RAMAN AND FOURIER TRANSFORM INFRARED SPECTROSCOPIC STUDIES OF THE INTERACTION BETWEEN GLYCOPHORIN AND DIMYRISTOYLPHOSPHATIDYLCHOLINE

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The question of the existence of an immobilized class of lipid adjacent to membrane protein is of current interest in membrane biophysics. The evidence for this so-called "boundary layer lipid" is contradictory. The conflicting results may be partially reconciled as a consequence of (*a*) the experiments being performed with different proteins; (*b*) the different time domains of the various spectroscopic techniques employed; and (*c*) the presence of possibly perturbing probe molecules.

The current study involved a vibrational spectroscopic determination of acyl chain order and mobility in dimyristoylphosphatidylcholine (DMPC)-glycophorin complexes. Since Raman and Fourier transform infrared (FTIR) spectroscopy monitor hydrocarbon chain conformation on a subpicosecond time scale without the use of probe molecules, the possible existence of an immobilized component can be examined in a time domain faster than magnetic methods and without the potential for system perturbation caused by probes.

RESULTS AND DISCUSSION

Glycophorin was extracted from lyophilized human erythrocyte ghosts and purified according to Marchesi and Andrews (1). Reconstitution into phospholipid vesicles according to the method of MacDonald and MacDonald (2) led to homogeneous preparations of unilamellar vesicles 0.1–0.15 μm Diam.

The C-H stretching region in the FTIR spectrum responds to alterations in phospholipid acyl chain order and mobility. The frequency position monitors conformational order while the bandwidth reflects the dynamic

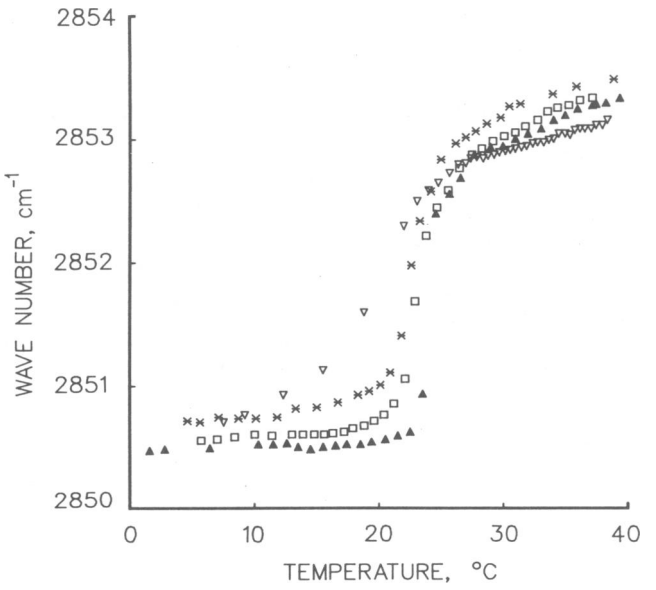

FIGURE 1 *A* Plots of frequency vs. temperature for the symmetric CH_2 stretching band in the infrared spectra of DMPC multibilayers (▲), and DMPC - glycophorin complexes (□, 200:1, *, 100:1, ▽, 50:1).

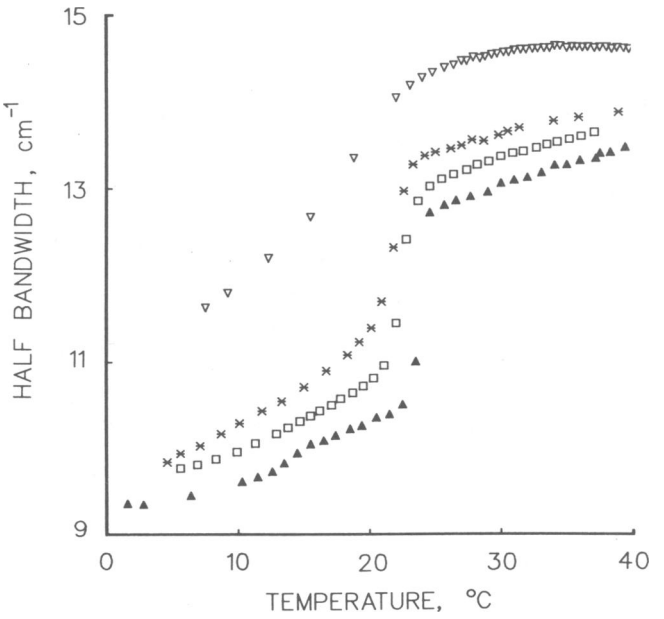

FIGURE 1 *B* Plots of half bandwidth vs. temperature for the symmetric CH_2 stretching band in the infrared spectra of DMPC multibilayers (▲), and DMPC -glycophorin complexes (□, 200:1, *, 100:1, ▽, 50:1).

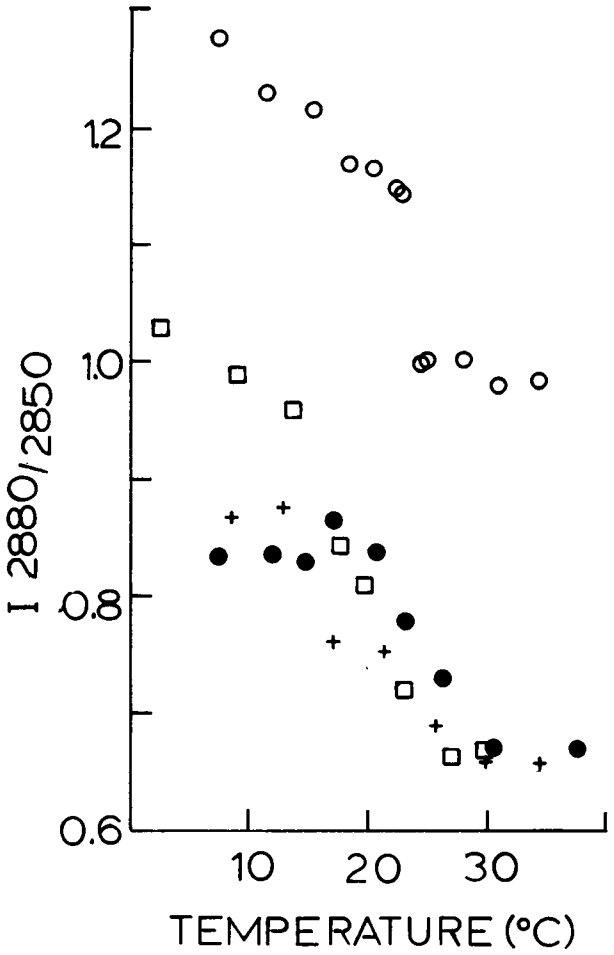

FIGURE 2 Temperature dependence of the Raman parameter I 2880/2850 for: DMPC multilamellar vesicles (○); DMPC sonicated unilamellar vesicles (●); 100:1 DMPC - glycophorin complexes (□); 50:1 DMPC - glycophorin complexes (+)

behavior. At temperatures removed from the lipid phase transition region in both the gel and liquid crystalline phases, the CH_2 symmetric stretching frequency near 2850 cm^{-1} remains approximately constant as protein is incorporated (Fig. 1 *A*), indicating that conformational order is not greatly affected by glycophorin. However, at any given temperature, the bandwidth of this vibration progressively increases as the amount of protein in the vesicle is increased (Fig. 1 *B*). This behavior shows increased acyl chain mobility induced by protein in both the gel and liquid crystalline phases. In the liquid crystalline phase, both pure DMPC and DMPC-glycophorin complexes exhibit increases in frequency and bandwidth with increasing temperature, indicative of a continuous increase in conformational disorder and acyl chain mobility. There is no indication in any of the melting curves of a second transition at higher temperatures which may be associated with boundary lipid. The data in Figs. 1 *A* and 1 *B* primarily demonstrate an abolished pretransition and a main transition that is progressively broadened and shifted to lower temperatures as protein is incorporated.

Evidence for the disruption of lipid packing by glycophorin is available from the Raman spectroscopic data. The peak height of the asymmetric methylene stretching band near 2880 cm^{-1}, in addition to responding to rates of motion about the long chain axis, is sensitive to interchain lateral interaction (3, 4). The temperature dependence of this spectral parameter (appropriately ratioed) is shown in Fig. 2 for two lipid:protein ratios as well as for DMPC in multilamellar and unilamellar form. The progressive reduction in I 2880/2850 as protein is introduced reflects a reduction in interchain vibrational coupling and an increase in acyl chain mobility. The lipids do not exist in a well-packed array even at low temperatures where they are highly ordered.

The primary conclusion from the current investigation is that there is no evidence for an immobilized (higher melting) lipid class in the DMPC/glycophorin system as monitored via the acyl chain vibrational spectrum. The data show a broadened phase transition which is progressively shifted to lower temperatures as protein is incorporated. The addition of protein results in little change in lipid conformational order but significantly increases the mobility of both the gel and liquid crystalline phases.

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