PROTEIN-LIPID INTERACTIONS: GLYCOPHORIN AND DIPALMITOYLPHOSPHATIDYLCHOLINE

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Received November 13,1975

SUMMARY: The choline methyl groups of dipalmitoylphosphatidylcholine were enriched with ^{13}C, and glycophorin extracted from human erythrocytes was included in bilayers of this phospholipid. At temperatures below the transition temperature, the ^{13}C nuclear magnetic resonance spectra have two components, one sharp and one broad. The sharp signal is attributed to relatively "fluid" lipids in the immediate vicinity of the glycoprotein. In a defined ternary mixture consisting of ^{13}C-labeled dipalmitoylphosphatidylcholine, dielaidoylphosphatidylcholine and glycophorin, the sharp ^{13}C resonance signal disappears below the transition temperature of the mixture, indicating that the unsaturated lipid is preferentially associated with the glycoprotein under these conditions.

MATERIALS AND METHODS: Glycophorin was isolated from human erythrocytes and incorporated into phospholipid bilayers as described by Grant and McConnell (1). The synthesis of ^{13}C enriched DPPC, DEPC, the phase diagram of these two lipids, and various assays are given in a previous paper (2). In preliminary experiments, it was noticed that protein-containing vesicles undergo enhanced fusion. The vesicles were centrifuged at 200,000g for 24 hours on a flat support. The spectra were taken using the centrifuged pellet with a Bruker 360 operating at 90.5 MHz (2), using a delay of 16μsec before collecting the magnetization. Freeze-fracture and

Abbreviations used are: DPPC, dipalmitoylphosphatidylcholine; DPPC* is DPPC enriched 90% with ^{13}C in the choline methyl groups; DEPC, dielaidoylphosphatidylcholine; DEPC* is DEPC enriched 90% with ^{13}C in the choline methyl groups; Tc is the chain melting transition temperature of a phospholipid.

electron microscopy (1) were performed on the centrifuged pellet and showed large extended layers. Samples with and without protein were prepared and examined under the same conditions.

RESULTS AND DISCUSSION: The ^{13}C nuclear magnetic resonance spectra of DPPC* containing one mole percent of glycophorin (molecular weight assumed equal to 50,000) are shown in Fig. 1 at three temperatures in the vicinity of $Tc \simeq 41^{\circ}C$. In contrast to DPPC* alone (see Fig. 2), the resonance spectrum of DPPC* in the presence of one mole percent glycophorin shows both a sharp and a broad component at temperatures below Tc; these two components remain distinct at temperatures as low as $31^{\circ}C$. Freeze-fracture electron microphotographs of the samples showed clearly that the glycophorin-associated particles are distributed randomly throughout the bilayer membrane. (For similar freeze-fracture microphotographs, see Grant and McConnell (2)). Thus, the sharp ^{13}C choline methyl signal is associated with DPPC* molecules in the vicinity of one, or at most a few, glycophorin molecules (certainly not "patches" containing large numbers of glycophorin molecules). The relative sharpness of the ^{13}C choline methyl signal shows that motional freedom of the glycophorin-associated DPPC* choline methyl groups is greater than the choline methyl groups in the bulk lipid phase below Tc. This suggests, but does not prove, that the overall motional freedom of the glycophorin-associated DPPC* is higher than the bulk DPPC* for temperatures $T < Tc$. (The sharp signal might be due to an enhanced mobility of the choline methyl group and not be related to enhanced motions of other groups in the DPPC* molecule. On the other hand, a preference of glycophorin for fluid lipids has already been demonstrated in that when fluid and solid lipid phases of DPPC and DEPC coexist, glycophorin is

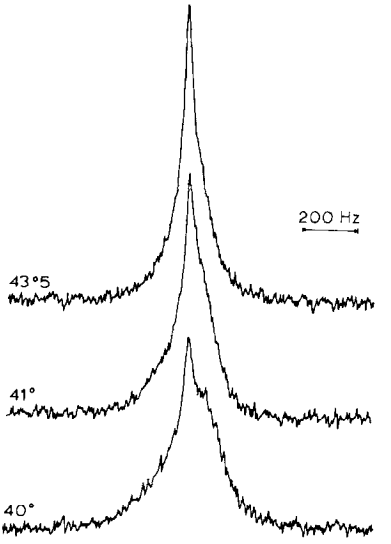

Figure 1. ^{13}C NMR signal from ^{13}C enriched choline methyl groups of DPPC in bilayer containing glycophorin. 6 mg of DPPC* were used. The spectra were taken at 90.5 MHz with 4000 pulses, and without spinning the sample.

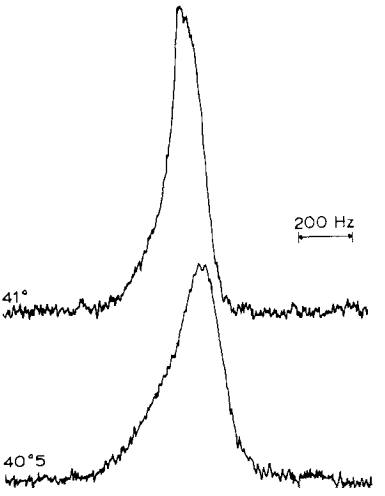

Figure 2. ^{13}C NMR signal from DPPC*. In all reported spectra, protons were decoupled using a broad-band decoupler with 5 watts of power.

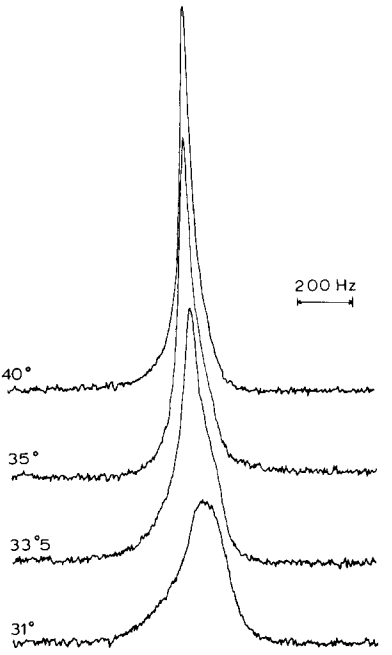

Figure 3. ^{13}C NMR signal from DPPC* in a DPPC:DEPC 83:17 mixture. The bilayer contains one mole percent of glycophorin.

found predominantly localized in the fluid phase (1)).

A surprising feature of the 40°C spectrum (and spectra at other lower temperatures) seen in Fig. 1 is that the sharp signal is so well resolved. One might have anticipated that exchange of DPPC* molecules between the environment of glycophorin and the bulk DPPC* would be so fast that the sharp signal would be strongly broadened. Since there is no extensive polymerization of the glycophorin (with a possible trapping of "fluid" lipids), we can only conclude that the glycophorin-associated DPPC* molecules do not exchange rapidly with available bulk DPPC* molecules under the phase transition.

It will also be seen in Figs. 1 and 2 that glycophorin produces a narrowing of the ^{13}C resonance signal in bilayers of DPPC* at temperatures greater

than Tc. The charged glycophorin molecules may give rise to a repulsion of adjacent bilayers, possibly enhancing motional narrowing, and/or possibly giving a more uniform orientation of the bilayer planes relative to the applied field.

The obliteration of the sharp ^{13}C choline methyl signal in the presence of 17 mole percent DEPC must be due to one of two possible effects. Either the glycophorin-associated phospholipid tends to be unsaturated, thus displacing DPPC*, or the presence of DEPC in the lipid mixture enhances the rate of DPPC* lipid exchange between the fluid and solid phase. The second possibility is consistent with a previously reported study of ^{13}C choline nuclear resonance spectra of binary mixtures of DPPC* and DEPC; and DPPC and DEPC* (2).

The present study has a bearing on previous studies involving lipid-lipid and lipid-protein interactions. Brûlet and McConnell (2) have reported an experimental and theoretical investigation of the ^{13}C nuclear resonance of DPPC* in binary mixtures of DPPC* and DEPC as a function of temperature in relation to the DPPC-DEPC phase diagram. This study was hampered to some extent by the large ^{13}C line widths for both "fluid" phase and "solid" phase lipids, and in no case was it possible to resolve signals from the solid and fluid phases. In the present work, glycophorin in the membranes produces the above-mentioned sharpening of the ^{13}C signal; in the DEPC-DPPC* mixture containing glycophorin one can detect overlapping but nonetheless distinguishable signals from ^{13}C in the solid and fluid phases at a temperature 33.5°C, when roughly half the ^{13}C is expected to be in the fluid phase and half the ^{13}C is expected to be in the solid phase.

Our results also have a bearing on the temperature dependence of sugar transport into bacterial unsaturated fatty acid auxotrophs grown on simple

unsaturated fatty acid, such as elaidic acid (3). When such cells are grown at, e.g., 37°C, and sugar transport assayed at various temperatures, it is found that the transport rates show characteristic changes at temperatures corresponding to the onset and completion of lateral phase separations of the membrane lipids. But surprisingly, transport does not stop completely even at temperatures where various physical measurements indicate that the bulk of the membrane lipids are in the solid ("frozen") state. Clearly this effect may arise if protein-associated lipids remain in a "fluid state" at temperatures well below the freezing temperature of the bulk phospholipids.

The present work also bears on still another problem in membrane biophysics, namely the relation between the lateral diffusion of a membrane protein and the "viscosity" of the membrane lipids. Clearly, if a membrane protein is surrounded by "fluid" lipids it can undergo lateral diffusion, even though the bulk of the lipids are in a crystalline solid state - a process merely requiring the melting and freezing of lipids on two sides of the membrane protein. Although this rate may be low relative to a completely fluid bilayer, it is certainly not determined by the "viscosity" of the solid phase.

ACKNOWLEDGEMENTS: This research has been sponsored by the National Science Foundation (Grant BMS 75 02381).

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