Synergistic effects of Ca\(^{2+}\) and wheat germ agglutinin on the lamellar-hexagonal (H\(_{II}\)) phase transition of glycoporphin-containing egg-phosphatidylethanolamine membranes

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Glycoporphin has been reconstituted into egg phosphatidylethanolamine (egg-PtdEtn) membranes. Stable vesicles were obtained at a molar ratio of 8 \(\times 10^{-2}\) of inserted protein/lipid. This macroscopic change from lipid aggregates to lipid vesicles was followed by density gradient centrifugation. Vesicles formed in the presence of protein enclose the dye calcein and are stable with time and temperature. Membrane aggregation does not occur, as was demonstrated by energy-transfer experiments. The phase transition from the fluid lamellar L\(_{a}\) phase to the inverted hexagonal H\(_{II}\) phase observed at 29°C in pure egg-PtdEtn membranes is suppressed and finally disappears in the presence of glycoporphin. The transition enthalpy decreases linearly from \(\Delta H = 4\) kJ/mol in pure lipids to zero at a protein/lipid molar ratio of 1:1000. Ca\(^{2+}\) ions and wheat germ lectin act synergistically on the phase behavior of vesicles containing glycoporphin and phosphatidylethanolamine. Differential scanning calorimetry scans show that the lamellar-to-hexagonal phase transition is reinduced. The membranes aggregate and exchange lipid as could be demonstrated by energy transfer experiments. The dye calcein is released but only if the temperature exceeds the lamellar-to-hexagonal phase transition temperature of the pure egg-PtdEtn.

During the last decade considerable attention has been focussed on nonbilayer structures in reconstituted and in biological lipid membranes. Nonlamellar structures, such as the inverted hexagonal (H\(_{II}\)) phase, are thought to play an important role in membrane processes like membrane fusion, membrane contact, transblayer transport, vesicle stability and also protein function [1-5]. There are certain naturally occurring lipids, particularly phosphatidylethanolamines, that assume the hexagonal arrangement under certain conditions on dispersion in water. The polymorphic phase change from the lamellar to the hexagonal membrane structure is a function of the lipid composition [6], lipid chain length [7, 8], pH and divalent cation concentration [9], degree of hydration [10] and temperature as well as pressure [11]. These inverted intrabladder micelles, visible as particles and pits in freeze-fracture micrographs [12], have been investigated on a molecular basis by the anisotropy of the phospholipid \(^{31}\)P-NMR signal [13], by \(^{2}\)H-NMR [14, 15] and by infrared spectroscopy [16]. The macromolecular properties of the H\(_{II}\) phase and the lamellar-to-hexagonal phase transition were detected by differential scanning calorimetry (DSC) [8, 17, 18] and X-ray diffraction [8, 19-21]. A fluorimetric detection of the bilayer-to-hexagonal phase transition in diluted membrane suspension has been introduced which allows the analysis of the transition kinetics [22]. A recent review summarizes the actual knowledge of the structure of the inverted hexagonal (H\(_{II}\)) phase and of the non-lamellar phase transitions of lipids [23].

In addition to the application of different experimental techniques, models were developed postulating two types of inverted micellar structures [24]. Spherical inverted micelles (lipid particles) sandwiched between the lipid monolayers of a bilayer membrane are opposed to inverted micellar intermediates which are transient intermediates within an interbilayer attachment site necessary for the process of membrane aggregation, membrane fusion or the lamellar-to-hexagonal (H\(_{II}\)) phase transition [25, 26]. Siegel states that inverted micellar intermediates formed at the interface of interacting bilayers may either (a) revert or (b) transform into an interlamellar attachment or (c) into a H\(_{II}\)-phase precursor formed via coalescence of inverted micellar intermediates in a pearl-string fashion. Beside this monolayer-encapsulated H\(_{II}\) tube, which is called a rod micellar intermediate, Siegel proposes a second type of intermediate [25]. The coalescence of inverted micellar intermediates may also lead to the formation of line defects which then elongate into structures consisting of two opposed halves of H\(_{II}\) tubes. Formations of interlamellar attachments, rod micellar intermediates, as well as line defects, ILA, RMI as well as LD depends on the density of inverted micellar intermediates within the bilayer membrane. The formation of H\(_{II}\) precursors is accompanied by intramembranous lipid mixing and in vesicle systems by leakage of aqueous content.
Lipid membrane structure and lipid phase behavior is regulated by proteins. Since membrane fusion or transbilayer movement of lipids are inconsistent with a continuous bilayer membrane, it is reasonable to assume that proteins might regulate the lamellar/non-bilayer equilibrium. Gramicidin, a hydrophobic linear pentadecapeptide favors the transition from the lamellar to the hexagonal H$_2$ phase in phosphatidylethanolamine (PtdEtn) membranes [27]. Cytochrome c induces the H$_2$ phase as well and probably also an inverted micellar structure in cardiolipin-containing membranes [27]. Poly(lysine), however, inhibits the calcium-induced lamellar-to-H$_2$ transition in cardiolipin membranes [29]. Rhodopsin, the main protein from the photoreceptor membrane, also stabilizes the bilayer structure [30]. The importance of a non-bilayer phase for protein function has been shown by Jensen and Schützbach [5]. The glycosyl carrier mannosyltransferase II exhibits maximal activity in the presence of a non-bilayer phase.

The aim of the present paper was to investigate the effect of glycoporphin, the major integral sialoglycoprotein of the erythrocyte membrane, on the lamellar-to-hexagonal phase transition. Glycoporphin modulates the lateral lipid distribution in bilayer membranes by a strong interaction with cholesterol [31]. Phosphatidylethanolamines, in contrast to phosphatidylcholines and especially phosphatidylglycerols, are prevented from a motionally restricted domain surrounding the hydrophobic core of the glycoprotein [32]. As derived from $^{31}$P-NMR experiments by Tarashi et al. [33], a stabilization of the bilayer phase has to be expected in phosphatidylethanolamine membranes. This is interesting, since similarities have been found between glycoporphin and gangliosides with respect to the interaction of their oligosaccharide residues with membrane surfaces [34]. Gangliosides in return coexist with PtdEtn in cellular membranes and are especially enriched in membranes which have frequent fusion activities [35]. The question arises of whether glycoconjugates regulate membrane interaction processes by their capability to stabilize bilayer structures by electrostatic repulsion between negatively charged sialic acid groups and/or by the capability to keep membrane surfaces hydrated, which has been observed in PtdEtn membranes containing different gangliosides [36].

**EXPERIMENTAL PROCEDURES**

**Materials**

Egg phosphatidylethanolamine (egg-PtdEtn), Percoll and the density markers were obtained from Sigma (Deisenhofen, FRG). Dimyristoylglycerophosphoethanolamine (Myr$_2$GroP-Etn) was from Fluka (Neu-Ulm, FRG). N-(7-Nitrobenz-2-oxa-1,3-diazol-4-yl)-dimyristoylglycerophosphoethanolamine (Nbd-PtdEtn) was purchased from Avanti Polar Lipids (Birmingham, USA). Fluorescein 5-isothiocyanate and eosin 5-isothiocyanate were obtained from Molecular Probes (Eugene, USA). Fluorescein- and eosin-labeled phospholipids were synthesized according to Waggoner and Stryer from the isothiocyanate derivative of the dye and Myr$_2$GroP-Etn [37]. The purity of the lipids was controlled by thin-layer chromatography using reagents sensitive for phosphate, amines or just for organic substrates.

Sepharose 4B was obtained from Pharmacia (Freiburg, FRG). Glycoporphin was isolated and purified from human erythrocytes as described earlier [32]. The purity of the isolated protein was checked by SDS/polyacrylamide gel electrophoresis using silver and periodic acid/Schiff stain, by protein determination [38, 39] and by sialic acid determination using the resorcinol method of Svennerholm [40]. Phospholipids were quantified by phosphate determination according to Ammon and Hinsberg [41].

**Vesicle preparation and reconstitution**

Vesicles were prepared by successive freezing and thawing of sonicated samples [42]. Dried lipid films, containing the fluorescence probes if needed, were sonicated in 10 mM Tris/HCl pH 7.2 supplemented by 150 mM NaCl and 0.2 mM EDTA in the presence of glycoporphin with a Branson sonifier (5 min, power 20 mW, 25°C) until a clear suspension was formed. The lipid concentration was 10 mM. The suspensions obtained were slowly frozen from 4°C to -20°C in a refrigerator, slowly thawed again by keeping them at 4°C and incubated at 40°C for 1 h. This cycle was repeated three times yielding fused vesicles with an average diameter around 80 nm. All incubation steps were performed under an argon atmosphere. The vesicles were freed from surface-adsorbed glycoporphin by successive centrifugation and resuspension. Before measurements, vesicles were incubated again for 1 h at 40°C. The pelleted vesicles were assayed for phosphate and sialic acid before and after measurements. The analytical values were corrected for the remaining intermediate volume between the vesicles as described earlier [32]. Vesicles formed in the presence of glycoporphin were characterized by electron microscopy using the freeze-etching technique.

**Calorimetry**

DSC measurements were performed with an AT-PC computer-controlled Microcal MC-2 scanning calorimeter with an adiabatic differential-power-scanning twin system which corresponds to the Privalov type [43]. The two chambers were filled with the sample or the corresponding buffer solution: 10 mM Tris/HCl pH 7.2 containing NaNCl and 0.2 mM EDTA. The scan rate was 30 K/h, scans were taken between 5–60°C. Phase transition curves were corrected for their baseline and analyzed with a deconvolution program. Phase transition enthalpies were determined and related to the phosphate content.

**Density gradient centrifugation**

The homogeneity of the prepared vesicles was checked by density gradient centrifugation in 20% Percoll solution containing 10 mM Tris/HCl pH 7.2, 150 mM NaNCl and 0.2 mM EDTA. Density markers at $\rho = 1.016, 1.033, 1.048$ and 1.062 g/ml were added. The gradient was formed within 1 h in a Ti-60 rotor at 19000 rpm and at 5°C. Vesicle suspensions layered on top of the gradient were centrifuged in an SW-28 rotor for 15 min at 2800 rpm and 5°C.

**Nbd-PtdEtn fluorescence**

Lipid samples were labeled with 0.1 mol% Nbd-PtdEtn. The phosphatidylethanolamine concentration was 0.1 mM in 10 mM Tris/HCl pH 7.2, 150 mM NaNCl and 0.2 mM EDTA. The fluorescence measurements were carried out in thermostatted quartz cuvettes (10 x 10 mm) with a probe volume of 2 ml. The Nbd fluorescence was excited at 470 nm and the emission intensity was recorded at 530 nm. During microinjection of the concentrated calcium chloride or the wheat
glycophorin into egg-PtdEtn membranes

germ lectin solution, sedimentation was prevented by vigorous stirring.

Energy transfer experiments

Two different lipid samples containing the same given mole fraction of protein were labeled with either 1 mol% fluorescein-phosphatidylethanolamine or with 1 mol% eosin-phosphatidylethanolamine as an energy-transfer pair. After incubation for 1 h at 0°C, the two lipid samples were mixed again at 0°C in 10 mM Tris/HCl pH 7.2, 150 mM NaCl and 0.2 mM EDTA to make up a final concentration of 0.1 mM egg-PtdEtn. Stirring the sample in the quartz cuvette (10 × 10 mm) prevented the sedimentation of the lipid membranes. A probe volume of 2 ml was titrated by micro-injection of concentrated calcium chloride or wheat germ lectin solutions. The donor (fluorescein) was excited at 450 nm. The intensities of the donor and of the acceptor (eosin) emission, were measured at 520 nm and 548 nm, respectively, and were recorded by an AT-PC. The intensity ratio I acceptor /I donor is an estimate of the energy transfer efficiency and was measured as function of temperature with a scan rate of 60 K/h.

Leakage experiments

Vesicles were prepared in buffered solution as before but containing 50 mM calcein. At that dye concentration fluorescence self-quenching occurs. The vesicles filled with the dye were separated from non-included calcein by gel permeation chromatography using Sepharose 4B. For leakage measurements, the chromatographed vesicles were diluted by a factor of 10 with an equiosmolar buffer. Calcein was excited at 460 nm, the fluorescence intensity was recorded at 520 nm. Temperature scans were performed with a rate of 30 K/h.

RESULTS

Reconstitution of glycophorin-containing egg-PtdEtn vesicles

Glycophorin was reconstituted into egg-phosphatidylethanolamine membranes by the freeze-and-thaw technique. Stable vesicle suspensions were obtained in the presence of glycophorin. The vesicular protein content, measured as mole fraction of glycophorin (XG), increases with increasing amount of glycophorin (XG0) added to the lipid suspension before the freeze-and-thaw cycles (Fig. 1). However, only about 10% of the added glycophorin became inserted into the membranes, which is slightly lower but comparable to Myr2GroPCho membranes.

The PtdEtn-vesicle preparations obtained have been characterized by density gradient centrifugation using a Percoll gradient (Fig. 2). In the absence of protein, lipid aggregates concentrated in a sharp density band at 1.029 g/ml. Upon increasing the amount of glycophorin, this distinct band became broadened and converted into a diffuse density distribution with a concentration of 0.2 mg/ml. Scans were performed with a rate of 30 K/h.

Differential scanning calorimetry

Thermotropic phase transition curves of egg-PtdEtn in aqueous suspensions are shown in Fig. 3 in the absence (Fig. 3a) and in the presence of glycophorin (Fig. 3b and c).
transition curves are baseline corrected and standardized to the lipid concentration. (b) Transition enthalpy change as a function of the glycoprotein mole fraction xG.

**Fig. 4.** Lp, phase at

The low-temperature gel-like Lp, phase converts into the fluid tringular phase in arbitrary intervals of glycophorin concentrations exceeding xG = 10^-3. Thus one glycophorin molecule prevents a thousand egg-PtdEtn molecules from undergoing the lamellar-to-hexagonal phase transition and therefore keeps the membrane in a metastable bilayer phase.

Addition of wheat germ lectin together with Ca^{2+} ions reinduces the lamellar-to-hexagonal phase transition, which now appears at a temperature 8°C below the corresponding phase transition temperature of the pure egg-PtdEtn (Fig. 3d). This reduction of the lamellar-to-hexagonal phase transition temperature is caused by the Ca^{2+} ions (M. Hein, A. Post and H. J. Gall, unpublished results). Wheat germ lectin or Ca^{2+} ions alone do not reinduce the lamellar-to-hexagonal phase transition in the glycophorin-containing PtdEtn vesicles.

**Nbd-PtdEtn fluorescence**

The synergistic effect of Ca^{2+} and wheat germ agglutinin could also be demonstrated by measuring the fluorescence intensity of the lipid fluorophore Nbd-PtdEtn. A transition from the lamellar to the hexagonal phase is accompanied by a drastic increase of the fluorescence quantum yield [22]. Fig. 5 shows the results for egg-PtdEtn membranes containing 0.1 mol% Nbd-PtdEtn and xG = 8.4 x 10^-4 at 40°C, where the protein-containing membrane is in the lamellar and the protein-free membrane is in the hexagonal phase. The protein-containing lipid suspension is stable with time and exhibits a constant and low fluorescence intensity, which is not influenced by either 5 mM Ca^{2+} or 0.02 mg/ml wheat germ agglutinin added separately. However, if 5 mM Ca^{2+} is added together with 0.02 mg/ml of wheat germ agglutinin, a spontaneous fluorescence intensity increase was observed, clearly demonstrating the transition of the lipid bilayer membrane into the hexagonal phase.

**Energy transfer experiments**

Vesicles doped with either fluorescein- or eosin-labeled phosphatidylethanolamine were used to investigate the temperature-induced aggregation of the membranes, which is a precondition for the formation of the hexagonal phase. In the absence of glycophorin, PtdEtn membranes exhibit an increase in the energy transfer rate at temperatures where the lamellar-to-hexagonal phase transition occurs (Fig. 6, curve a). A subsequent temperature decrease hardly reduces the energy transfer efficiency (curve b). When glycophorin-containing PtdEtn vesicles were used, neither this clear temperature-induced increase in the energy transfer rate nor a hysteresis phenomenon was observed (curves c and d). However, if Ca^{2+} (5 mM) is added again together with wheat germ lectin (0.02 mg/ml), transition curves were obtained which are
with 50 mM calcein, a hydrophilic fluorescence dye that can-rescence intensity with time at 40°C, a temperature where the creases when the dye leaks out of the vesicles and dilutes into has been studied by leakage experiments. Vesicles were filled within the vesicle interior at that dye concentration but increases when the dye leaks out of the vesicles and dilutes into the aqueous bulk phase.

At a vesicular mole fraction of glycoporphin X₀ of $8 \times 10^{-4}$, we obtained egg-PtdEtn vesicles that exhibit a constant fluorescence intensity with time at 40°C, a temperature where the hexagonal phase exists in pure egg-PtdEtn membranes. This experiment again shows that stable vesicles are formed. Addition of Ca²⁺ ions (5 mM) or wheat germ lectin (0.02 mg/ml) alone did not cause any destabilization. However, the joint addition of both Ca²⁺ and wheat germ lectin causes a rapid and drastic fluorescence increase at temperatures above the lamellar-to-hexagonal phase transition temperature of the protein-free lipid membranes. The leakage kinetics at three selected temperatures of 0°C, 20°C and 40°C are shown in Fig. 7, where the time-dependent fluorescence intensity $F_0$ is related to $F_\infty$, the fluorescence intensity obtained after complete dye leakage. This final fluorescence level ($F_\infty$) is identical to that obtained after complete vesicle disruption caused by ultrasonic treatment. The straight lines in Fig. 7 yield the time constants $\tau_{1/2} = 130 \text{ s}$ at 40°C, $\tau_{1/2} = 340 \text{ s}$ at 20°C and $\tau_{1/2} = 2400 \text{ s}$ at 0°C.

These results already show that the ability of Ca²⁺ ions, together with wheat germ lectin, to destabilize glycoporphin-containing egg-PtdEtn vesicles strongly depends on temperature. Fig. 8 shows temperature scans of the vesicles in the absence of Ca²⁺ and/or wheat germ lectin (control, curve a) and in the presence of Ca²⁺ together with wheat germ lectin (curve b). The fluorescence decrease observed with temperature for the control sample is caused by the increased self-quenching rate with increasing temperature and confirms the thermal stability of the glycoporphin-containing vesicles. In the presence of Ca²⁺ and wheat germ lectin, the fluorescence used as a marker for the vesicle leakage increases between 20 – 30°C in agreement with the energy transfer data. The fluorescence intensity in these experiments may not be used as a quantitative measure since, in this automatic temperature scan with a scan rate of 30 K/h, the release at a given temperature does not correspond to the quantitative values given in Fig. 7. Fig. 8 demonstrates only that a release starts at the lamellar-to-hexagonal phase transition.

**Dye leakage**

The stability of glycoporphin-containing PtdEtn vesicles has been studied by leakage experiments. Vesicles were filled with 50 mM calcein, a hydrophilic fluorescence dye that cannot pass the membrane. The fluorescence is highly quenched within the vesicle interior at that dye concentration but increases when the dye leaks out of the vesicles and dilutes into the aqueous bulk phase.

We obtained egg-PtdEtn vesicles that exhibit a constant fluorescence intensity of $8.3 \times 10^{-4}$, filled with 50 mM calcein at temperatures above 20°-27°C. This indicates the calcein release from the vesicles now passing through the thermotropic lamellar-to-hexagonal lipid phase transition.

**DISCUSSION**

The transition between the lamellar and the inverted hexagonal (H₂) membrane structure is of importance for several biological processes. According to Siegel et al. [24 – 26] and Verkleij et al. [44], vesicle fusion starts with the adhesion of the corresponding membrane surfaces followed by the formation of spherical inverted micelles within cusp-like interbilayer attachment sites. This structural organization is considered to be a transient intermediate in the process of membrane fusion but also in the lamellar-to-hexagonal phase transition. These intermediates may revert to the bilayer structure, thus reforming the original planar membrane interface. Lipids may be exchanged via the merged interfaces but the aqueous interior of the vesicles is not mixed in this process. Beside this, spherical micelles may fuse to form the inverted cylinders of the hexagonal H₂ phase. The presence of spherical as well as cylindrical micelles may be the precondition for the membrane fusion.

Despite a wealth of data on inverted phase-forming lipids [23, 45, 46], we still do not clearly understand the ability of proteins, especially glycoproteins, to modulate the structural organization of membranes containing non-bilayer competent lipids. This, however, is of common interest since hormones like insulin or epidermal growth factor bind to specific receptors on the cell surface which are then internalized by endocytosis, possibly involving intermediate inverted micellar structures [47]. Moreover, non-bilayer lipid structures are thought to be involved in special semi-fused interbilayer cell—cell connections the so-called tight junctions or zona occludens [2, 47]. Peripheral proteins like ZO-1 or cingulin are involved in this contact phenomenon [48], acting as mediators between the cytoplasmic cell surface and the cytoskeleton.

Beside the natural occurrence of non-bilayer structures, stabilized PtdEtn vesicles recently became of interest due to their potential use as drug carriers [49]. Immunoliposomes...
were formed out of phosphatidylethanolamine and an antibody which has been anchored with its Fv fragment to the membrane by a covalently linked fatty acid residue. Only five modified protein molecules/vesicle were necessary to stabilize the bilayer phase. Specific binding of the corresponding antigen segregates the antibodies within the plane of the membrane. The protein-depleted membrane areas form unstable intermediates and the vesicle content, for example a drug or a concentrated dye solution, leaks out; leakage may be monitored by the fluorescence increase, caused by the dye, as a measure for the amount of antigen present in the solution.

The aim of this paper was to study the stability of glycophorin-containing egg-PtdEtn vesicles under the influence of Ca\(^{2+}\) and wheat germ lectin. Glycophorin has been chosen as an example of an intrinsic glycoprotein which has been repeatedly reconstituted into different lipid membranes [32, 33, 50, 51]. The protein is anchored by a membrane-spanning hydrophobic \(\alpha\)-helical part which by itself already stabilizes bilayer structures [33]. The extensive oligosaccharide residue which comprises about 60\% of the molecular mass is preferentially oriented to the outside of the vesicles. Thus small amounts of this protein are expected to cover large areas of the vesicle surface by a hydrophilic oligosaccharide gel which may keep the PtdEtn membrane in a fully hydrated state. Hydration, however, which is the most prominent barrier to the formation of molecular contacts between opposing bilayers [52], acts on the lamellar—hexagonal phase equilibrium by stabilization of the lamellar phase [10]. In addition, charge regulation resulting form the negatively charged sialic acid groups prevents vesicle aggregation which is a necessary precondition for the formation of the hexagonal phase. Since glycophorin is the primary cell membrane receptor for wheat germ agglutinin, lectin-induced clustering of receptor molecules may occur [53] and hexagonal phase formation will be observed in glycophorin-stabilized PtdEtn membranes [47].

In this paper we report on a synergistic effect of Ca\(^{2+}\) and wheat germ agglutinin in egg-PtdEtn vesicles with a very low amount of glycophorin (protein/lipid ratio \(<\) 1:1000). We used fused small unilamellar vesicles prepared by the freeze-and-thaw technique. Vesicles were carefully washed to strip off surface-adsorbed protein. By this procedure, we obtained only the above-mentioned low glycophorin incorporation, whereas other authors reached lipid/glycophorin molar ratios of 25:1 [54]. However, the same discrepancy has been reported in phosphatidylcholine vesicles [32]. At this low protein content, vesicles are formed as was shown by density gradient centrifugation (Fig. 2) and by electron microscopy (data not shown).

The egg-PtdEtn undergoes two thermotropic transitions from a lamellar gel to the fluid lamellar phase and from the fluid lamellar to the hexagonal (H\(_{II}\)) phase. The first occurs between 10—20°C, the second between 30—40°C in our pure lipid vesicle preparations. It should be mentioned that the transition range strongly depends on the material that has been used and the reported transition temperatures range from 28°C [16] to 37°C [22] for the lamellar—hexagonal transition. Addition of low amounts of glycophorin does not influence the gel-to-fluid transition but strongly diminishes the lamellar-to-hexagonal transition. From the linear decrease of the transition enthalpy, which is \(\Delta H = 3.9\ \text{kJ/mol}\) in pure egg-PtdEtn membranes, with increasing glycophorin content we extrapolate to a molar protein/lipid ratio of about 1000:1 for the disappearance of the transition. This clearly demonstrates that the lamellar phase of egg-PtdEtn is already stabilized by a very low protein content comparable to the one reported by Ho et al. [49] for acylated antibodies.

Binding of the effector molecule wheat germ agglutinin did not cause dye release (Fig. 5) or exchange of lipid material (Fig. 6). The same is valid for 5 mM Ca\(^{2+}\) in the absence of the lectin. However, if both effectors are added to glycophorin-containing membranes at a temperature where the pure lipid membrane is in the hexagonal (H\(_{II}\)) phase, strong dye release was observed, clearly demonstrating the synergistic effect of Ca\(^{2+}\) and wheat germ agglutinin (Fig. 5). DSC measurements reveal the reappearance of the lamellar-to-hexagonal phase transition with a reduced phase transition temperature due to the known effect of Ca\(^{2+}\) ions [6].

Temperature-dependent energy transfer experiments, as well as dye leakage measurements, support the DSC results and clearly show an increase in lipid mixing and membrane permeability at the recovered phase transition in the presence of Ca\(^{2+}\) and wheat germ agglutinin. The leakage kinetics shown in Fig. 7 is increased by a factor of 8 if the temperature is raised from 0°C to 20°C, which means that the system

\[\text{Ca}^{2+} + \text{WGL} \rightarrow \text{H}_{II} \text{ phase} \]
passes through the lamellar-to-hexagonal phase transition, which occurs at a reduced temperature in the presence of Ca\(^{2+}\). A further increase of the temperature from 20°C to 40°C only results in an additional twofold acceleration. Patching of the glycophorin might occur in the fluid phase.

Fig. 9 summarizes the results. The multivalent wheat germ lectin specifically binds to sialic acids. Vesicle aggregation under the influence of lectin unshielding parts of the vesicle surface from protein and oligosaccharide residues. Ca\(^{2+}\) ions may interact with these unshielded membrane areas, causing the dehydration and the aggregation of the vesicles and the formation of the hexagonal phase which destabilizes the vesicles.

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