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LIPID-PROTEIN INTERACTIONS OF THE HUMAN ERYTHROCYTE CONCANAVALIN A RECEPTOR IN PHOSPHOLIPID BILAYERS

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The interaction of the human erythrocyte concanavalin A receptor (a subpopulation of Band 3) with phospholipids has been investigated using differential scanning microcalorimetry of reconstituted vesicles prepared by detergent dialysis. The mean diameter of dialyzed phospholipid vesicles jumps dramatically on inclusion of the concanavalin A receptor and then increases linearly with the fraction of protein in the bilayer. The glycoprotein has a dramatic effect on the phospholipid gel to liquid-crystalline phase transition, and ΔH decreases linearly with increasing mole fraction of protein up to a protein/lipid mole ratio of around 1:1160. Extrapolation of this data indicates that each concanavalin A receptor is able to perturb about 685 molecules of dimyristoylphosphatidylcholine, withdrawing them from the main phase transition. The cooperativity of phospholipid melting is profoundly disrupted by small amounts of glycoprotein, with the cooperative unit dropping to less than half its initial values at a protein/lipid mole ratio of 1:3800. A break occurs in the ΔH curve as the protein/lipid mole ratio is increased above 1:1160, and ΔH then increases linearly with increasing amounts of concanavalin A receptor in the bilayer. This phenomenon may be interpreted in terms of protein-protein aggregation which occurs in the phospholipid bilayer above a certain critical mole fraction of concanavalin A receptor, resulting in perturbed phospholipids being returned to the phase transition. In addition, the hydrophilic domains of the glycoprotein may exist in two different conformations depending on the protein concentration in the bilayer, and these may differ in their ability to interact with phospholipid headgroups at the membrane surface.

Introduction

The arrangement of membrane proteins within the bilayer matrix and the interaction of these proteins with lipid molecules such as phosphatidylcholine and cholesterol has been the subject of considerable study in recent years. Differential scanning calorimetry has proved to be an extremely valuable technique for studying the thermotropic behaviour of lipids both in native membranes and in model systems [1,2], providing detailed information on the interactions of many membrane proteins with the lipid bilayer matrix (for a review, see Ref. 3 and references contained therein). Glycosylated membrane proteins are known to act as important receptors for external agents at the cell surface (e.g., lectins, hormones, viruses, toxins) and many questions remain to be answered concerning both their behaviour in the lipid bilayer and their interactions with other membrane components. The possible conse-

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Abbreviations: DMPC, L-α-dimyristoylphosphatidylcholine; DPPC, L-α-dipalmitoylphosphatidylcholine; PC, phosphatidylcholine; PS, phosphatidylserine; DSC, differential scanning calorimetry; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

quences of these interactions on the structure and function of the membrane and on the receptor properties of the glycoprotein itself remain largely unexplored. Recent studies on human erythrocyte glycophorin have revealed that the carbohydrate headgroup, as well as the membrane-spanning peptide domains, may influence the interactions of the glycoprotein with surrounding lipid molecules [4].

The concanavalin A receptor of the human erythrocyte membrane is a subpopulation (approx. 20%) of the Band 3 group of proteins, which has been relatively well characterized at the molecular level, both from the point of view of structure and anion transport function. Band 3 has a molecular weight of 95 kDa, of which some 5–7% is carbohydrate present as a single asparagine-linked oligosaccharide chain, and has been reconstituted into lipid bilayers by several groups (see, for example, Refs. 5–8). Considerable progress has been made in elucidating the structure and arrangement of Band 3 by Rothstein and coworkers, who have proposed a model for the assembly of the Band 3 transmembrane segments within the bilayer matrix [9]. The concanavalin A receptor reconstituted into lipid bilayer membranes provides an excellent model for studying the interactions of glycosylated proteins with membrane lipids since it is structurally fairly representative of the type of receptor glycoprotein found at cell surfaces (glycophorin may be a somewhat atypical glycoprotein).

In the studies described in this paper, we have used differential scanning calorimetry to study the thermotropic behaviour of large unilamellar vesicles containing reconstituted concanavalin A receptor glycoprotein. Our goal was to attempt to evaluate the effects of a large glycosylated membrane protein on the properties of membrane phospholipids. The results we have obtained show that the concanavalin A receptor causes a dramatic perturbation of the bilayer phospholipids, which may be related to both the peptide and carbohydrate domains of the glycoprotein.

Materials and Methods

The concanavalin A receptor glycoprotein was isolated from human erythrocyte ghosts by affinity chromatography on concanavalin A- Sepharose 4B (Pharmacia) as described previously [10]. Egg PC, DMPC, DPPC and PS (bovine brain) were obtained from Sigma Chemical Co., and were used as supplied (purity greater than 98–99%).

Lipid-protein reconstitution. The concanavalin A receptor glycoprotein was reassembled into large unilamellar phospholipid vesicles using the detergent dialysis technique described previously [10]. The glycoprotein in 25 mM dodecyltrimethylammonium bromide solution was added to the appropriate phospholipid mixture solublized in 200 mM dodecyltrimethylammonium bromide/5 mM Hepes-buffered saline (pH 7.4). The lipid-protein mixture was then dialyzed extensively against Hepes-buffered saline (pH 7.4) for 48 h at 4°C and the resulting phospholipid vesicles harvested by ultracentrifugation. Phospholipid recoveries were measured by inclusion of tracer quantities of di[1-^{14}C]palmitoyl-L-α-phosphatidylcholine (Amersham, specific activity 80–120 mCi/mmol) in the reconstitution mixture, followed by liquid scintillation counting of the recovered vesicles. Protein recoveries were determined using the method of Peterson [11]. Reconstituted concanavalin A receptor samples with varying lipid/protein ratios were prepared independently using several different glycoprotein preparations.

Quasi-elastic light scattering. Quasi-elastic light scattering measurements were carried out using a helium-neon laser (wavelength 632.8 nm), a quantum photometer and a 64 channel autocorrelator (Langley-Ford model 1096). A small aliquot of vesicles was diluted to 2 ml with phosphate-buffered saline (pH 7.4) and light scattering was recorded at an angle of 90° in a thermally jacketed sample chamber maintained at 20°C. Measurements were made at several sample ('bin') times and analysis of the resulting autocorrelation functions was carried out using the method of cumulants. The average hydrodynamic diameter of the vesicles was computed assuming they were spherical (Stokes radius).

Differential scanning calorimetry. Calorimetric data were obtained using a Microcal MC1 high sensitivity differential scanning calorimeter with a built-in calibration pulse. Reconstituted vesicles (around 4 mg phospholipid) in a total volume of 100–150 μl of 10 mM Hepes-buffered saline (pH 7.4) were thermally analyzed at a scanning rate of

approx. 0.75 K/min. Samples were prewarmed above the gel to liquid-crystalline phase transition temperature prior to calorimetric analysis. Each sample was scanned three times, with consistent results, and the calorimetric parameters averaged. Peak areas were determined by weighing paper cut-outs of the DSC scans and converting to areas from a standard curve, and the enthalpy of transition was obtained from the area under the peak and the amount of phospholipid in each sample. The phase transition temperature T_m was defined as the temperature at the peak maximum and the sharpness of the transition was expressed as the temperature width at half-maximum height, $\Delta T_{1/2}$.

Results and Discussion

Effect of the concanavalin A receptor on phospholipid vesicle size

The detergent dialysis procedure used to prepare reconstituted concanavalin A receptor results in the formation of large unilamellar vesicles containing the glycoprotein, which is reassembled in a symmetric fashion [10]. Concanavalin A receptor reconstituted by detergent dialysis from solutions of dodecyltrimethylammonium bromide shows large intramembranous particles (about 100 Å in diameter) on freeze-fracture electron microscopy, indicating insertion of the protein across the hydrophobic interior of the membrane [5,12]. Dialyzed vesicles of phospholipid alone (4:1, w/w egg PC/PS) are relatively small and have a mean diameter of 0.1 μm as measured by quasi-elastic light scattering techniques. Inclusion of even small amounts of the concanavalin A receptor in the bilayer (15:1, w/w) results in a substantial increase in the mean diameter of the vesicle population to 0.27 μm and as the protein content is further increased, there is a linear increase in vesicle size (see Fig. 1). Another integral membrane protein has also been shown to affect vesicle size; Goodwin et al. [13] reported that sonicated vesicles of DPPC showed a large increase in diameter on addition of the erythrocyte sialoglycoprotein glycophorin, but noted no further change in size with increasing ratios of protein to lipid.

The size of the reconstituted vesicles used in our studies is presumably determined during their formation by detergent dialysis, and careful consider-

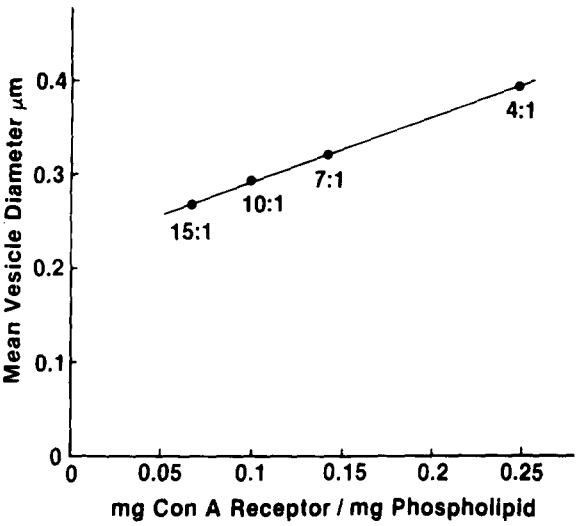

Fig. 1. Quasi-elastic light scattering data showing the effect of concanavalin A receptor content on the mean diameter of reconstituted large unilamellar vesicles of 4:1 egg PC/PS. Lipid/protein weight ratios for the various preparations are indicated for reference.

ation of the events taking place during vesicle formation may help to explain the observed increase in diameter with concanavalin A receptor content. During the process of dialysis, mixed micelles of phospholipid, protein and detergent (dodecyltrimethylammonium bromide) are converted to closed bilayer structures as detergent is removed. There is little experimental data available on the mechanism of vesicle formation from mixed micelles or the intermediate structures produced during dialysis, and the possible role played in this process by integral membrane proteins has not been explored. Recent theoretical considerations [14] have suggested that flat disc-shaped lipid micelles with detergent molecules around their circumference fuse into progressively larger structures as detergent is removed, then begin to curve and finally close to form unilamellar bilayer vesicles. It was proposed that the shape of the vesicle finally produced depends only on the balance between E_h, the unfavourable interaction energy of the lipid hydrocarbon chains with water, and E_c, the elastic curvature energy. Working within this model, it is possible to see how introduction of a very hydrophobic membrane protein into the mixed micelles might change these com-

plex interactions. If the protein could effectively change the curvature elasticity of the disc-like micelles, this could result in the formation of a vesicle of larger radius of curvature during detergent removal.

The effect of the concanavalin A receptor on vesicle size could also result from fusion between vesicles. Insertion of certain membrane proteins (e.g. lipophilin) into phospholipid vesicles has been shown to greatly increase the tendency of vesicles to fuse, and it is possible that the concanavalin A receptor acts in a similar fashion.

Perturbation of the phospholipid gel to liquid-crystalline phase transition by the concanavalin A receptor

In order to examine the effect of the concanavalin A receptor on the thermotropic properties of phospholipid bilayers, the glycoprotein was reconstituted into DMPC vesicles at various lipid/protein ratios and the main gel to liquid-crystalline phase transition was examined by differential scanning calorimetry. Fig. 2 shows representative DSC scans of pure DMPC and of the lipid-protein recombinants (ranging from 28 : 1 to less than 3 : 1, w/w lipid/protein) for qualitative comparison. The inclusion of the concanavalin A receptor in the DMPC bilayers has relatively little effect on the phase transition temperature T_m, which rises by only about 2 °C as the protein mole ratio is increased to $3 \cdot 10^{-3}$ (about 320 phospholipids per protein, 2.4 : 1, w/w). Integral membrane proteins, in general, do not appear to have a consistent effect on T_m, with large decreases being noted for cytochrome *P*-450 [15] and relatively little change seen for other proteins such as bacteriorhodopsin [16], glycophorin [17], cytochrome b_5 [18] and vesicular stomatitis virus glycoprotein [19]. A useful comparison of DSC data for reconstituted systems containing membrane proteins is presented by Silvius in Ref. 3. At low protein mole fractions, the concanavalin A receptor causes dramatic changes in the shape of the phase transition, producing a peak which is skewed towards the high temperature end of the transition. This is apparently a result of the superposition of a broad component, centred at a slightly higher temperature, on the sharp peak characteristic of pure DMPC. As the protein mole fraction is further increased, the sharp component declines

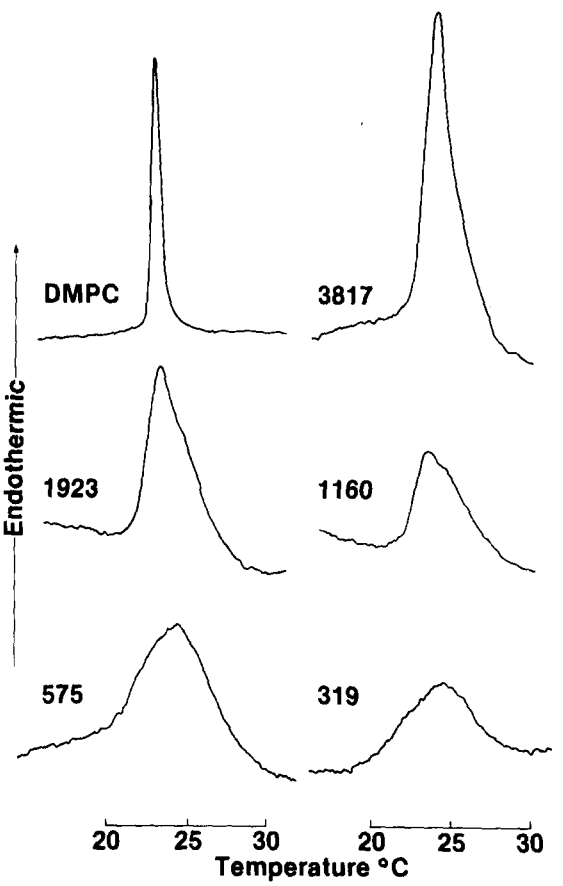

Fig. 2. Calorimetric heating curves for large unilamellar vesicles containing the human erythrocyte concanavalin A receptor at various indicated mole ratios of DMPC to protein. Vesicles were suspended in 10 mM Hepes-buffered saline (pH 7.4) and thermally analyzed at a heating rate of around 0.75 K/min. These scans are not normalized and are for qualitative comparison only.

rapidly until only a broad peak is present at a protein mole fraction of about $0.86 \cdot 10^{-3}$ (1160 phospholipids per protein). These shape changes may be quantitated by measuring the sharpness of the phase transition, expressed as the temperature width at half-maximal height $\Delta T_{1/2}$. As shown in Fig. 3, $\Delta T_{1/2}$ increases dramatically up to a protein mole fraction of about $0.86 \cdot 10^{-3}$, when only a broad component remains on the DSC scans, and then rises more slowly. This broad component does not arise as a result of sample inhomogeneity since Sepharose 2B gel filtration chromatography, electron microscopic and quasi-elastic light scattering measurements all indicate a highly homoge-

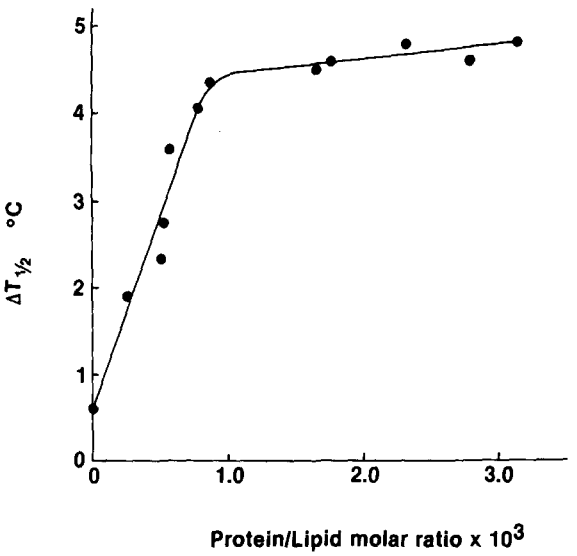

Fig. 3. Effect of increasing mole fractions of concanavalin A receptor on the width of the main gel to liquid-crystalline phase transition $\Delta T_{1/2}$ for samples reconstituted with DMPC.

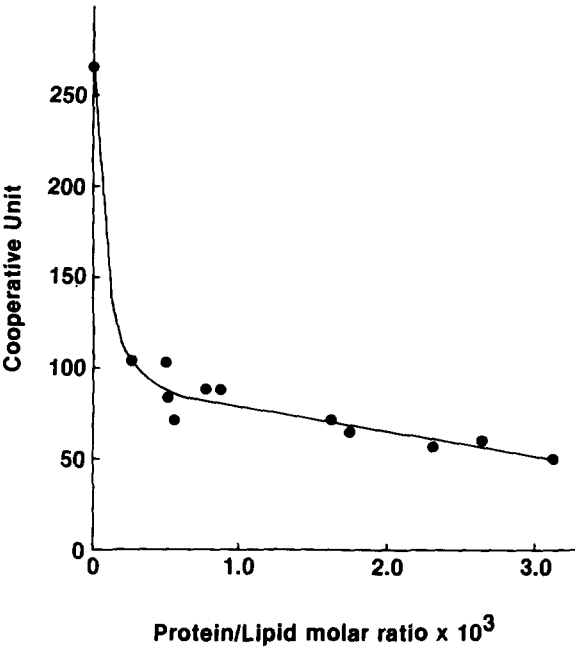

Fig. 4. Variation of the cooperative unit (CU) for the main gel to liquid-crystalline phase transition of DMPC with the mole fraction of concanavalin A receptor glycoprotein in the bilayer.

neous vesicle population over a wide range of lipid/protein ratios [10]. It seems likely that the broad component observed in the DSC scans is due to the presence of a lipid phase whose thermotropic properties are perturbed by the presence of the glycoprotein but which can still undergo a gel to liquid-crystalline phase transition. Thus this lipid phase melts at a slightly higher temperature than pure DMPC and its cooperativity is markedly disrupted (see below). When the protein mole fraction of $0.86 \cdot 10^{-3}$ is reached, most of the bilayer lipid has perturbed thermotropic properties. A complete explanation of the shape of the gel to liquid-crystalline phase transition must await the determination of the phase diagram for DMPC-concanavalin A receptor bilayers.

The van't Hoff enthalpy ΔH_{vH} can be calculated from the T_m and $\Delta T_{1/2}$ values of the transition using the relationship

$$\Delta H_{vH} \cong 4RT_m^2/\Delta T_{1/2}$$

and using the ratio $\Delta H_{vH}/\Delta H$, we can determine the cooperative unit CU, in molecules. The cooperative unit is a measure of the degree of cooperativity of the phospholipid phase transition, and although the absolute value of cooperative unit is markedly dependent on phospholipid purity, comparison between samples reconstituted with the same phospholipid can give a useful evaluation of protein effects on transition cooperativity. The size of the cooperative unit for the pure DMPC used in these experiments was found to be 270, only slightly less than that reported by Mabrey and Sturtevant [20]. Inclusion of even small amounts of concanavalin A receptor in the DMPC bilayer causes the cooperative unit to drop to around 100, and increasing amounts of protein result in further decreases in the cooperative unit to about 50 (see Fig. 4). Thus this particular membrane protein markedly disrupts the cooperativity of the phospholipid phase transition.

Lipid-protein and protein-protein interactions in phospholipid vesicles containing the concanavalin A receptor

Fig. 5 shows a plot of the phase transition enthalpy ΔH, (calculated from the sample size and the area under the DSC peaks) against the concanavalin A receptor content of the vesicles. At low protein concentrations (more than 1160 phos-

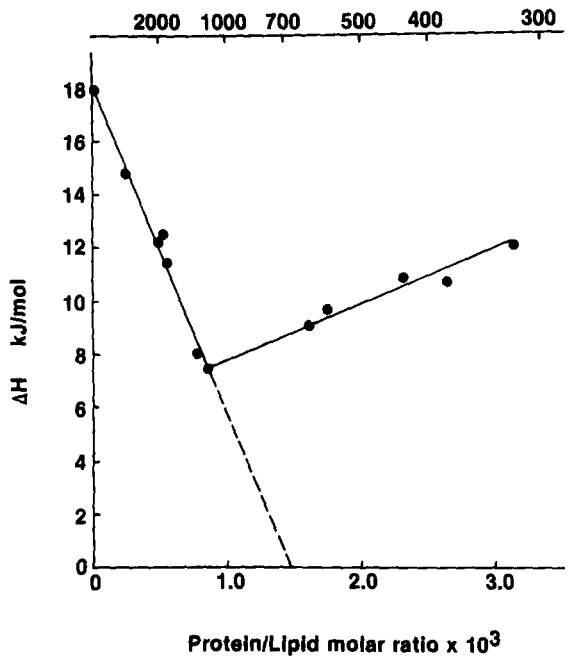

Fig. 5. Effect of the concanavalin A receptor glycoprotein on the total enthalpy change per mole of phospholipid for the main gel to liquid-crystalline phase transition of DMPC. Each point on the plot represents a different reconstituted sample, prepared independently using several different glycoprotein preparations as described in Materials and Methods. The data were calculated from heat capacity curves such as those shown in Fig. 2 and each point shows the mean value of ΔH for three consecutive scans of the same samples.

pholipids per protein) we observe a linear decrease in ΔH from 18.25 kJ/mol for pure DMPC (in agreement with the data of Goodwin et al. [13]) indicating that the membrane protein is preventing some of the phospholipid molecules from participating in the phase transition. The number of phospholipids N removed from the gel to liquid-crystalline phase transition by one protein molecule can be estimated using the equation [17]

$$\Delta H/\Delta H_0 = 1 - N(P/L)$$

where ΔH_0 is the enthalpy change for the pure phospholipid and P/L is the protein/lipid mole ratio in the bilayer. By extrapolation of the first part of the plot in Fig. 5 to $\Delta H = 0$, a value for N of around 685 is obtained for DMPC. Thus each molecule of concanavalin A receptor is capable of perturbing or influencing a very large number of phospholipids; the largest value of N previously reported is 350 for reconstituted cytochrome P-450 in DMPC bilayers [15]. Other integral membrane proteins in reconstituted systems interact with a much smaller number of phospholipids; for example, vesicular stomatitis virus glycoprotein (270), glycophorin (80–100), bacteriophage M13 coat protein (70–100), bacteriorhodopsin (19–22), cytochrome b_5 (14) (for a more extensive listing, see Ref. 3). Other researchers have visualized N as the number of concentric shells of phospholipid which a single α-helical membrane-spanning polypeptide chain can remove from the phase transition. The large number of phospholipids able to be influenced by a single concanavalin A receptor may not be unexpected in light of the fact that this glycoprotein has at least 5 separate 'hydrophobic' segments which span the bilayer [9], each of which could perturb phospholipid molecules in its vicinity. The overall amino acid composition of these five segments is not markedly nonpolar, but they behave physically as if highly hydrophobic and can only be extracted from the erythrocyte membrane by organic acids or detergents [9]. Rothstein and coworkers have postulated that the hydrophilic residues of these five segments are internalized to form an aqueous pore, which may play a vital role in the anion transport function of Band 3, while the hydrophobic residues contact the phospholipid acyl chains. This peptide assembly may be able to influence a relatively large area of membrane and may explain the large number of phospholipids which a single concanavalin A receptor molecule can perturb relative to other integral membrane proteins. An additional explanation may be that the hydrophilic portion of this protein may take part in lipid-protein interactions with phospholipid headgroups at the membrane surface and thus remove them from the phase transition. Rüppel et al. [4] have already shown that at low protein mole fractions, the glycosylated headgroup of erythrocyte glycophorin spreads out on the bilayer surface in a 2-dimensional pancake-like conformation. This structure can interact with many more phospholipids than the upright headgroup conformation found at higher protein concentrations (300 vs. 100). Since the concanavalin A receptor has a hydrophilic portion which includes several different peptide domains

and a fairly large carbohydrate chain (4750–6650 Da), it seems reasonable to assume that this glycoprotein could enter into substantial hydrophilic interactions at the bilayer surface, and that these might contribute to the large number of phospholipids perturbed. The possible involvement of hydrophilic lipid-protein interactions in the complex relationship between ΔH and protein concentration is discussed in more detail below. Band 3 makes up about 25% of the total protein of the erythrocyte membrane, which results in a relatively high lipid-Band 3 ratio of approx. 3 : 1 (w/w) in the intact cell. Obviously a large fraction of the lipids in the erythrocyte membrane will be perturbed by this glycoprotein and this interaction may dramatically affect their ability to undergo both lateral and transverse diffusion.

At protein mole fractions above $0.86 \cdot 10^{-3}$ (less than 1160 phospholipids per protein), a 'break' occurs in the plot shown in Fig. 5, and ΔH then increases with protein concentration. Thus it appears that phospholipids are being released from the glycoprotein at higher protein mole fractions and returned to the phase transition so that they participate in melting once again. However, these released phospholipids do not possess the thermotropic properties of pure DMPC and their melting behaviour is still considerably disrupted by the presence of the glycoprotein; the cooperative unit remains low (Fig. 4) and the shape of the transition is still broad (Fig. 2). Freeze-fracture electron microscopic studies have shown that the concanavalin A receptor gives rise to intramembranous particles at lipid/protein ratios as high as 1 : 1 (w/w) [21] and is therefore reconstituted in a membrane-spanning fashion at protein concentrations higher than the break point of 8.5 : 1 (w/w) lipid/protein. Unilamellar vesicles containing the reconstituted glycoprotein are also capable of binding lectins such as succinyl-concanavalin A at their surface in a quantitative fashion, up to a lipid/protein ratio of 2 : 1 (w/w) [10]. Both these pieces of evidence argue against a fundamental change in the arrangement of the glycoprotein in the bilayer which would account for the break in the ΔH curve. This behaviour may however be explained if the concanavalin A receptor forms clusters or aggregates in the bilayer above a certain critical protein concentration (less than 1160 phospholipids per protein, 8.5 : 1, w/w). If protein-protein contacts occur in these aggregates, the number of phospholipids perturbed by each protein would not remain constant, but would drop with increasing protein/lipid ratios. This would result in an increase in the measured ΔH as the protein formed clusters in the bilayer and released phospholipids previously removed from the phase transition, which could then participate in melting.

There is considerable evidence in the literature supporting the idea that the concanavalin A receptor (Band 3) forms protein aggregates, both in detergent solutions and in a bilayer matrix. Lukacovic et al. have noted that Band 3 shows a marked tendency to form high molecular weight aggregates in Triton X-100 solution and reported that β-mercaptoethanol could act as a stabilizing agent [8]. Freeze-fracture electron microscopy studies on the concanavalin A receptor in lipid bilayers have shown that the intramembranous particles due to this glycoprotein appear relatively homogeneously dispersed at low protein/lipid ratios [5,12] although some 'jumbled' lipid structures containing particles were found. If the protein concentration in the bilayer is increased to 1 : 1 (w/w) the intramembranous particles become densely packed and appear to deform the bilayer structure [21]. Studies on the rotational diffusion of Band 3 have also been interpreted in terms of self-association of the protein within the erythrocyte membrane itself [22]. Thus the available information suggests that strong protein-protein interactions exist between individual concanavalin A receptor molecules and this supports our conclusions that the glycoprotein forms clusters or aggregates in the bilayer above a critical protein concentration. These protein-protein interactions may be hydrophobic in nature, involving contacts between the nonpolar surfaces of the transmembrane peptide assembly proposed by Rothstein.

Although protein aggregation seems most likely to play the dominant role in the thermotropic behaviour of concanavalin A receptor-phospholipid recombinants, it is also possible that another type of phospholipid-protein interaction may come into play at high protein mole fractions, and this factor may contribute to the dramatic change in slope of the ΔH plot in Fig. 5. Substantial hydrophilic interactions at the membrane surface be-

tween phospholipid headgroups and the aqueous segment of glycoproteins have already been shown for glycophorin in lipid bilayers at low protein mole fractions [4] and have also been proposed for vesicular stomatitis virus glycoprotein [19]. This type of surface interaction might also play a role in the complex relationship between the concanavalin A receptor and membrane phospholipids. At lipid/protein ratios less than 8.5 : 1 (w/w) the glycoprotein headgroup region may fold back down to the bilayer surface in a conformation resulting in maximum interaction with phospholipid headgroups. In this way, a single glycoprotein is able to perturb membrane lipids by both hydrophobic interactions within the bilayer matrix and hydrophilic interactions at the bilayer surface, resulting in a large value for N. A conformational change in the headgroup region at the critical lipid/protein ratio of 8.5 : 1 (w/w), such that the new conformation has a much reduced ability to participate in surface interactions with phospholipids, would result in the release of lipids which were previously bound. Rüppel et al. [4] have suggested that such a conformational change in the carbohydrate-bearing headgroup would be favoured for steric reasons, since it allows a higher protein packing density within the bilayer.

Effects of protein-protein interactions on the behaviour of the concanavalin A receptor

Binding of concanavalin A and succinyl-concanavalin A to the receptor glycoprotein shows positively cooperative characteristics at low lectin concentrations in both the intact erythrocyte [23] and reconstituted systems [10,24]. We have shown that these cooperative properties are not dependent on either rapid lateral mobility of the receptor itself or dimer-tetramer equilibrium of the lectin, and it seems likely that a lectin-induced conformational change in nearby receptors is involved [10]. Our lectin binding studies were carried out in 4 : 1 (w/w) PC/PS mixtures rather than in pure phospholipids such as the DMPC used for the calorimetric experiments, but we have confirmed that the ΔH vs. protein mole fraction curve shows the same general two-slope behaviour in mixtures of 4 : 1 (w/w) DPPC-PS (Chicken, C.A. and Sharom, F.J., unpublished results). Thus at the 4 : 1 (w/w) lipid/protein ratios used in the lectin binding studies, the receptor is also most likely to exist as protein aggregates. It thus seems logical to ask whether the observation of cooperativity is related to receptor clustering in the bilayer. Lectin binding to a single glycoprotein within a larger aggregate could relatively easily lead to transmission of a perturbation to adjacent receptors resulting in a conformational change in the headgroup regions of these nonbound receptors. Given the fact that Band 3 in native erythrocyte membranes is also present as aggregates [22] this clustering may also be responsible for the positive cooperativity of concanavalin A binding to the intact cell.

We have previously observed that the inclusion of the concanavalin A receptor in lipid bilayers containing glycophorin results in a dramatic reduction in liposome agglutinability by glycophorin-directed immunoglobulins, without affecting the ability of glycophorin to bind these agents [5]. It was proposed that the concanavalin A receptor modified glycophorin arrangement or presentation, thus preventing the correct pattern of antibody crosslinking necessary for agglutination to occur. If glycophorin molecules were tightly associated with concanavalin A receptor aggregates in the bilayer, either by specific association or by physical 'trapping', then this immobilization might greatly reduce the ability of glycophorin to participate in immunoglobulin crosslinking by preventing the correct surface arrangement of glycoproteins from being achieved.

Acknowledgements

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