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The influence of lipid composition and lectin-glycophorin interaction on the rotational diffusion of glycophorin in vesicles, as measured by time-resolved phosphorescence depolarization

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The rotational mobility of glycophorin in various lipid vesicles was studied, using time-resolved measurements of the depolarization of laser flash excited phosphorescence of glycophorin labelled with the triplet probe erythrosin. With the exception of dimyristoylphosphatidylcholine at the phase transition no phosphorescence depolarization decays were observed in the 1–300 μs time interval following the laser flash. Instead, a constant anisotropy level was observed, with two distinct values depending on the experimental system. In liquid-crystalline bilayers of dioleoylphosphatidylcholine, bovine brain phosphatidylserine and dimyristoylphosphatidylcholine, the anisotropy was 0.01. This was increased to 0.03 upon addition of wheat germ agglutinin which aggregates glycophorin. In the case of gel state dimyristoylphosphatidylcholine and liquid-crystalline dioleoylphosphatidylethanolamine the anisotropy also amounted to 0.03. Experiments with glycerol to vary the viscosity of the medium, and theoretical considerations, exclude the possibility that these different anisotropy levels are related to differences in motional properties of the entire protein/lipid vesicles. These results strongly suggest that the anisotropy level of 0.03 corresponds to slowly rotating glycophorin (rotational relaxation time > 0.3 ms) while the anisotropy level of 0.01 corresponds to fast rotating glycophorin (rotational correlation time < 1 μs). The difference in glycophorin mobility is discussed in terms of aggregation state of the protein, lipid composition of the vesicle bilayer and membrane viscosity. The observed differences in rotational mobility of glycophorin in glycophorin/dioleoylphosphatidylcholine vesicles, glycophorin / bovine heart phosphatidylserine vesicles as compared to glycophorin / dioleoylphosphatidylethanolamine vesicles are not in quantitative agreement with the relative size of the intramembrane particles in these systems as revealed by freeze-fracture electron microscopy.

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Abbreviations: DOPC, 1,2-dioleoyl-*sn*-glycero-3-phosphocholine; DOPE, 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine; DMPC, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine; DSC, differential scanning calorimetry; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine.

Introduction

Interactions between lipids and proteins play important roles in biomembrane structure and function. To gain detailed insight into the molecular aspects of such interactions model systems containing the protein of interest are commonly studied. Glycophorin, the major sialoglycoprotein of the human erythrocyte membrane [1] is suitable

for such studies, since this intrinsic membrane protein is chemically well characterized [2–4], it can be purified in sufficient quantities with very little contamination by native erythrocyte lipids [5] and it can be incorporated in unilamellar vesicles without the use of detergents [6]. In such reconstituted systems, glycophorin greatly affects both the static and dynamic properties of the lipid bilayer.

Influences on lipid packing are indicated by differential scanning calorimetry [7] and by Raman studies which show that incorporation of glycophorin in a gel state PC bilayer causes an increase of acyl chain mobility and disrupture of interchain lateral interaction [8,9]. Glycophorin can also influence the macroscopic organization of the lipids since glycophorin is able to stabilize PE molecules, which at physiological temperature adopt the hexagonal (H_{II}) phase, into a bilayer organization [5].

These interactions, described above, are caused by interaction of the hydrophobic membrane spanning fragment of the protein as suggested by differential scanning calorimetry measurements [10]. Possibly, the hydrophilic sugar containing part of the protein glycophorin may also play a role in glycophorin-lipid interactions [11]. Glycophorin is also able to disturb the lipid dynamics of the membrane, since incorporation of glycophorin induces an enhanced transbilayer movement of PC [12] and lyso-PC [13]. This effect is accompanied by an increased bilayer permeability [7,14] which is strongly lipid dependent [14,15].

That the aggregation state of glycophorin is dependent on the type of lipid used in the reconstitution is suggested by freeze-fracture electron microscopy on glycophorin-lipid vesicles. Glycophorin/phosphatidylcholine and glycophorin/phosphatidylserine vesicles show, on the fracture faces, particles with a diameter of 40–50 Å [16], while in contrast glycophorin/phosphatidylethanolamine vesicles show particles with a diameter of about 100 Å [5]. If the size of these particles is related to the aggregation state of the protein, then it can be expected that the rotational diffusion of the protein aggregate will differ in these different lipid systems, since the rotational mobility is inversely proportional to the square of the radius of the cylinder which models the transmembranous segment of the membrane protein or aggregate [2].

In order to investigate whether the rotational mobility of the protein is dependent on the lipid composition and physical state of the bilayer, we report in this study phosphorescence depolarization measurements of erythrosin-labelled glycophorin incorporated in different lipid systems. This technique has already been successfully applied to the study of the rotational mobility of different membrane proteins in both biological and model membranes [18–21].

It will be shown that the gel to liquid-crystal phase transition of DMPC and the interaction with wheat germ agglutinin greatly affects the rotational mobility of the protein. Furthermore, in the liquid-crystalline state the rotational diffusion of glycophorin in PE is much slower than in PC and PS.

Experimental

Reagents

1,2-Dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) and 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC) were synthesized according to established methods [23]. 1,2-Dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE) was prepared as described before [22]. Phosphatidylserine was purified from bovine brain according to a procedure which will be described elsewhere. All phospholipids were pure as judged by one spot on high performance thin-layer chromatography using the appropriate solvent systems. Glycophorin was purified from human erythrocytes according to the method of Ref. 5. Erythrosin-5-isothiocyanate was purchased from Molecular Probes Inc. Wheat germ agglutinin was obtained from Boehringer Mannheim and neuraminidase from Koch-Light Ltd. (U.K.). Trypsin (type X1, diphenyl carbamyl chloride treated to remove chymotrypsin activity) and *N*-acetyl-D-glucosamine were from Sigma. Sephadex G-25 medium was purchased from Pharmacia. All other chemicals were of analytical grade.

Labelling of glycophorin

Glycophorin was labelled with erythrosin according to the procedure of Ref. 21. Glycophorin (0.1 μmol, 5.2 mg) and erythrosin-5-iso-

thiocyanate (0.1 μmol) were dissolved in 1 ml 0.1 M $Na_2B_4O_7$ (pH = 8.0). After 2 h incubation, in the dark, at room temperature, the pH of the mixture was adjusted with concentrated HCl to pH = 7.0. In order to separate non-covalently bound erythrosin-5-isothiocyanate and labelled glycophorin, the mixture was eluted twice with the same buffer over a Sephadex G-25 medium column (equilibrated in 0.1 M $Na_2B_4O_7$ (pH = 7.0); 6 ml wet bed volume) at room temperature in the dark. The final void volume fraction (3 ml) was dialyzed overnight at 4°C, in the dark, against 5 litres distilled water, and freeze-dried. In this way, a complete separation between erythrosin-5-isothiocyanate and labelled glycophorin was obtained. By reaction with glycophorin the absorbance maxima of erythrosin-5-isothiocyanate shifted 10 nm, from 530 to 540 nm. The concentration of bound erythrosin was determined by reading the absorbance of erythrosin glycophorin in 0.1 M $Na_2B_4O_7$ (pH = 7.0) at 540 nm and comparing the value with absorbances of standard solutions of erythrosin-5-isothiocyanate under the same conditions. This labelling procedure resulted in 0.8 mol erythrosin/mol protein.

Incorporation of glycophorin in lipid vesicles

Glycophorin was incorporated into unilamellar lipid vesicles by the method of MacDonald and MacDonald [6]. A mixture of 0.65 mg erythrosin-labelled glycophorin and 5 μmol lipid (lipid/glycophorin, mole ratio 400 : 1) was dissolved in chloroform/methanol/water (150 : 75 : 1, v/v/v) and dried by evaporation for at least 2 h at high vacuum. The mixed glycophorin lipid film was resuspended in 0.5 ml 100 ml NaCl, 0.2 mM EDTA, 0.2 mM NaN_3, 10 mM Tris-HCl (pH 7.4) at 0°C (in the case of DOPC and DOPE) or 30°C (in the case of bovine brain PS and DMPC). The formed vesicles were centrifuged, first for 10 min at $10000 \times g$ (at 4°C) in order to remove large structures containing little or no protein [7]; then the supernatant was centrifuged at $37500 \times g$ for 30 min (at 4°C) to collect the unilamellar glycophorin-containing vesicles. In order to remove non-incorporated labelled glycophorin, the pellet of these vesicles was washed twice by resuspending in 1 ml in cold buffer followed by centrifugation $37500 \times g$ for 30 min. In the case of DOPC, DMPC and bovine brain PS the vesicles have a 400 : 1 phospholipid/protein molar ratio, in agreement with previous studies with unlabelled glycophorin [7]. The glycophorin/DOPE vesicles had a lipid to protein molar ratio of 25 : 1, also in agreement with studies with unlabelled glycophorin [5].

Permeability measurements, by means of an efflux assay [14,15] showed that the glycophorin/DOPC, glycophorin bovine brain PS and glycophorin/DMPC vesicles have a very high permeability for glucose, and the glycophorin/DOPE vesicles are only slightly leaky for glucose. These permeability characteristics were found to be identical for labelled and unlabelled glycophorin/lipid vesicles. The morphology of labelled and unlabelled glycophorin/lipid vesicles as revealed by freeze-fracture electron microscopy was also identical. In the case of glycophorin/DOPC, glycophorin/bovine brain PS and glycophorin/DMPC vesicles on the fracture faces small (40–50 Å diameter) particles were found, while glycophorin/DOPE vesicles showed larger (about 100 Å diameter) particles [5,16].

Measurement of phosphorescence depolarization

The apparatus used the T-format geometry described previously, wherein two photomultipliers on opposite sides were equipped with polarizer filters orientated parallel (∥) or perpendicular (⊥) to the plane of polarization of the laser flash [19,21]. The dye-laser (model LN 102, Nitromite laser model LN 100, PRA Photochemical Research Associates Inc.) was operated with 0.15 mM Coumarin 6 in methanol and had a repetition rate of about 15 Hz. The laser flash duration was 300 ps. An extra-cavity Glan-Taylor prism, which could be rotated, polarized the laser output to the sample. The output energy received at the sample was 10–20 μJ per flash at 540 nm. Photomultiplier tubes were protected by a red-pass filter (Schott R6 695, 3 mm thick) and additional filters (Schott KV 550, 3 mm thick) placed between the sample and each of the red pass filters. The signals coming from the photomultiplier tubes were averaged by a Nicolet model 1170 signal averager, giving 12-bit resolution and a minimal address time of 1 μs. For each experiment about 300 traces were averaged. The sample (usually 1 ml with 10^{-6} M probe) was deoxygenated with

argon (10 min at room temperature in the dark) prior to phosphorescence measurements. The closed sample tube was immersed in water in a small Dewar flash in the laser-flash apparatus. The water temperature was adjusted with ice or heating, and measured with a thermistor and meter.

Results

Analysis of anisotropy decay curves

Before proceeding to the experimental data, it is first necessary to consider the form that such data might take. For a randomly orientated suspension of membranes in a photoselection experiment, the time-dependent anisotropy parameter $r(t)$ is defined as [24]:

$$r(t) = \frac{S_{\parallel}(t) - S_{\perp}(t)}{S_{\parallel}(t) + 2S_{\perp}(t)} \quad (1)$$

where $S_{\parallel}(t)$ and $S_{\perp}(t)$ are the measurement signals made in polarization planes either parallel ($\parallel$) or perpendicular ($\perp$) to the plane of the photoselection flash, and at time t after that flash. If D is the uniaxial rotational diffusion coefficient for rotational diffusion about an axis normal to the plane of the membrane, then [25]:

$$r(t) = A_1 \exp(-Dt) + A_2 \exp(-4Dt) + A_3$$

where A_1, A_2 and A_3 are variables dependent on the orientations of the dipole moments used for photoselection and measurement. Because these orientations are not usually known, and may be environmentally sensitive in the case of phosphorescence emission, it follows that the analysis of phosphorescence anisotropy decay curves is complex. For example, there are three sets of circumstances where the measured anisotropy may appear to be time independent. Firstly, and least likely, the dipole moment orientations may be such that the two experimental terms in Eqn. 2 are zero, leaving $r(t)$ to be equal to the time independent term A_3. Secondly, if the time resolution of the photoselection flash or measuring system is slow compared to the rotational relaxation time under study, then only the time-independent term A_3, the so-called residual anisotropy or r_∞ value, will be detected. Thirdly, if the molecules under study do not undergo significant rotational diffusion during the lifetime of the photoactivated state used for photoselection, then $r(t)$ will remain as its zero-time value, r_0.

We expect that the last two sets of circumstances would obtain in this present work. In the case where glycophorin was embedded in lipid in the gel-phase, the rotational relaxation time was expected to be very much greater than the triplet lifetime of erythrosin. On the other hand, because the polypeptide chain of glycophorin crosses the lipid bilayer only once [2], it was anticipated that the rotational relaxation time of glycophorin embedded in liquid crystal phase lipid would be no more than 1–2 μs. This latter expectation has previously been realized: Jovin et al. [18] reported that the rotational relaxation time of erythrosin labelled glycophorin reconstituted into DMPC vesicles and measured above the lipid phase transition was in the range 1–2 μs. This is too fast to be resolved by the digital data collection system used in our equipment, where each channel was sampled for every other microsecond.

At 0°C, the half-time for the phosphorescence decay of erythrosin-labelled glycophorin dissolved in 90% (w/w) glycerol was about 200 μs, falling to 160 μs for erythrosin-labelled glycophorin incorporated in DOPC vesicles. For all systems tested the decay was faster at higher temperature. At 0°C we were able to measure anisotropy of the phosphorescence extending to 500 μs after the laser flash, falling to 300 μs at 30°C.

Influence of the physical state of phospholipid on the rotational diffusion of glycophorin in vesicles

Fig. 1 shows recordings of phosphorescence anisotropy measurements made over the period 0–500 μs following laser-flash photoselection of erythrosin-labelled glycophorin incorporated into DMPC vesicles. Below the gel-to-liquid crystalline phase transition [23°C, [26]) the anisotropy parameter r was time-independent with a value of approx. 0.03. Between 21 and 25°C the initial anisotropy parameter r_0 fell dramatically, and at 30°C was 0.01 or less (Fig. 2). Our interpretation is that at the gel-to-liquid phase transition, the rotational diffusion of erythrosin-labelled glycophorin changed from being too slow to measure (anisotropy time-independent for 500 μs) to being too

fast to measure (rotational relaxation time < 1–2 μs). However, at 22°C, just below the gel-to-liquid crystalline transition temperature, we consistently

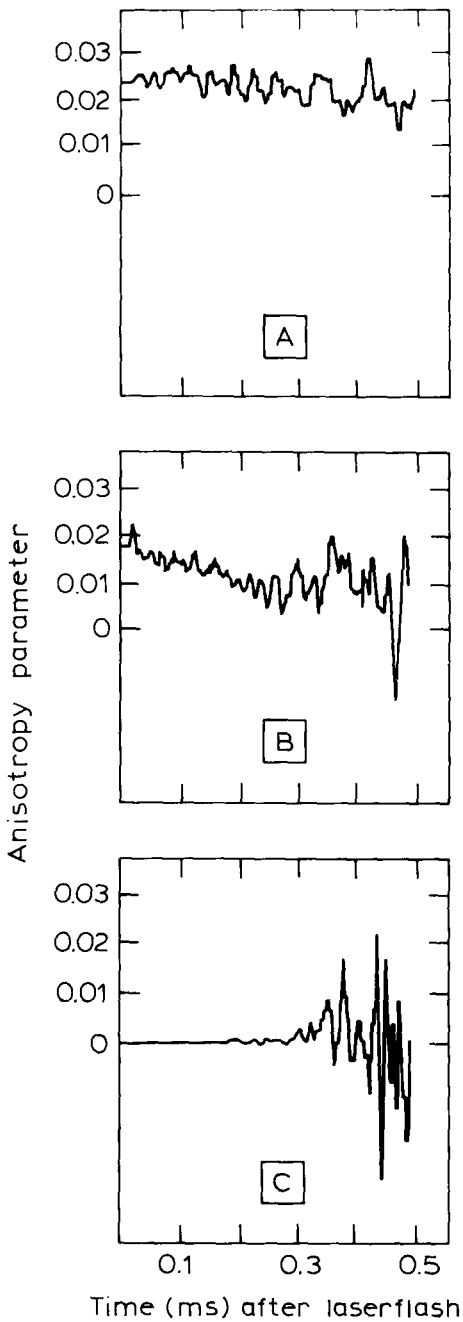

Fig. 1. Phosphorescence anisotropy traces of erythrosin-labelled glycophorin reconstituted in DMPC vesicles. (A) At 0°C; (B) at 22°C; (C) at 30°C. Reconstitution of glycophorin and measurement of phosphorescence depolarization are described in Experimental.

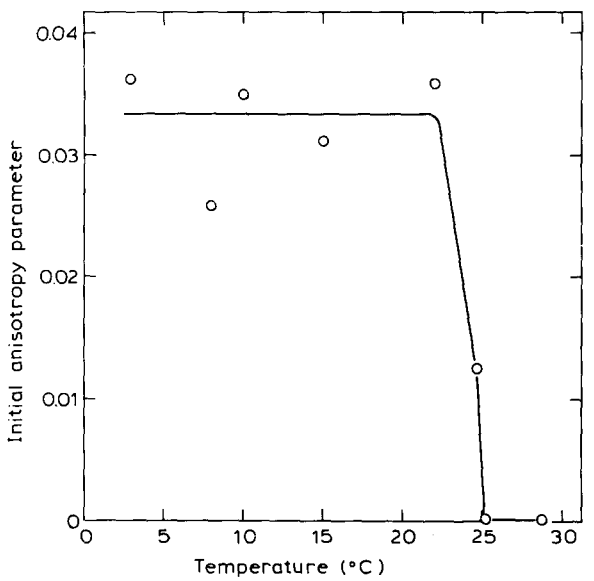

Fig. 2. Initial anisotropy of phosphorescence depolarization traces of erythrosin-labelled glycophorin reconstituted in DMPC vesicles, as a function of temperature. Reconstitution of glycophorin and measurement of phosphorescence depolarization are described in Experimental.

observed a slow but incomplete decay of anisotropy extending over 100–200 μs. A different temperature dependency was described by Vaz et al. [27], who showed that the lateral diffusion coefficient of fluorescein labelled glycophorin in DMPC liposomes changed 200-fold at 15°C, some 9 degrees C beneath the transition temperature of the lipid. However, it should be noted that these experiments [27] involved pretreatment of the vesicles by drying and rehydration to obtain large multilayered vesicles. Jovin et al. [18] have previously described the effects of temperature on the rotational diffusion of glycophorin incorporated into DMPC vesicles. Our own results are in agreement broadly with theirs, despite the use of different molar lipid-to-protein ratios.

Lipid dependency of the rotational mobility of glycophorin

We made time-resolved measurements of phosphorescence anisotropy on vesicles reconstituted from erythrosin-labelled glycophorin and DOPC, DOPE and bovine brain PS as phospholipids, over the temperature range 0–25°C. The results are summarized in Fig. 3 and Fig. 4. As with DMPC,

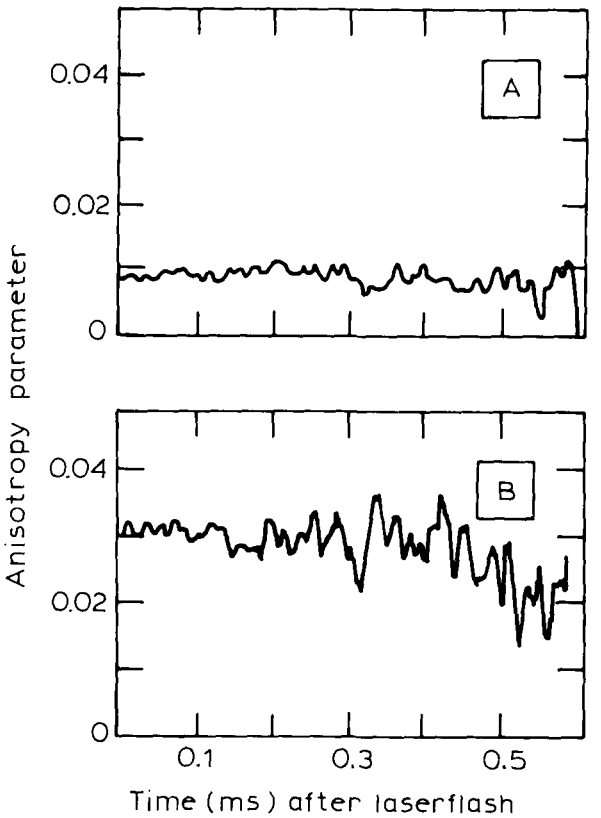

Fig. 3. Phosphorescence anisotropy traces at 0°C of erythrosin labelled glycophorin reconstituted in DOPC vesicles (A) or DOPE vesicles (B). Reconstitution of glycophorin and measurement of phosphorescence depolarization are described in Experimental.

we found that the anisotropy was generally time independent, and was either high or low. For DOPE, the anisotropy was high ($r = 0.03$) and similar to that observed with gel-phase DMPC, despite the fact that DOPE was above its transition temperature. For DOPC, the anisotropy was low ($r \leq 0.01$) and similar to that observed with liquid-crystal phase DMPC. If we assign the higher anisotropy values to immobile glycophorin and the lower anisotropy to mobile glycophorin, then the contrasting results with DOPE and DOPC show how phospholipid head-group can affect the rotational mobility of a membrane protein (see below).

The anisotropy for erythrosin-labelled glycophorin in bovine brain PS was 0.015 at temperatures below 5°C, and fell to 0.008 for temperatures above 10°C. This behaviour reflects that of bovine brain PS which has a gel-to-liquid crystal transition temperature in the range 0–10°C [28]. Incubation of glycophorin/bovine brain PS vesicles with 2 mM or 10 mM $CaCl_2$ for 30 min at 37°C caused the anisotropy, measured at 20°C, to increase from 0.008 to 0.035. This effect, presumably

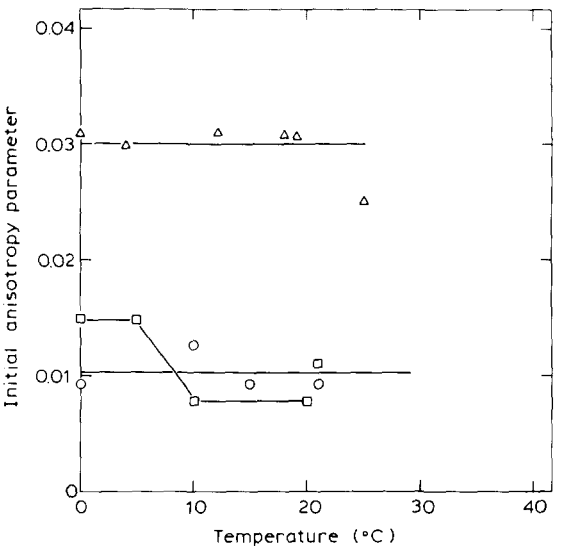

Fig. 4. Initial anisotropy of phosphorescence depolarization traces of erythrosin-labelled glycophorin reconstituted in DOPC (○——○), bovine brain PS (□——□) or DOPE (△——△) as function of temperature.

TABLE I

EFFECT OF ADDITION OF WHEAT GERM AGGLUTININ ON THE INITIAL PHOSPHORESCENCE ANISOTROPY OF VARIOUS GLYCOPHORIN/LIPID VESICLES

Glycophorin-lipid vesicles were incubated during 10 min at 37°C in the dark with a 10-fold molar excess of wheat germ agglutinin (WGA) to glycophorin prior to the phosphorescence depolarization measurements. Phosphorescence depolarization measurements were performed at 0°C for glycophorin/DOPC and glycophorin/DOPE vesicles and at 15°C for glycophorin/bovine brain PS vesicles and at 30°C for glycophorin/DMPC vesicles. The error in the anisotropy values was estimated to be about 0.005.

Lipid composition of glycophorin/lipid vesicle	r_0	
	−WGA	+WGA
DOPC	0.011	0.031
DOPE	0.031	0.031
Bovine brain PS	0.015	0.034
DMPC	0.010	0.050

arose from interaction of PS headgroups with Ca^{2+} leading to an increased lipid packing [29].

Effects of wheat germ agglutinin and other treatments

Glycophorin has a high content of *N*-acetylglucosamine and sialic acid. Accordingly it binds wheat germ agglutinin which is specific for these carbohydrate residues [30–33]. The effects of wheat germ agglutinin on phosphorescence anisotropy are summarized in Table I, from which it can be concluded that if anisotropy was low ($\simeq$ 0.010) in the absence of wheat germ agglutinin, then addition of wheat germ agglutinin increased the anisotropy to 0.030 or higher. However, wheat germ agglutinin did not increase the anisotropy if it was already high (0.03 or more). The simplest interpretatoin of these results is that wheat germ agglutinin will aggregate and therefore immobilize those glycophorin molecules which were not already immobilized, at least on the timescale of our measurements.

The possibility that rotational diffusion of lipid vesicles contributed to phosphorescence depolarization was explored by comparing the phosphorescence anisotropy of glycophorin vesicles suspended either in aqueous media or with the addition of 50% (w/w) glycerol. The viscosity of 50% (w/w) aqueous glycerol is 14.6-times that of water at 0°C and 6.0 times that of water at 20°C. Phosphorescence anisotropy was unaffected by 50% (w/v) glycerol over the range 0–20°C, so it is unlikely that vesicle tumbling was affecting our results.

Discussion

Phosphorescence depolarization measurements on erythrosin labelled glycophorin-lipid vesicles showed in general two phenomena. Firstly an anisotropy level of 0.01 or less was obtained for glycophorin in liquid crystalline DMPC, DOPC and bovine brain PS vesicles. Secondly an anisotropy level of 0.03 was obtained for glycophorin in liquid-crystalline DOPE, gel state DMPC and liquid-crystalline DOPC, after addition of wheat germ agglutinin. A similar initial anisotropy level was observed in liquid crystalline bovine brain PS after addition of wheat germ agglutinin or Ca^{2+}.

Depending on the orientations of the dipole moments used for photoselection and measurement (see Eqn. 2) the initial anisotropy can be anywhere between 0.4 and -0.2 [34,41], which might be an explanation for the low maximal initial anisotropy value of 0.03, as observed in this study. The absence of anisotropy decay from a higher value to 0.03 might be caused by motions of the probe which are faster than the time-resolution limit (2 μs) of the instrument and therefore not detected. Such probe motions can arise from: (i) free but limited motions of the phosphorescence probe at its site of attachment, which is expected to occur on a nanosecond time scale [35]; (ii) motion of the segment or domain of protein carrying the probe (segmental motion) which may occur in as little as 33 ns [36]; (iii) rocking of the whole protein in the lipid bilayer [37], which is possibly in the same time domain as rotational diffusion; (iv) tumbling of the glycophorin/lipid vesicles; (v) lateral diffusion of probe carrying protein around a curved bilayer. The rotational diffusion coefficient D_r arising from tumbling of spherical vesicles is given by the Stokes-Einstein relation $D_r = kT/8\pi a^3\eta$ where a is the radius of the sphere and η the viscosity of the aqueous medium. The corresponding rotational relaxation time τ_{rot} is $1/6\ D_r$. For glycophorin-DOPC vesicles (radius 50–250 nm), τ_{rot} ranges from 128 μs to 16 ms, whereas for the smaller glycophorin/DOPE vesicles (radius 15–75 nm) τ_{rot} ranges from 3 μs to 375 μs. These calculations suggest that vesicle tumbling could cause decay of anisotropy, particularly with the smaller DOPE vesicles. Nevertheless, several arguments oppose vesicle tumbling as a possible source for anisotropy decay. Firstly, the presence of distinct anisotropy levels which would not be present if isotropic motion caused the anisotropy decay; secondly, the lack of influence of increase of solvent viscosity on the observed anisotropy level, which is in agreement with a previous study on glycophorin/DMPC vesicles [18]; thirdly, the fact that the smaller vesicles (glycophorin/DOPE, 150–750 Å radius) show a higher anisotropy level (0.03) as compared to the larger glycophorin/DOPC vesicles (0.01) (500–2500 Å radius) [16], and fourthly, clustering of the glycophorin/DOPE vesicles, by addition of wheat germ agglutinin [5] does not increase the anisotropy level (Table I).

Anisotropy decay can also arise from lateral diffusion in the membrane plane of a vesicle. For instance lateral diffusion around a spherical vesicle results in an apparent rotational relaxation time τ_{rot} given by the relation of $\tau_{rot} = a^2/6D_L$ [38]. Vaz et al. [27] reported that D_L for glycophorin in DMPC vesicles is $2 \cdot 10^{-8}$ cm$^2 \cdot$ s^{-1} in the liquid-crystal phase, falling to $< 5 \cdot 10^{-11}$ cm$^2 \cdot$ s^{-1} below 15°C. Taking these values for D_L, the apparent rotational times arising from lateral diffusion could be as little as 14 μs for liquid-crystal phase vesicles of radius 15 nm (e.g. the smallest glycophorin/DOPE vesicles) or as great as 2 s or more for gel phase vesicles of radius 250 nm. For vesicles, in which DOPC above 0°C is in the liquid-crystalline state, having a radius 50–250 nm, τ_{rot} would be in the range from 2.0 to 52 μs. None of the possible apparent rotational relaxation times arising from lateral diffusion were short enough to cause phosphorescence depolarization too fast for detection. Accordingly we attribute the higher anisotropy values ($r = 0.03$) to immobile glycophorin, and the lower values ($r = 0.01$) to rotationally mobile glycophorin that has dissipated its anisotropy to the limit of its residual value (i.e., the A_3 term of Eqn. 2) within 1–2 μs following the laser flash. This interpretation, taken in conjunction with the results of Figs. 1–4, leads to the conclusion that the rotational diffusion of an integral membrane protein can be strongly influenced not only by the gel to liquid-crystal transition [39] but also by the nature of the phospholipid headgroup.

Comparison of the rotational relaxation time of glycophorin in DOPC vesicles (< 1 μs) and glycophorin in DOPE vesicles (> 300 μs) with the observed protein particle size, as revealed by freeze-fracture electron microscopy (in DOPC, 40–50 Å; in DOPE about 100 Å diamater; [5,16]) suggest that differences in the size of the protein particle is not the only factor responsible for the differences in rotational mobility between the two lipid species. This is because an increase of particle diameter by 2–3-fold would be expected to increase the rotational relaxation time by a factor of 4–9 [17], whereas the observed increase was at least 100-fold. Alternative factors which additionally may explain the low rotational mobility of glycophorin in DOPE vesicles as compared to DOPC vesicles are the presence of hydrogen bonds between the phosphatidylethanolamine headgroup and the protein, and the absence of such interactions in the glycophorin/PC bilayer. As previously suggested, the difference in hydrogen bond formation, might explain to a certain extent the lower permeability of glycophorin/DOPE vesicles as compared to glycophorin/DOPC vesicles [15].

Another explanation for the differences in rotational diffusion of glycophorin in these different lipid systems might be related to the protein concentration in the membrane. It has been suggested that the membrane viscosity increases upon increasing the membrane protein concentration [40]. Since glycophorin/DOPE vesicles have a higher protein/lipid ratio than glycophorin/DOPC vesicles, the decreased glycophorin mobility in DOPE vesicles might be partially caused by an increased membrane viscosity.

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