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LIPID PHASE STATES INFLUENCE GLYCOPHORIN RECONSTITUTION

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Summary

Reconstitution of glycophorin into dimyristoyl phosphatidylcholine and sphingomyelin vesicles was sub-maximal below the phase transition temperatures of these lipids. Reconstitution of glycophorin into diisostearoyl phosphatidylcholine and dioleoyl phosphatidylcholine liposomes was maximal within a range of temperatures below the phase transition temperatures of dimyristoyl phosphatidylcholine and sphingomyelin but above the phase transition temperatures of diisostearoyl phosphatidylcholine and dioleoyl phosphatidylcholine. These findings indicate a greater tendency for reconstitution of glycophorin into fluid as opposed to solid lipid phases.

The affinity of glycophorin for fluid, as opposed to solid, regions of phospholipid bilayers has been inferred from two kinds of evidence. (1) Intramembranous particles of glycophorin detected in freeze-fractured reconstituted vesicles were confined to fluid regions of the lipid bilayer [1]. Results obtained with the freeze-fracture technique can be misleading, however, insofar as the absence of particles from solid regions does not necessarily signify the absence of glycophorin from those regions [2]. (2) At temperatures below the phase transition of dipalmitoyl phosphatidylcholine (DPPC), two components could be resolved in NMR spectra of DPPC-glycophorin vesicles similar to those in Ref. 1: a sharp signal from the ^{13}C in the choline methyl groups of DPPC corresponding with 'fluid' DPPC adjacent to the glycophorin, and a broad signal, corresponding with the bulk solid DPPC [3]. Disappearance of the sharp signal on incorporation of dielaidoyl phosphatidylcholine (DEPC) into such vesicles was interpreted as either a stimulation of the exchange be-

Abbreviations used: DMPC, dimyristoyl phosphatidylcholine; DOPC, dioleoyl phosphatidylcholine; DISPC, diisostearoyl phosphatidylcholine; DPPC, dipalmitoyl phosphatidylcholine; DEPC, dielaidoyl phosphatidylcholine.

tween fluid and solid DPPC by DEPC or a displacement of the fluid DPPC by truly fluid DEPC which has a lower phase transition temperature than DPPC.

This report contains another kind of evidence that glycophorin tends to be excluded from solid phase lipid, based on a quantitative analysis of the temperature dependence of glycophorin reconstitution into lipid vesicles, according to the method of MacDonald and MacDonald [4]. Glycophorin was isolated according to the procedure of Marchesi and Andrews [5]. Reconstitution was accomplished by mixing 100—200 μg of glycophorin in small aliquots of aqueous solutions with 1 mg of phospholipid in chloroform: methanol, 2:1, evaporating the organic solvent at various temperatures, suspending the dried lipid-protein film in 2 ml of 0.14 M NaCl + 0.01 M Tris-HCl, pH 7.5, at room temperature to 40°C, and centrifuging for 30 min at 10 000 rev./min in a Sorvall SS34 rotor. Supernatants containing unincorporated glycophorin and pellets of glycophorin-reconstituted vesicles were assayed for sialic acid, which comprises 25% of glycophorin by weight [6], by the method of Warren [7]. Phospholipids used were DISPC synthesized in this laboratory [8], DOPC, DMPC and bovine brain sphingomyelin. The last three phospholipids were purchased from Sigma Chemical Co. and used without further purification.

Temperature is crucial for reconstitution during removal of the organic solvent but not during resuspension of the dried lipid-protein film, presumably because the formation of lipid-glycophorin complexes occurs only as the organic solvent is evaporating. Temperature control was contrived by immersing the round-bottomed flask containing glycophorin and appropriate lipid in a water bath at the desired temperature which was constantly monitored during evaporation of the organic solvent. This arrangement was not ideal because: (1) the temperature of the flask contents under vacuum could not be measured directly and (2) the evaporation of organic solvent is temperature dependent and reconstitutions at the lowest temperatures took up to six times longer than those at the highest temperatures. The time differences between reconstitutions of glycophorin and the same phospholipid species at different temperatures could not be eliminated. Although some variability in the results may reflect less than ideal temperature control, it was adequate for demonstrating the temperature dependence of glycophorin reconstitution with DMPC and with sphingomyelin.

The three figures indicate the percent of glycophorin incorporated into various phospholipid bilayers as a function of temperature. Means ± S.E. were calculated for groups of values within every 10°C-increment above 15°C. Incorporation into DMPC liposomes increases from 37 to 57% between 15 and 25°C and remains constant thereafter (Fig. 1). Incorporation into sphingomyelin liposomes increases from 7 to 51% between 15 and 35°C, after which it plateaus (Fig. 2). Both DMPC and sphingomyelin, the phase transition temperatures of which are 23 [9] and 20—37°C ([10] for red cell sphingomyelin), respectively, appear to accommodate glycophorin best above their phase transition temperatures. In contrast with incorporation into DMPC and sphingomyelin, incorporation into DISPC and DOPC decreases from 67 to 44% between 15 and 35°C, after which no change is seen (Fig. 3). Phase transition temperatures of DISPC [8] and DOPC [9] are below 0°C. The sig-

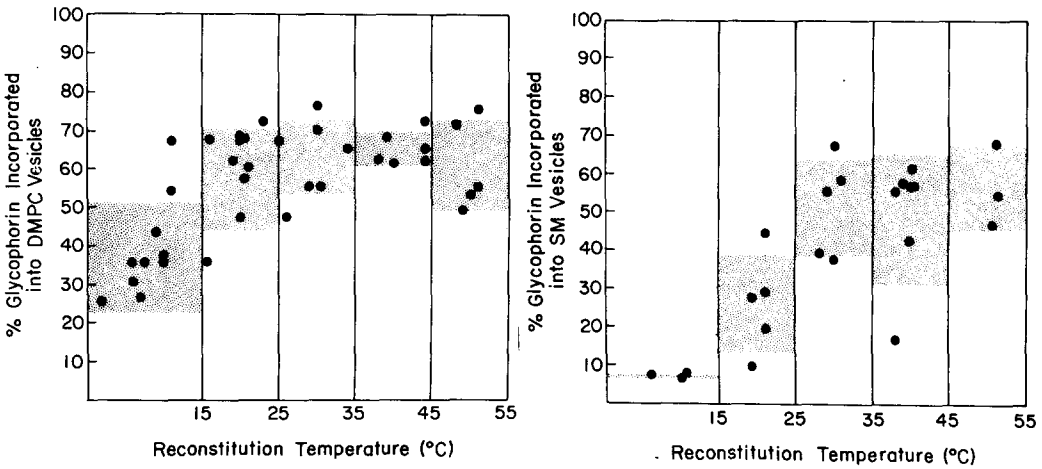

Fig. 1. Percent of glycophorin reconstituted into DMPC vesicles as a function of temperature. Reconstitution was accomplished as described in the text and unincorporated glycophorin remaining in the aqueous supernatant and glycophorin reconstituted with lipid vesicles were determined by assaying the sialic acid in both fractions.

Fig. 2. Percent of glycophorin reconstituted into sphingomyelin vesicles as a function of temperature. Reconstitution was accomplished as described in the text and unincorporated glycophorin remaining in the aqueous supernatant and glycophorin reconstituted with lipid vesicles were determined by assaying the sialic acid in both fractions.

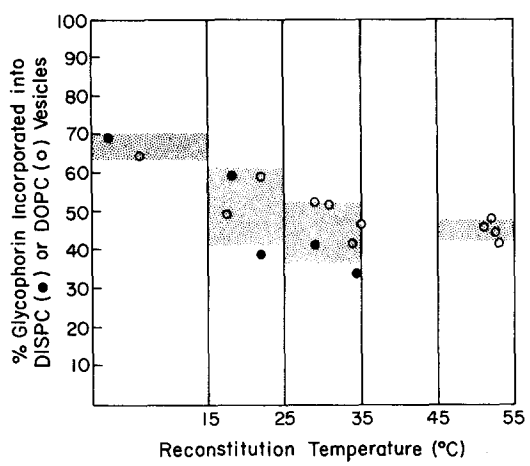

Fig. 3. Percent of glycophorin reconstituted into DISPC (●) or DOPC (○) vesicles as a function of temperature. Reconstitution was accomplished as described in the text and unincorporated glycophorin remaining in the aqueous supernatant and glycophorin reconstituted with lipid vesicles were determined by assaying the sialic acid in both fractions.

nificant incorporation of glycophorin into DISPC and DOPC liposomes at temperatures from 0 to 25°C rules out the possibility that glycophorin is unfit for reconstitution into lipid bilayers between those temperatures. Finally, Table I indicates that the mean total recovery of incorporated + unincorporated glycophorin ± S.E. was the same for each type of phospholipid.

Glycophorin being more readily reconstituted with phospholipid above, as opposed to below, its lipid phase transition temperature is not strictly analogous to the distribution of glycophorin within a bilayer composed of fluid and solid regions. With the type of reconstitution method followed

TABLE I

PERCENT RECOVERIES OF GLYCOPHORIN ± S.E. SUBSEQUENT TO RECONSTITUTION OF GLYCOPHORIN INTO VESICLES OF VARIOUS PHOSPHOLIPID COMPOSITIONS

Percent values equal (amount of glycophorin incorporated plus amount of glycophorin not incorporated) divided by the starting amount of glycophorin multiplied by 100. Amounts of glycophorin were determined by assaying its sialic acid.

Phospholipid	Glycophorin recovered (%)
Dimyristoyl phosphatidylcholine	83.0 ± 7.1
Sphingomyelin	86.4 ± 12.5
Diisostearoyl phosphatidylcholine	77.3 ± 5.1
Dioleoyl phosphatidylcholine	85.9 ± 10.0

here, the unincorporated glycophorin undergoes a presumably micellar type of aggregation [5] which allows it to remain in the aqueous phase instead of entering the lipid bilayer. Nevertheless, the tendency of glycophorin to associate with fluid lipid rather than undergo self-aggregation and the tendency of glycophorin to self-aggregate rather than to associate with solid lipid provide a reasonable basis for the conclusion that glycophorin prefers fluid to solid lipid. The relevance of these and other [1,3] findings for the behavior of glycophorin in intact red cells is uncertain. Recent evidence indicates that glycophorin is not aggregated in red cell membranes. (1) Freeze-fractured human red cells of blood group En(a–), which contain no glycophorin, do not differ from those of normal human red cells with respect to number and morphology of intramembranous particles [11]. (2) Freeze-fractured vesicles, produced by pH manipulation, high-salt washing and Triton treatment of red cells and containing glycophorin but no band III, are particle-free [2]. In contrast, glycophorin molecules reconstituted into vesicles in the previous [1,3] and current studies were more (8-nm particles [1]) or less (4—5-nm particles [12]) aggregated and may have displayed a phase preference because of their experimentally-induced state of aggregation.

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