for MgMP, trehalose changed the structural conditions of porphyrin binding to the liposomes. The amount of trehalose used did not have a general trend to modify the association constants of porphyrin derivatives either to liposomes or to HSA.

Keywords: Fluorescence; Human serum albumin; Liposome; Mesoporphyrin; Magnesium mesoporphyrin

1. Introduction

Experimental evidence shows that biologically active molecules maintain their structural stability for a longer time after freeze-drying in the presence of certain carbohydrates [1–3]. It was also shown that mono-, oligo- and polysaccharides are able to preserve the integrity of dehydrated membranes or liposomes, and to protect proteins against thermal denaturation and aggregation [4–12].

From among the studied carbohydrates, α-α trehalose has been found to act as one of the best stabilizers of structure and function in macromolecules, and to be very effective in preventing fusion and aggregation or in reducing leakage of entrapped material from vesicles or membranes during dehydration [13–18]. As a result of this beneficial effect, trehalose is frequently used by the pharmaceutical

Abbreviations: DMF, dimethylformamide; DMPC, 1,2-dimyristoyl-*sn*-glycero-3-phosphatidylcholine; DMPG, 1,2-dimyristoyl-*sn*-glycero-3-phosphatidylglycerol; HSA, human serum albumin; MgMP, magnesium mesoporphyrin IX; MP, mesoporphyrin IX; SUV, small unilamellar vesicle

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industry to increase the stability of macromolecular systems [12,18].

This effect of trehalose has been attributed to its hydrogen bond forming capacity, to trehalose-glass characteristics, to its solvation layer modification effect and to its large hydrated volume as compared to other carbohydrates [9–11,16–21].

Despite the vast number of studies in this field, there are no results in the literature confirming that trehalose-treated biological systems maintain the structural conditions required for their proper functioning in more complex biochemical processes. In this work, we examined whether the presence of trehalose influenced the binding properties or transport characteristics of proteins and liposomes. In our previous studies, we tested the partition of porphyrins between liposomes and human serum albumin (HSA), using a model system that was composed of small unilamellar liposomes (SUV) capable of binding/incorporating free base or magnesium (Mg) containing mesoporphyrin [22,23]. In the present study, we added trehalose to the solutions in a concentration suitable for rehydration [7], and tested its effect on the binding and transport of porphyrins taking advantage of the high fluorescence quantum yield of these molecules. We show that although the binding capability of liposomes is not generally affected by trehalose, the transport of porphyrins from liposomes to HSA and the structural conditions for their incorporation into the liposomes are indicative of an effect on the hydrogen bond network of trehalose with the surface of liposomes.

2. Materials and methods

2.1. Chemicals

1,2-Dimyristoyl-*sn*-glycero-3-phosphatidylcholine (DMPC), 1,2-dimyristoyl-*sn*-glycero-3-phosphatidylglycerol (DMPG), 1× crystallized and lyophilized HSA, mesoporphyrin IX (MP) dihydrochloride and α-α trehalose were purchased from Sigma, and MgMP (disodium salt) from Porphyrin Products (Logan, UT). All solvents (chloroform, methanol, ethanol and dimethylformamide (DMF)) were obtained from Merck. All chemicals in the samples were of spectroscopic grade.

2.2. Preparation of solutions and liposomes

The purification of the protein and the preparation of sonicated SUVs of DMPC and DMPC/DMPG (19:1 w/w) in 10^{-2} M sodium phosphate buffer at pH 7.4 were described earlier [22,23]. The porphyrins were dissolved in organic solvent (due to the different solubility and aggregation problems, we had to use different solvents: DMF for MP, and ethanol for MgMP) and added to the liposomes in a concentration of 5×10^{-8} M to prevent their aggregation even in aqueous phase. Incorporation was completed within 30 min of equilibrium binding. To determine the association constants and to measure the redistribution, HSA concentration was changed between 7×10^{-8} and 6×10^{-5} M. To avoid the problems due to the light scattering of SUVs in optical spectroscopy, the lipid concentration was kept below 10^{-3} M. When trehalose was used, the lipids were hydrated using the same phosphate buffer with 3×10^{-2} M trehalose. This trehalose concentration is in reality 3 times larger compared to the lipids in molar units. In w/w% this sugar-lipid concentration is about 1. Since for the stabilization of liposomes before freeze-drying the applied sugar-lipid w/w ratio is generally larger than 3 [13,14] and the trehalose concentration to stabilize the proteins and liposomes in an aqueous system at low temperature is a few M [1–3], this sugar concentration is relatively low and falls in the concentration range typical of trehalose-treated and freeze-dried/rehydrated liposome solutions [7]. In all experiments the same trehalose concentration was used.

2.3. Equipment

Absorption spectra were recorded on a Cary 4E UV-Visible Spectrophotometer. Fluorescence emission and excitation spectra and fluorescence anisotropy measurements were performed using an Edinburgh Analytical Instruments (Edinburgh, UK) CD900 luminometer equipped with a Xe 75 W light source.

For the fluorescence anisotropy measurements, the *G* factors [24] of the device were determined for each excitation wavelength and temperature using an emission wavelength of 584 nm for MgMP, and 624 nm for MP. Their equal values in the liposome

solutions and in the organic solvents at the same wavelengths and temperatures supported the view that the light scattering caused by the liposomes (10^{-3} M) in the fluorescence measurements was negligible under the applied experimental conditions. The appropriate G factors were used to calculate the anisotropy values at each wavelength and temperature.

The diameter of the liposomes was determined by light scattering measurements, using an ALV goniometer with a 35 mW He-Ne laser light source.

2.4. Determination of the binding constants

The association constants of porphyrins to HSA and to liposomes were determined by following the change of the fluorescence intensity of a 5×10^{-8} M porphyrin solution when titrated by HSA or by different liposomes. The association constants of porphyrins to HSA (K_p) were obtained from Scatchard plots using the following equation:

$$K_p\cdot(n\cdot[P]_{tot}-[HSA]_{bound}) = \frac{[HSA]_{bound}}{[HSA]_{free}} \quad (1)$$

where n equals the number of binding sites of HSA. $[P]_{tot}$, $[HSA]_{bound}$ and $[HSA]_{free}$ represent the total porphyrin, the HSA that binds porphyrin, and the free protein concentration in solution, respectively.

The equilibrium constant (K_L) for porphyrin-liposome binding was evaluated according to the equation:

$$\frac{1}{I-I_0} = \frac{1}{(I_{lip}-I_0)\cdot K_L}\cdot\frac{1}{[lip]} + \frac{1}{I_{lip}-I_0} \quad (2)$$

where [lip] represents the lipid concentration corresponding to the free liposomes in solution, I_0, I and I_{lip} are the fluorescence intensities in the absence and presence of liposomes and for total incorporation, as determined at the wavelength of maximum emission for porphyrins bound to liposomes. The details of the evaluation have been described previously [22,23].

The temperature of the sample was kept at 32°C, except when temperature dependence was studied.

2.5. Partition of porphyrins between liposomes and HSA

To monitor the partition of porphyrins, MP or MgMP completely bound to liposomes (DMPC or DMPC/DMPG) was titrated with HSA, and the fluorescence intensity of porphyrins was recorded stepwise. Assuming that the redistribution of porphyrins is the result of a competition between HSA and liposomes (i.e. the porphyrin-liposome and the porphyrin-HSA complex are formed independently from each other through the aqueous phase), the partition can then be described by the following formula [22,25]:

$$I/I_{lip} = \frac{K_L(1-I_{prot}/I_{lip})}{K_L + K_p\dfrac{[protein]}{[lipid]}} + I_{prot}/I_{lip} \quad (3)$$

K_L and K_p are the equilibrium constants for the binding of porphyrins to liposomes and to protein, respectively, I, I_{prot} and I_{lip} are the fluorescence intensities of porphyrins in the actual step, whilst totally bound to the protein and fully incorporated in liposomes, respectively.

According to Eq. 3, the relative fluorescence inten-

Table 1
The association constant of porphyrins to HSA and to liposomes

Porphyrin	Ligand	K (M^{-1})	
		10 mM pH 7.4 phosphate buffer	30 mM trehalose in 10 mM pH 7.4 phosphate buffer
MP	HSA	$(2.5\pm0.7)\times10^7$ [a]	$(3.5\pm0.6)\times10^7$
	DMPC liposome	$(1.3\pm0.8)\times10^5$ [b]	$(4.0\pm0.8)\times10^4$
	DMPC/DMPG (19:1) liposome	$(3.2\pm0.6)\times10^4$ [a]	$(4.4\pm0.5)\times10^4$
MgMP	HSA	$(1.7\pm0.5)\times10^7$ [a]	$(1.4\pm0.6)\times10^7$
	DMPC liposome	$(1.2\pm0.7)\times10^4$	$(2.9\pm0.8)\times10^4$
	DMPC/DMPG (19:1) liposome	$(1.5\pm0.6)\times10^4$ [a]	$(2.7\pm0.4)\times10^4$

[a] See [22].
[b] See [23].

sity as a function of the [HSA]/[lipid] ratio of the system is hyperbolic and a theoretical curve can be obtained if the association constants and the porphyrin fluorescence intensities whilst totally bound to HSA and fully incorporated in liposomes are known.

3. Results

The absorption and emission spectra of MP and MgMP did not show any difference in the presence of 3×10^{-2} M trehalose compared to the spectra recorded in liposome or in HSA containing solutions in the absence of the sugar. Both the increases in the fluorescence intensity of porphyrins in HSA or in liposomes and the spectral shifts caused by HSA and liposomes remained the same [22].

3.1. Partition of porphyrins between liposomes and HSA

To follow the redistribution of porphyrins between the liposomes and HSA, we determined the equilibrium constants for each system. The association constants are summarized in Table 1. These data show that the amount of trehalose used does not have a general trend to modify binding of porphyrins either to HSA or to liposomes.

On the basis of Eq. 3 we plotted the relative fluorescence intensities as a function of the HSA-lipid concentration ratio for each system. We fitted these data according to the theoretical function of the partition reaction, using the association constants and fluorescence intensities whilst the porphyrins are totally bound to liposomes (I_{lip}) and to HSA (I_{prot}). The MgMP distribution curves obtained from the experimental data and from the calculations show equally good fits for both liposomes in the absence of trehalose (see Fig. 5 in [22]), and a similar fit was also obtained in the presence of trehalose. Fig. 1 shows the experimental data (mean of three independent measurements) and the calculated curves for the MP distribution in the presence and absence of trehalose. The partition of MP without trehalose in the case of the DMPC/DMPG liposome system does not follow the independent partition function as discussed in our previous works [22,23]. In Fig. 1, we compare these earlier data from [22] with those of the present studies. When trehalose is added to the system, the data follow the theoretical curve of independent partition for both liposomes.

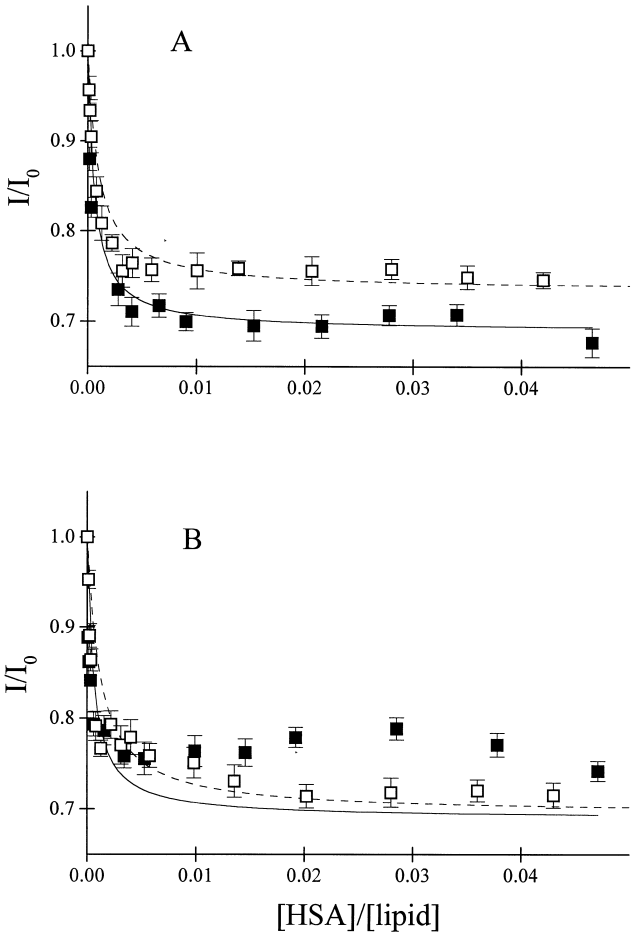

Fig. 1. Relative fluorescence intensity of 5×10^{-8} M MP as a function of the relative molar concentrations of HSA, and phospholipids for DMPC liposomes and HSA (A) and DMPC/DMPG liposomes and HSA (B) in the absence (■) (from [22]) and in the presence of trehalose (□) at 32°C. The theoretical curve corresponds to Eq. 3 with K_L, K_p values and the fluorescence data in the absence (———) and in the presence (- - -) of 3×10^{-2} M trehalose. MP was excited at 399 nm with emission recorded at 625 nm.

3.2. Temperature effect on the fluorescence intensity of porphyrins incorporated into liposomes

As the quantum yield of fluorescence is sensitive to the surroundings of the excited fluorophore, we used the fluorescence intensity to monitor the effect of trehalose on the location of porphyrins within the

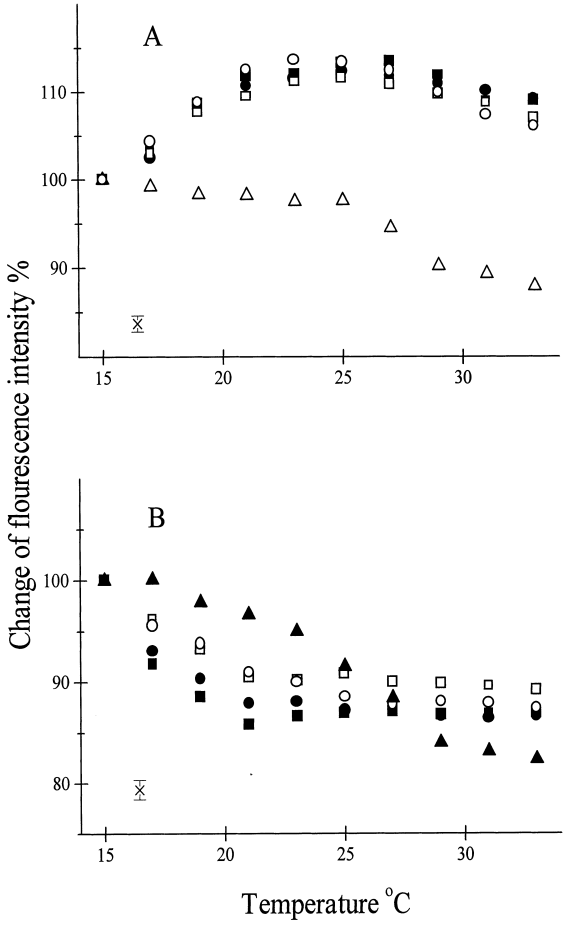

Fig. 2. The relative change of 5×10^{-8} M MP (A) and 5×10^{-8} M MgMP (B) fluorescence intensity as a function of temperature in DMPC (squares) and in DMPC/DMPG liposomes (circles) in the absence (solid symbols) and in the presence (open symbols) of 3×10^{-2} M trehalose and in organic solvents (DMF for MP and ethanol for MgMP) (triangles). (The solvents used for control measurements are different due to the different solubility and aggregation of the porphyrins.) The error bars (LHS) represent the highest standard deviation in the given series of measurements. The excitation wavelength was 399 nm for MP and 410 nm for MgMP, emission was recorded at 623 nm for MP and at 584 nm for MgMP. The fluorescence intensity at 15°C was taken as 100%.

liposomes. According to our previous measurements, the phase transition of the studied SUV liposomes occurs at approx. 22°C [26]. We performed an experiment in which the temperature of the sample was raised from 15 to 33°C by increments of 2°C and measured the fluorescence emission spectra. The excitation wavelength for MP was 399 nm and 410 nm for MgMP in the case of both liposomes. Since the fluorescence intensity depends not only on the environment of the fluorophore but also on the temperature, for comparison and to exclude false conclusions due to temperature dependence, we measured the fluorescence intensity change of both porphyrin derivatives in organic solvents as well.

The increase of temperature caused a gradual red shift (max. 2 nm) in the emission spectrum of MP in the case of both liposomes. In the DMF solution the temperature did not modify the maximum wavelength of MP emission. There was no change in the maximum emission wavelength of the MgMP spectra either in liposomes or in the ethanol solutions. Fig. 2 shows the relative change of fluorescence intensity (with the intensity at 15°C taken as 100%) at 623 nm for MP and 584 nm for MgMP in the organic solvents and in DMPC and DMPC/DMPG liposomes, in the absence and in the presence of trehalose. (Data points are the mean of three independent measurements. The standard deviations were within 3% and are not plotted for clarity's sake.) The change of the fluorescence intensity in the organic solvents is similar for both porphyrins. Increasing temperature results in a decrease in the fluorescence intensity. Below 22°C this decrease is relatively small, only a few percent; above it the intensity suddenly decreases to approx. 80% of the original value.

The change in MP fluorescence intensity in the liposomes as a function of temperature is clearly different from that of MgMP. It is also obvious that the presence of trehalose does not significantly influence the temperature effect in the case of MP incorporation. The fluorescence intensity of MP increases with temperature, and above the liposome phase transition temperature it starts to decrease slightly. In the case of MgMP, the fluorescence intensity decreases as the temperature increases, and the decrease is more pronounced than that in the ethanol solution. This decrease, however, is significantly smaller, especially in the case of DMPC liposomes, when trehalose is present in the solution. Above the phase transition temperature this effect of trehalose becomes negligible.

3.3. Fluorescence anisotropy measurements

Porphyrins incorporated in liposomes are known to show a well measurable fluorescence anisotropy [27–29]. We measured the fluorescence anisotropy

of the porphyrins incorporated in the liposomes to monitor the effect of trehalose on the incorporation process. The porphyrins were excited in the Soret region (in the 396–408 nm range for MP and between 403 and 415 nm for MgMP), the emission wavelength was 624 nm for MP and 584 nm for MgMP. The temperature dependence of anisotropy was measured in the same range as the intensity measurements.

For comparison, we measured the anisotropy of porphyrins in organic solvents (DMF and ethanol) as well. As expected, it was zero in the given temperature interval.

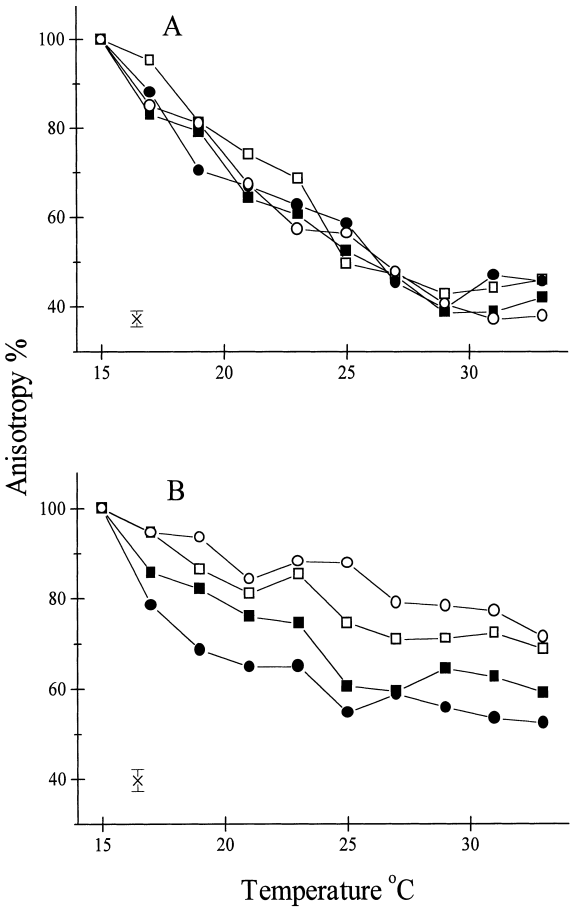

Fig. 3. The relative change of 5×10^{-8} M MP (A) and 5×10^{-8} M MgMP (B) fluorescence anisotropy as a function of temperature in DMPC (squares) and in DMPC/DMPG liposomes (circles) in the absence (solid symbols) and in the presence (open symbols) of 3×10^{-2} M trehalose. The error bars (LHS) represent the highest standard deviation in the given series of measurements. The porphyrins were excited in the Soret band, at 399 nm for MP and at 411 nm for MgMP, with the emission recorded at 624 nm and at 584 nm, respectively. To calculate the r values the appropriate G factors were used at each temperature. The anisotropy at 15°C was taken as 100%.

The anisotropy of porphyrins did not depend on the lipid compositions of the liposomes at 15°C in the given excitation range, but the presence of trehalose reduced its absolute value. The anisotropy, measured for MP at 15°C, with 404 nm excitation in the absence of trehalose yielded $r=0.08$, while in the presence of trehalose under the same conditions it yielded $r=0.06$. A similar effect was observed for the anisotropy of MgMP. At 15°C, with 411 nm of excitation, the r value was 0.10 in the absence and 0.08 in the presence of sugar, in both liposomes. As the optical rotation of the sugar modifies the absolute anisotropy values, the data with and without trehalose are not comparable. To exclude this disturbing effect, we normalized the anisotropy values and considered only their relative changes to draw further conclusions. The change of anisotropy as a function of temperature is shown in Fig. 3. (Data points are the mean of three measurements. The standard deviation was less than 5%; error bars are not shown for clarity's sake.) The anisotropy of MP decreases by approx. 60% until 29°C where it levels off (Fig. 3A). The decrease of MP anisotropy with increasing temperature did not depend on the lipid composition or on the presence of trehalose in the system.

The effect of temperature on the anisotropy of MgMP is not so pronounced as in the case of MP. For the metalloporphyrin, however, the trehalose effect is different (Fig. 3B). The presence of trehalose protects the system against anisotropy decrease with increasing temperature in both liposomes. The effect is markedly stronger in the case of the DMPC/DMPG vesicles.

4. Discussion

The identical spectral properties of MP (the fluorescence intensity enhancement, the magnitude of spectral shift) in the presence and in the absence of trehalose in HSA, DMPC and DMPC/DMPG liposomes suggest that the immediate environment of this porphyrin when bound to HSA or to liposomes is not modified by the disaccharide. Comparing the

association constants (Table 1) in samples with and without trehalose, differences can be observed in the case of the MP, but there is no general tendency in the change. The binding constants for MgMP in the absence and in the presence of trehalose lead to the same conclusion. The spectral characteristics of MgMP, however, are not sensitive enough to draw firm conclusions concerning the changes of the surroundings of this metalloporphyrin [22].

4.1. Effect of temperature

The control measurements in the organic solvents demonstrate the temperature dependence of *fluorescence intensity* for both porphyrins. In the case of MP the tendency of the change in the organic solvent and in the liposome solution is opposite (Fig. 2A), so we can separate the effect of the temperature and the effect of the environment on the fluorescence intensity. For MgMP the decrease of intensity with increasing temperature was observed both in ethanol and in the liposome solution, but the change of relative intensity in the presence of vesicles was larger below the phase transition temperature (Fig. 2B). This suggests that the effect of temperature and the effect of porphyrin surrounding on the fluorescence intensity are superimposed.

The identical change of the relative fluorescence intensity of MP in the samples studied suggests that MP is located at the same position in both liposomes in the presence and in the absence of trehalose, respectively (Fig. 2A). The enhancement of fluorescence intensity and the 2 nm red shift observed with increasing temperature indicate the motion of the porphyrin to the more hydrophobic and deeper segments of the chains [30]. The fact that MP fluorescence is not sensitive to effects influencing the liposome surface (such as the head group composition of lipids or the presence of trehalose) strengthens our previous conclusion that the location of MP is within the deeper, hydrocarbon chains of the liposomes [22,23].

In the case of MgMP, the simultaneous effect of temperature and environment on the fluorescence intensity makes it difficult to draw firm conclusions. The more pronounced decrease in fluorescence intensity in the liposome solutions below the phase transition temperature of vesicles, however, suggests a change of interactions between the head groups of liposomes and MgMP. This might result in moving the porphyrin closer to the hydrophilic surface region of the vesicle compared to metal free MP, as suggested in our previous work [22]. The direction of the fluorescence intensity change for MgMP with increasing temperature (Fig. 2B) is in concert with this model since the increasing disorder makes the environment of the molecules more and more hydrophilic in the region of the head groups. The more hydrophilic surrounding can be the result of either the migration of MgMP toward the surface of the liposomes or the penetration of water due to hydration as a consequence of increasing fluidity of the bilayer, or the superposition of these two effects [31]. Below the phase transition temperature (approx. 22°C for SUVs), the relative change of fluorescence intensity is slightly greater in the case of DMPC liposomes compared to the DMPC/DMPG vesicles. This shows that the structural conditions for binding MgMP are not identical. The presence of trehalose decreases the effect below the phase transition temperature and keeps the intensity higher in DMPC above this temperature. This suggests that the presence of the disaccharide in the system modifies the binding parameters for MgMP, and this modification is mediated by DMPG. The result is an even more hydrophilic environment for the porphyrin.

The *anisotropy* data (Fig. 3) are consistent with the results of the fluorescence intensity measurements. Similarly to the fluorescence intensity measurements, neither the MP anisotropy was influenced by the modifications of the surface of liposomes. The stronger decrease of the relative anisotropy from 15 to 29°C compared to that of MgMP corresponds to a difference in the change of rigidity of the porphyrin environment. This is in concert with the distinct location of the fluorophores within the vesicles. The anisotropy of MP is influenced by the rotational motion and chain isomerism of the hydrocarbon chains inside the bilayer, while the anisotropy of MgMP is only affected by the less significant change of rotational motion of the head groups (about the P-O bond to the glycerol backbone) with increasing temperature [31]. We attribute the fact that the anisotropy of MP does not show a sharp phase transition of the liposome bilayer to the broad phase transition temperature range of SUV liposomes [26].

In the case of MgMP, the anisotropy decreases with temperature up to the phase transition, and becomes constant above it. The change is more pronounced in DMPC/DMPG, an indication that the structural parameters of the binding are different in the two liposomes.

The presence of trehalose has a marked effect on the MgMP anisotropy. The stabilization of anisotropy can be explained in different ways. There is evidence that the presence of sugar (above 0.3 M) might modify the membrane structure after rehydration of freeze-dried vesicles [11,17,32]. In the case of our experiments the trehalose concentration was 10 times smaller, and there was no drastic dehydration of the membrane either. We conceive that under the applied experimental conditions, this effect of the trehalose can be excluded.

Another possible explanation of the observed change might be the modification of the membrane head group hydration shell. There are experiments supporting the view that the presence of trehalose can cause osmotic dehydration of uni- and multilamellar liposomes, respectively, resulting in an increase in the main phase transition temperature of the vesicles. This effect, however, weakens with decreasing sugar concentration, and was not observed below 0.5 M of trehalose concentration [5,13]. We concluded to also rule out this factor as a possible explanation.

Timasheff et al. interpret the protein stabilizing effect of trehalose and other sugars as a result of preferential interaction (preferential exclusion or preferential binding) [21,33]. The formation of such contacts does not imply any kind of specific interaction at sites of the sugar to the protein molecule. We cannot exclude preferential binding of trehalose to the liposome surface in our system, but concerning the concentration dependence of this effect (in the cited experiments the trehalose concentration varied from 0.1 to 0.9 M, and the measured preferential binding parameter was very small at the lower sugar concentrations), we do not believe that the 20% difference in MgMP anisotropy is due to this weak effect.

We can interpret our observation as a result of a stronger effect like the hydrogen bond formation of trehalose, which might enhance the rigidity of the liposome head group region. The existence of hydrogen bonds is also supported by NMR and FTIR studies in dried and hydrated form of the liposomes [9,10,14]. Although in our experiments the trehalose concentration was one or two orders lower than the one usually used, we assume that, even at these conditions, trehalose can coordinate to the membrane surface, and reduces the rotational motion of head groups.

The molar ratio of water and trehalose in our system was about 500. The experimental concentration of lipids was 10^{-3} M, from which we can estimate the liposome concentration using the average liposome diameter (50 nm) obtained by light scattering and the molecular size of the phospholipids. We get an approx. 4×10^{-8} M liposome concentration, thus the trehalose concentration is some six orders of magnitude larger. From these water/trehalose and trehalose/liposome concentration ratios, and on the basis of the relatively large partial negative charge of the oxygen atoms of the head groups which makes them a good target for binding, it seems probable that the trehalose may form hydrogen bonds on the surface of the liposomes [34]. In addition, the propionate side chains of porphyrin can behave as H-bond acceptors when the fluorophore is close to the surface of the liposomes. The stabilizing effect of hydrogen bonds would explain the much less pronounced temperature effect on the anisotropy of MgMP in the presence of the disaccharide. Comparison of the effect on the two types of liposomes shows that the presence of DMPG in the liposome is a mediator of this stabilization, which is not surprising since the ability of the phosphatidylglycerol head groups to form hydrogen bonds is well known [34]. Its presence alone, however, is not sufficient to fix the orientation of excited state MgMP; the presence of trehalose is also necessary.

4.2. *Effect of trehalose on the partition of porphyrins*

It is well known that molecules bound by liposomes close to their head groups may modify the interaction of the vesicles with other compounds, such as proteins for example [23,26,34–40]. In an earlier study [22], we showed that MgMP incorporated near the liposome surface is able to prevent the binding of HSA to liposomes. It is obvious accord-

ingly that the effect of trehalose on the redistribution of MgMP could not be detected in this system.

The partition of the free base porphyrin between the DMPC/DMPG liposomes and HSA deviates from the model described by Eq. 3 in the absence of trehalose (Fig. 1). This result proposed to take into account a kind of interaction between the liposome surface and HSA in the case of negatively charged liposomes [23]. The inhibiting effect of HSA on the MP redistribution disappears in the presence of trehalose (Fig. 1). This supports the assumption that trehalose is at least partly bound to the surface of the liposomes. We believe that the hydrogen binding capacity of the surface is monopolized by the sugar, which makes the binding conditions unfavorable for the protein.

In conclusion, this work shows that trehalose – even at relatively low concentration – may bind to the surface of DMPC and DMPC/DMPG liposomes. In partition phenomena that involve the interaction of lipid membranes and proteins, this effect may significantly modify the transport properties of these particles.

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