Fain, J. N. (1968) *Endocrinology 83*, 548–554.

Fairclough, P., Malathi, P., Preiser, H., & Crane, R. K. (1979) *Biochim. Biophys. Acta 553*, 295–306.

Geiger, P. J., & Bessman, S. P. (1972) *Anal. Biochem. 49*, 467–473.

Goldin, S., & Rhoden, V. (1978) *J. Biol. Chem. 253*, 2575–2583.

Hart, W. M., Jr., & Titus, E. O. (1973) *J. Biol. Chem. 248*, 4674–4681.

Kahlenberg, A., & Zala, C. A. (1977) *J. Supramol. Struct. 7*, 287–300.

Kasahara, M., & Hinkle, P. C. (1976) *Proc. Natl. Acad. Sci. U.S.A. 73*, 396–400.

Kasahara, M., & Hinkle, P. C. (1977) *J. Biol. Chem. 252*, 7384–7390.

Laemmli, U. K. (1970) *Nature (London) 227*, 680–685.

Laurent, T. C., & Killander, J. (1964) *J. Chromatogr. 14*, 713–730.

LeTarte, J., Jeanrenaud, B., & Renold, A. E. (1969) *Biochim. Biophys. Acta 183*, 357–365.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951) *J. Biol. Chem. 193*, 265–275.

Rodbell, M. (1966) *J. Biol. Chem. 241*, 130–139.

Shanahan, M. F., & Czech, M. P. (1977a) *J. Biol. Chem. 252*, 6554–6561.

Shanahan, M. F., & Czech, M. P. (1977b) *J. Biol. Chem. 252*, 8341–8343.

Sogin, D. C., & Hinkle, P. C. (1978) *J. Supramol. Struct. 8*, 447–453.

Sogin, D. C., & Telford, J. N. (1978) *Fed. Proc., Fed. Am. Soc. Exp. Biol. 37*, 1567.

Steck, T. L. (1974) *J. Cell Biol. 62*, 1–19.

Wardzala, L. J., Cushman, S. W., & Salans, L. B. (1978) *J. Biol. Chem. 253*, 8002–8005.

Zoccoli, M., Baldwin, S. A., & Lienhard, G. E. (1978) *J. Biol. Chem. 253*, 6923–6930.

Carbon-13 Nuclear Magnetic Resonance Studies on the Interaction of Glycophorin with Lecithin in Reconstituted Vesicles[†]

Hideo Utsumi, Budi D. Tunggal, and Wilhelm Stoffel*

ABSTRACT: Glycophorin, the MN blood group substance, is a major intrinsic glycoprotein in erythrocyte membranes. The interaction of glycophorin with phosphatidylcholine, ^{13}C-labeled in specific positions in reconstituted unilamellar vesicles, was investigated by using the ^{13}C NMR technique. 1-Palmitoyl-2-([14-^{13}C]linoleoyl)-*sn*-glycero-3-phosphocholine was synthesized and used as a probe. At 37 °C the spin–lattice relaxation time (T_1) of vesicle bilayers consisting of this phospholipid was 0.74 s in the absence of glycophorin. The incorporation of glycophorin decreased the T_1 to 0.63 s, indicating that the bulk lipid molecules are somewhat immobilized by glycophorin. In addition to the reduction in time, a broad component $(\Delta H_{1/2} = \sim 40 \text{ Hz})$ superimposing the sharp resonance was observed in the ^{13}C NMR spectrum of reconstituted vesicles. The T_1 of the broad component was 0.32 s, suggesting that the lipid molecules contributing to the broad component may be more restricted than that of the sharp component. In order to quantify the broad component, a computer simulation was performed. The intensity of the broad component estimated from the simulation depended linearly on the concentration of glycophorin. Therefore, the broad component is considered to be contributed by a phospholipid domain surrounding the glycophorin molecules, a so-called "boundary lipid". The relationship between the broad component and the stoichiometry of the reconstituted vesicles allows the conclusion that about 30 lipid molecules are immobilized by one glycophorin monomer.

Recently, many investigations on the molecular motions of membrane lipids have provided evidence that lipid molecules show motions within the membrane, e.g., trans–gauche isomerization in the acyl chains, rotational motion, and lateral and transverse diffusion. These motions are affected by the incorporation of membrane proteins. In 1973, Jost et al. concluded from their results of spin-labeling studies on a cytochrome *c* oxidase–lecithin complex that a "boundary lipid" exists around membrane proteins (Jost et al., 1973). They are less mobile than the bulk lipids. Dahlquist et al. (1977) have also provided evidence for a boundary lipid using ^2H NMR.[1] Other groups, however, deny the existence of boundary lipids (Oldfield et al., 1978; Seelig & Seelig, 1978). The discrepancy might arise from differences in experimental techniques or

experimental systems. ^{13}C NMR has some advantages over spin-labels in studying protein–lipid interactions, since there is no perturbation of the probe.

Glycophorin is a major intrinsic glycoprotein of the erythrocyte membrane. It may play a role as a membrane receptor, since glycophorin is well-known to have MN blood group characteristics. The extensive work of Marchesi's group has characterized glycophorin thoroughly. It seems to exist as a dimer in the membrane, with its C-terminal end at the cytoplasmic side of the membrane, and its N-terminal end, containing the sugar residues, exposed to the exterior of the cell (Marchesi et al., 1976). The interaction of glycophorin with lipids in the membrane is, however, not well understood from a dynamic standpoint, although several investigations have

[1] Abbreviations used: NMR, nuclear magnetic resonance; CD, circular dichroism; HDL, high-density lipoprotein; NaDodSO$_4$, sodium dodecyl sulfate; Tris, 2-amino-2-(hydroxymethyl)-1,3-propanediol; EDTA, ethylenediaminetetraacetic acid.

been performed with the reconstituted glycophorin–phospholipid system (Grant & McConnell, 1974; Redwood et al., 1975; MacDonald & MacDonald, 1975; van Zoelen et al., 1978a,b).

The effects of glycophorin on lipid molecular motion in reconstituted membranes as studied by ^{13}C NMR are reported. The existence of the boundary lipid suggested from line-shape analysis is discussed.

Materials and Methods

Materials. Packed erythrocytes in ACD–adenine–anticoagglutine were kindly provided by Professor Dr. J. Krüger (Abteilung für Bluttransfusion, Universität zu Köln). [14-^{13}C]Linoleic acid was synthesized as described by Stoffel (1964, 1965). 1-Palmitoyl-2-([14-^{13}C]linoleoyl)-*sn*-glycero-3-phosphocholine was synthesized by acylation of 1-palmitoyl-*sn*-glycero-3-phosphocholine with the acyl chloride of [14-^{13}C]linoleic acid according to the method described by Stoffel et al. (1972) and was stored as a chloroform solution at –20 °C. Soybean [N-^{14}CH$_3$]lecithin was synthesized according to Stoffel et al. (1972). All phospholipids showed a single spot on thin-layer chromatograms. Other reagents were of commercial origin and were used without further purification.

Preparation of Glycophorin. Ghosts were prepared according to Dodge et al. (1963). Crude glycophorin was isolated from ghosts according to the method described by Kathan et al. (1961). The crude glycophorin was purified by chromatography on Sepharose 6B as follows: The crude glycophorin was dissolved in a solution containing 10 mM Tris, 0.2% NaDodSO$_4$, 1 mM EDTA, and 0.02% sodium azide and applied to a column of Sepharose 6B (90 × 2 cm) equilibrated with the same buffer at 45 °C. The fractions containing glycophorin were dialyzed against distilled water and lyophilized. Disc electrophoresis was carried out according to Weber & Osborn (1969). The purified glycophorin showed a single band on both NaDodSO$_4$–polyacrylamide gels stained with Coomassie blue and with periodate–Schiff reagent.

Reconstitution of Glycophorin with ^{13}C-Labeled Phospholipid. The reconstitution of glycophorin with phospholipid was performed by the method according to MacDonald & MacDonald (1975). Glycophorin (30–60 mg) in 1 mL of distilled water and 30–60 mg of ^{13}C-labeled phospholipid containing trace amounts of ^{14}C-labeled phospholipid were mixed in 75 mL of chloroform–methanol (2:1) solvent. The mixture was dried in a rotary evaporator to form a lipid–protein film. Tris buffer, pH 7.4 (2.5 mL, 10 mM), containing 20% D$_2$O and 0.02% sodium azide was added to the lipid film, and the mixture was vigorously agitated on a Vortex mixer. Of the resulting vesicle suspension, 2 mL was used for the NMR measurement. The suspension (0.5 mL) was subjected to a Sepharose 2B column in order to evaluate the protein to lipid ratio in the vesicles.

Stoichiometry of Glycophorin and Phospholipid in the Reconstituted Vesicles. The suspension (0.5 mL) was subjected to a Sepharose 2B column (90 × 1.6 cm) equilibrated with 10 mM Tris-HCl, pH 7.4, containing 0.02% sodium azide at room temperature in order to separate the reconstituted vesicles from free glycophorin. Glycophorin was measured with the Lowry method (Lowry et al., 1951), sialic acid was assayed by the method according to Diringer (1972), and phospholipid was determined from the radioactivity of soybean [N-^{14}CH$_3$]lecithin admixed to the ^{13}C-labeled phospholipid.

^{13}C NMR Measurement and Line-Shape Analysis. Proton noise-decoupled ^{13}C NMR spectra were obtained at 22.63 MHz with a Bruker WH-90 pulse spectrometer operating in the Fourier transform mode. The proton decoupling power was 5 W.[2] An internal deuterium field frequency lock was

used. The free induction decay signals were obtained following a 24-μs pulse by using a 180°-t-90° pulse sequence, where t is the delay time between the 180 and 90° pulses (Freeman & Hill, 1970; Vold et al., 1968). The suspension (2 mL) was transferred in an argon atmosphere into samples tubes of 10 mm o.d. sealed with a Teflon stopper in order to avoid the liquid–vapor interchange. The temperature was controlled with a Bruker ST 100/700 (variable-temperature controller). The calculations of line shape were performed by using the equation described under Results. The resulting spectra were obtained with a Houston Plotter (COMPLOT), controlled by a 12K Varian computer (SS-100 MS).

Results

Reconstitution of Glycophorin with ^{13}C-Labeled Phospholipid. Glycophorin reconstituted with phospholipid vesicles according to MacDonald & MacDonald (1975) are unilamellar vesicles, with diameters between 4000 and 5000 Å (MacDonald & MacDonald, 1975; van Zoelen et al., 1978a). The ratio of protein to lipid in the vesicles depends on the preparation. In order to estimate this ratio, aliquots of each sample were subjected to Sepharose 2B column chromatography. Figure 1 represents the profile of the chromatography. The total phospholipid and most of the glycophorins were eluted together near the void volume, indicating that the peak near the void volume represents the reconstituted vesicles. Only a small part of glycophorin was recovered as free glycophorin. The stoichiometry of glycophorin and phospholipid recovered in the first peak was measured, representing reconstituted vesicles.

NMR Measurements. The difference between NMR spectra of ^{13}C-labeled phospholipid vesicles in the absence and presence of glycophorin is illustrated in Figure 2. The NMR spectrum without glycophorin contained two resonance lines, one at 27 ppm due to labeled ^{13}C in the linoleoyl chain and the other (indicated with B in Figure 2) at 29.5 ppm, which arises from the natural abundance of ^{13}C in the palmitoyl chain from C$_4$ to C$_{13}$ and in the linoleoyl chain from position 4 to 7 and position 15. On the other hand, in the spectrum of the vesicles with glycophorin, a broad component ($\Delta H_{1/2} = 40$ Hz) was observed at 27 ppm superimposed on a sharp component ($\Delta H_{1/2} = $ ca. 1.5 Hz) at 27 ppm. In NMR spectroscopy, line width depends on spin–spin relaxation time, which should be influenced by the degree of internal motion of molecules. Therefore, the appearance of two distinct components in the spectrum of reconstituted vesicles strongly suggests the existence of at least two different phases in the lipid bilayer, which might be correlated with "boundary lipids" and "bulk lipids". If the internal mobilities in the two phases are different, some differences should be observed in the spin–lattice relaxation times of each component. Therefore, the spin-lattice relaxation times were measured with the inversion recovery Fourier transform method (Freeman & Hill, 1970).

Figures 3 and 4 show the partially relaxed NMR spectra of ^{13}C-labeled phospholipid vesicles in the absence and presence of glycophorin, respectively. As described above, the spectra without glycophorin contained two signals (indicated by A and B in Figure 3). The two signals had different dependencies on delay times; e.g., the signal A had a reversed magnetization at a longer delay time than the signal B. From the partially relaxed spectra the spin–lattice relaxation times (T_1) of signals A and B were estimated to be 0.74 and 0.28 s, respectively.

---

[2] Signal intensity and line width depended on the amplitude of proton decoupler in the range of incomplete decoupling. No appreciable change was observed, however, above 5 W of the power.
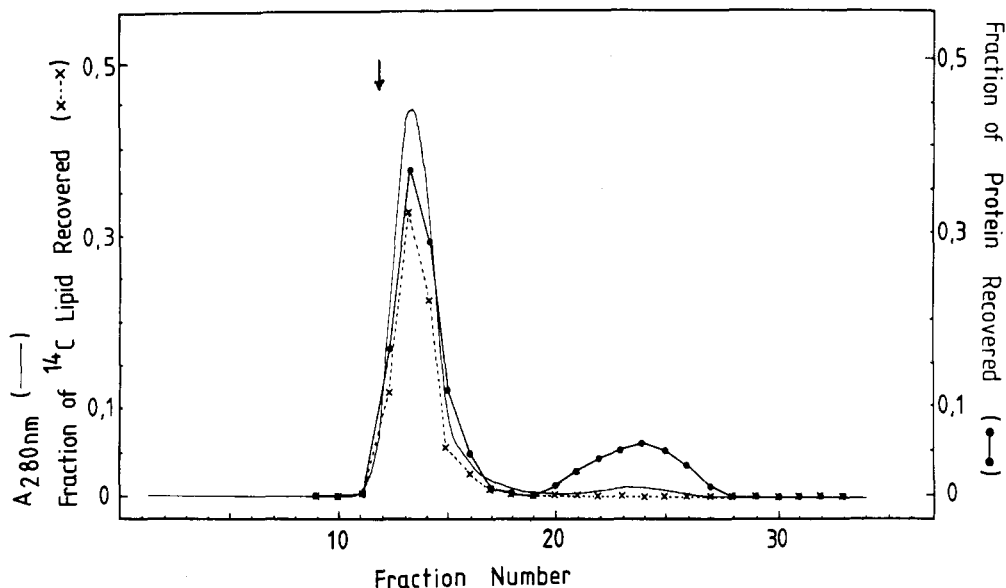
FIGURE 1: Elution profile of the reconstituted vesicle suspension on a Sepharose 2B column. A mixture of glycophorin (30 mg) and 1-palmitoyl-2-([14-^{13}C]linoleoyl)-sn-glycero-3-phosphocholine (30 mg) in chloroform-methanol-water (50:25:1) was dried in a rotary evaporator, and the resulting dried film was hydrated in 2.5 mL of 10 mM Tris-HCl, pH 7.4, containing 20% D$_2$O and 0.02% sodium azide. The solid line indicates the absorbance at 280 nm; crosses and closed circles indicate phospholipid glycophorin recovered. Concentrations were determined by the measurement of the radioactivity of soybean [N-^{14}CH$_3$]lecithin and by protein determination with the Lowry method (Lowry et al., 1951). The arrow indicates the void volume estimated with Blue Dextran 2000.
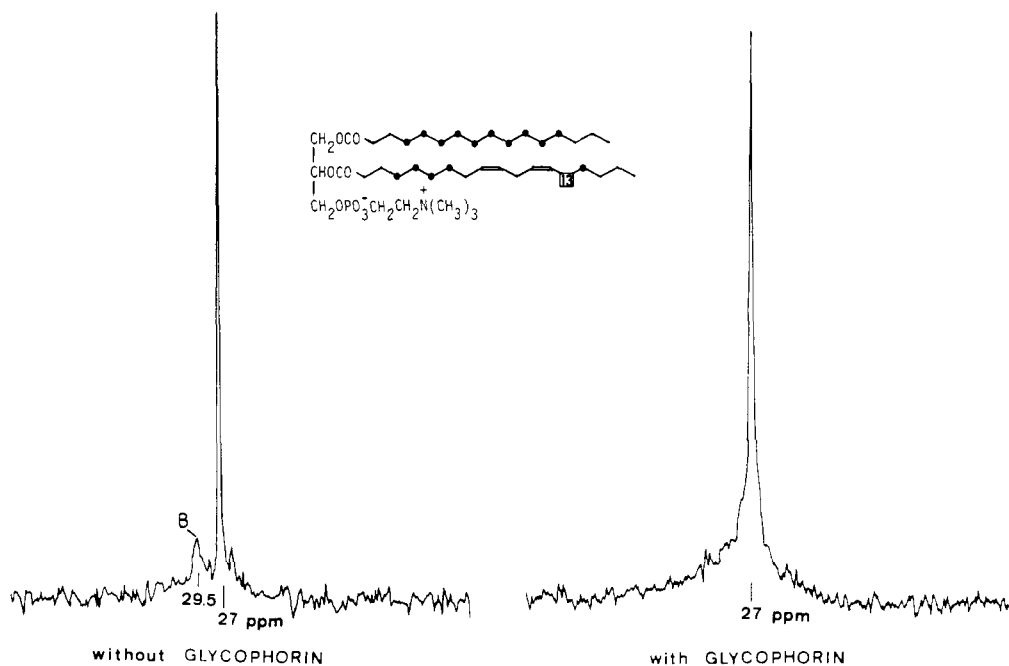

FIGURE 2: NMR spectra of 1-palmitoyl-2-([14-^{13}C]linoleoyl)-sn-glycero-3-phosphocholine vesicles in the absence and presence of glycophorin. The NMR spectra were obtained at 22.63 MHz with a Bruker WH-90 pulse spectrometer. Operating temperature was 37 ± 1 °C. The chemical shifts were related to tetramethylsilane. B indicates a resonance line due to the natural aboundance of ^{13}C in the palmitoyl chain from C$_4$ to C$_{13}$ and in the linoleoyl chain from C$_4$ to C$_7$ and C$_{15}$ (shown with closed circles in the chemical structure). The number of scans was 8000.

These T_1's are consistent with earlier results (Stoffel et al., 1974).

On the other hand, the two components shown in Figure 2 could be well distinguished in the partially relaxed spectra of the reconstituted vesicles with glycophorin (Figure 4). The most interesting spectrum was obtained at a delay time of 0.4 s. The broad component was still positive, while the sharp component had a reversed magnetization. At a delay time of 0.2 s, the broad component almost disappeared and only the sharp component was observed, indicating that the broad component has a shorter spin–lattice relaxation time than the sharp component.

In order to quantify the broad component, we calculated the theoretical spectra. The broad component seemed to be asymmetrical by the contribution of signal B due to the natural abundance of ^{13}C. The contribution was estimated to be 20% of the labeled ^{13}C from the NMR spectrum shown in Figure 2. Therefore, three components should be considered for the simulation: the sharp signal A, the symmetrical broad signal at 27 ppm, and the signal B at 29.5 ppm.[3]

---

[3] The contribution of another component at 29.5 ppm due to the naturally abundant ^{13}C in "boundary lipids" was neglected, as its influence on the broad component seems to be small.
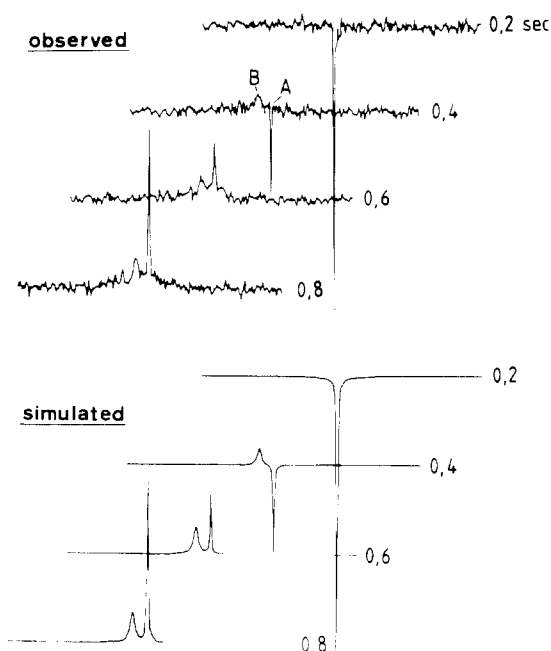
FIGURE 3: Partially relaxed ^{13}C NMR spectra of 1-palmitoyl-2-([14-^{13}C]linoleoyl)-sn-glycero-3-phosphocholine vesicles and the simulated spectra. The spectra were recorded at 22.63 MHz with a Bruker WH-90 pulse spectrometer operating in the Fourier transform mode. A 180°-t-90° pulse sequence was employed, where t is the delay time between 180 and 90° pulses. The operating temperature was 37 ± 1 °C. A and B indicate the resonance lines due to the label and the natural abundance of ^{13}C, respectively. Delay times (t) are shown to the right of each tracing. The simulated spectra were calculated with the equation described under Results. They were plotted with a Houston plotter.

Under our experimental conditions for NMR measurements, it is reasonable to assume the following. (1) Each resonance line may obey a Lorentzian distribution as represented by the equation

$$f(w)_i = \frac{T_{2i}^{eff}}{\pi(1 + (T_{2i}^{eff}(w - w_{0i}))^2)} \quad (1)$$

where T_{2i}^{eff} is the effective spin–spin relaxation time[4] of each signal i, w_{0i} is the resonance frequency of each signal i, and w is the radio frequency.

(2) The signal intensity of each resonance line should depend on the delay time according to the exponential equation

$$I(t)_i = 1 - 2 \exp(-t/T_{1i}) \quad (2)$$

where T_{1i} is the spin–lattice relaxation time[4] of signal i and t is the delay time.

Neglecting the contribution to the NMR spectrum other than the three components, an equation can be derived from eq 1 and 2 for the theoretical spectrum at each delay time

$$G(w)_t =$$

$$\sum_i n_i(1 - 2 \exp(-t/T_{1i}))\left(\frac{T_{2i}^{eff}}{\pi(1 + (T_{2i}^{eff}(w - w_{0i}))^2)}\right)$$

where i is the sharp signal A and B and the symmetrical broad signal at 27 ppm, and n_i is the fraction of each signal i.

The resulting simulated spectra are shown in Figure 4. The theoretical spectra of vesicles without glycophorin as a control
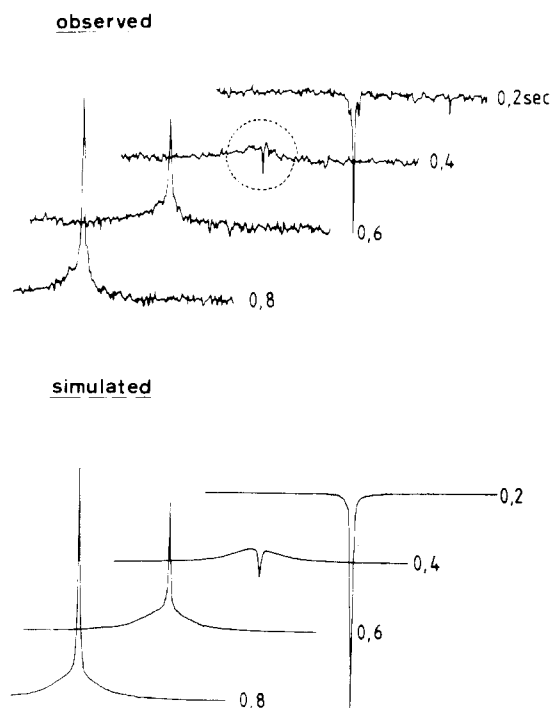

FIGURE 4: Partially relaxed ^{13}C NMR spectra of 1-palmitoyl-2-([14-^{13}C]linoleoyl)-sn-glycero-3-phosphocholine vesicles with glycophorin and the simulated spectra. The NMR spectra and the simulated spectra were obtained as described in Figure 3. Numbers on the right of each tracing indicate the delay times (t).

Table I: Parameters of the Line-Shape Analysis for ^{13}C NMR Spectra of 1-Palmitoyl-2-([14-^{13}C]linoleoyl)-sn-glycero-3-phosphocholine Vesicles[a]

% (w/w) of glycophorin[b]	sharp component		broad component		fraction of broad component
	T_1	T_2^{eff}	T_1	T_2^{eff}	
0	0.74	0.35	c		
27	0.63	0.20	0.32	0.009	0.50
37	0.64	0.14	0.32	0.009	0.61
44	0.53	0.15	0.29	0.008	0.72

[a] T_1's and T_2^{eff}'s are given in seconds. The contributions of naturally abundant ^{13}C were taken into account in the simulations ($T_1 = 0.28$ s, $T_2^{eff} = 0.10$ s, and fraction = 0.20). They are not included in the table. [b] Concentrations of glycophorin in the reconstituted vesicles were obtained from elution profiles of the Sepharose 2B column as described in Figure 1. [c] To obtain the best fitted spectra in the control (glycophorin-free vesicles), 20% of a broad component with a T_1 of 0.74 s and a T_2^{eff} of 0.008 s was taken into account for the simulation of the spectra in Figure 3. ^{13}C NMR spectra used for the analysis were observed at 37 °C. The standard deviations are within the range of 2% for T_1 of the sharp component, 5% for T_1 of the broad component, 10% for T_2^{eff}, and 5% for the fraction of the broad component.

are represented in Figure 3. The simulated spectra fitted very satisfactorily the observed spectra. The results of the line-shape analysis are summarized in Table I.

In vesicles 1-palmitoyl-2-([14-^{13}C]linoleoyl)-sn-glycero-3-phosphocholine had a spin–lattice relaxation time (T_1) of 0.74 s and an effective spin–spin relaxation time (T_2^{eff}) of 0.35 s (T_1 and T_2^{eff} of signal B were 0.28 and 0.10 s, respectively). The T_1 of the sharp component decreased to 0.63 s after the incorporation of glycophorin (27% of glycophorin). In addition to the T_1 reduction, the incorporation of glycophorin introduced a broad component, which had a T_1 of 0.32 s and T_2^{eff} of 0.009 s in the reconstituted vesicles. The shorter T_1 of the broad component indicates that the lipid molecules contributing to

---

[4] T_{1i} and T_{2i}^{eff} were roughly estimated from the partially relaxed spectra and were corrected through the simulation.
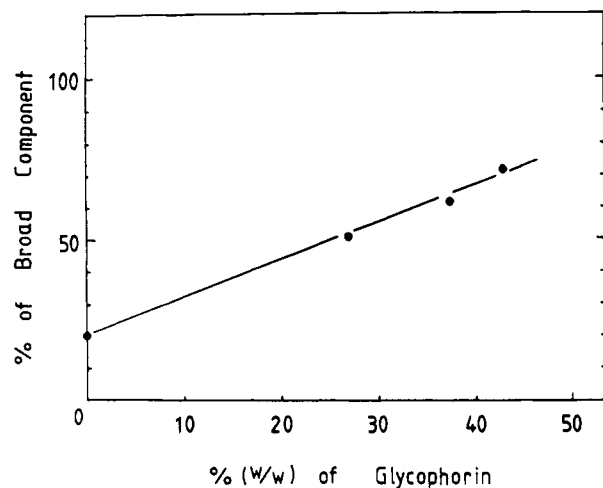
FIGURE 5: Relationship between the intensity of the broad component and the concentration of glycophorin. The intensity (area of resonance line) of the broad component was evaluated from the computer simulation. The concentration of glycophorin was determined as described in Figure 1.

the broad component are less mobile than those contributing to the sharp component, since the dipole–dipole interaction of the ^{13}C-labeled nucleus with two neighboring protons is the major mechanism for spin–lattice relaxation.

The percentage contribution of the broad component to the total signal depended upon the concentration of glycophorin. Figure 5 shows the relationship between the percentage of the broad component and the concentration of glycophorin. The broad component increased linearly with increasing concentration of glycophorin. From their relationship, we calculated the number of boundary lipid molecules. One glycophorin monomer with a molecular weight of 35 500 (Marchesi et al., 1976) affected about 30 lipid molecules.

For the simulation of the NMR spectra without glycophorin, we took into account the contribution of a broad component which had a T_1 of 0.74 s, a T_2^{eff} of 0.008 s, and a fraction of 0.2 (see Table I). It should be emphasized that the T_1 of 0.74 s was quite different from that of reconstituted vesicles and that the T_1 was the same as that of the sharp component. It might have arisen from some inhomogeneity of the magnetic field. Another possible source might be incomplete decoupling of the protons bound to the ^{13}C-labeled nucleus, since the proton noise decoupler in the WH-90 pulse spectrometer is designed for relatively narrow band NMR studies. However, the spin–lattice relaxation time was not influenced by the degree of decoupling, although the line width and the signal intensity depended on it. As described under Materials and Methods, the amplitude of the proton decoupler above 5 W did not change the spectra appreciably. Therefore, it is reasonable to conclude that the possibility of incomplete decoupling does not disturb the observation of two components at 27 ppm with different T_1's.

Discussion

The NMR spectroscopic results presented here indicate the presence of two kinds of lipid domains in reconstituted vesicles of 1-palmitoyl-2-([14-^{13}C]linoleoyl)-sn-glycero-3-phosphocholine with glycophorin. These were segregated into two different components, a sharp one and a broad one.

The sharp component of reconstituted glycophorin-containing vesicles had a slightly shorter T_1 than that of vesicles in the absence of this membrane-intrinsic protein, indicating that the bulk lipid molecules in reconstituted vesicles are immobilized to some extent by the glycophorin incorporated. It
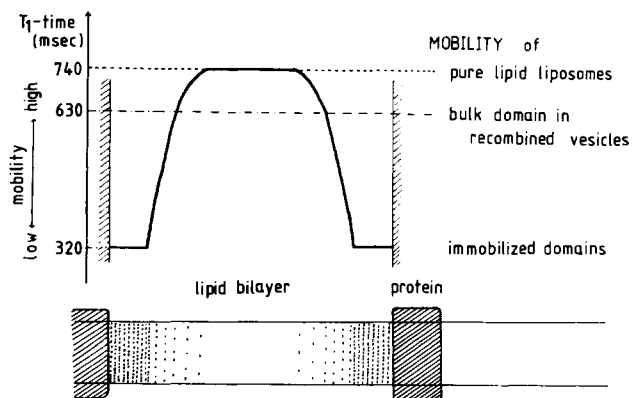

FIGURE 6: Diagrammatic representation of the glycophorin-lecithin interaction. The solid line indicates the suggested distribution of lipid molecular motions in reconstituted membranes with glycophorin.

is worthwhile mentioning that the reduction of the T_1 is apparently smaller than those by other proteins; e.g., T_1 of the same phospholipid with apo-HDL is 0.44, while broad components cannot be observed in the apo-HDL-phospholipid complex (Stoffel et al., 1974, 1979).

The appearence of a broad component was stoichiometrically correlated to the amount of glycophorin incorporated. Therefore, it may be concluded that the broad component arises from a phospholipid domain adjacent to glycophorin molecules, which has been named "boundary lipid" by Jost et al. (1973).

The number of lipid molecules directly immobilized by glycophorin was estimated at about 30 per 1 glycophorin monomer. This value is higher than those calculated by van Zoelen et al. (1978b), who estimated 9 phosphatidylserine molecules from ^{31}P NMR data. The discrepancy might be due to either the difference in the phospholipid species applied or the experimental technique. One might, however, consider that the small intrinsic part of glycophorin cannot interact with so many lipid molecules in the time scale of NMR frequency, since CD studies on glycophorin indicate an α-helix structure for the intrinsic part of glycophorin in NaDodSO$_4$ solution (Schulte & Marchesi, 1979). Other theoretical methods for predicting protein conformations based on primary structure suggest a β structure for the intrinsic part (Green & Flanagan, 1976; W. Stoffel et al., unpublished experiments). The β-sheet structure is well-known for its tendency to form dimers in a hydrophobic medium. Glycophorin exists in a dimeric form in the lipid bilayer (Marchesi et al., 1976). Therefore, it seems possible that the intrinsic region might have a conformation in lipid bilayers different from the helix suggested in NaDodSO$_4$ solution. The amino acid residues in the β-sheet conformation, on the other hand, would cause an enlargement of the protein perimeter.

A broad component is not observed in reconstituted vesicles of ^{13}C-labeled phospholipid with apo-HDL, while large reductions of T_1 are measured (Stoffel et al., 1974, 1979). A broad component has also been observed in the complex of [11-^{13}C]dioleoyllecithin with β-hydroxybutyrate apodehydrogenase or deuterium-labeled phospholipid with cytochrome c oxidase, but a small increase of the T_1 or a small decrease of the quadrupole splitting is also observed (Fleischer et al., 1979; Dahlquist et al., 1977).

The presumed interaction of phospholipid with glycophorin can be visualized as shown in Figure 6. When glycophorin is present in a lipid bilayer, the lipid molecules around glycophorin are immobilized by the steric hindrance and interaction with glycophorin. As the distance between protein and

lipid molecules of the bulk phase increases, these interactions decrease. As a result, the mobility reflected in the T_1 of the bulk lipids in reconstituted vesicles is influenced only by an exponentially decreasing steric hindrance.

Oldfield et al. (1978) and Seelig & Seelig (1978) concluded from ^2H NMR studies that no boundary lipid exists. However, Oldfield's group has recently observed a broad component due to the boundary lipid at low temperature in the system of cytochrome c oxidase with deuterium-labeled dimyristoyl-phosphatidylcholine (Kang et al., 1979). Therefore, the number of boundary lipid may depend on the temperature, as suggested by Dahlquist et al. (1977).

Kang et al. (1979) also suggested the possibility that lipid molecules around cytochrome oxidase (boundary lipids) may be less ordered than the bulk lipids. Lipid molecules are known to undergo some types of motion in the membrane. T_1 shows no dependency on order parameter. It is a function of correlation time in tumbling of the $^{13}C–H$ group. The discrepancy might depend on the motion observed.

References

Dahlquist, F. W., Muchmore, D. C., Davis, J. H., & Bloom, M. (1977) *Proc. Natl. Acad. Sci. U.S.A. 74*, 5435–5439.

Diringer, H. (1972) *Hoppe-Seyler's Z. Physiol. Chem. 353*, 39–42.

Dodge, J. T., Mitchell, C. D., & Hanahan, D. J. (1963) *Arch. Biochem. Biophys. 100*, 119–130.

Fleischer, S., McIntyre, J. O., Stoffel, W., & Tunggal, B. D. (1979) *Biochemistry 18*, 2420–2429.

Freeman, R., & Hill, H. D. W. (1970) *J. Chem. Phys. 53*, 4103–4105.

Grant, C. W. M., & McConnell, H. M. (1974) *Proc. Natl. Acad. Sci. U.S.A. 71*, 4653–4657.

Green, N. M., & Flanagan, M. T. (1976) *Biochem. J. 153*, 729–732.

Jost, P. C., Griffith, O. H., Capaldi, R. A., & Vanderkooi, G. (1973) *Proc. Natl. Acad. Sci. U.S.A 70*, 480–484.

Kang, S. Y., Gutowsky, H. S., Hsung, J. C., Jocobs, R., King, T. E., Rice, D., & Oldfield, E. (1979) *Biochemistry 18*, 3257–3267.

Kathan, R. H., Winzler, R. J., & Johnson, C. A. (1961) *J. Exp. Med. 113*, 37–45.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951) *J. Biol. Chem. 193*, 265–267.

MacDonald, R. J., & MacDonald, R. C. (1975) *J. Biol. Chem. 250*, 9206–9214.

Marchesi, V. T., Furthmayr, H., & Tomita, M. (1976) *Annu. Rev. Biochem. 45*, 667–698.

Oldfield, E., Gilmore, R., Glaser, M., Gutowsky, H. S., Hsung, J. C., Kang, S. Y., King, T. E., Meadows, M., & Rice, D. (1978) *Proc. Natl. Acad. Sci. U.S.A. 75*, 4657–4660.

Redwood, W. R., Jonsons, V. K., & Patel, B. C. (1975) *Biochim. Biophys. Acta 406*, 347–362.

Schulte, T. H., & Marchesi, V. T. (1979) *Biochemistry 18*, 275–280.

Seelig, A., & Seelig, J. (1978) *Hoppe-Seyler's Z. Physiol. Chem. 359*, 1747–1756.

Stoffel, W. (1964) *Justus Liebigs Ann. Chem. 673*, 26–36.

Stoffel, W. (1965) *J. Am. Oil Chem. Soc. 42*, 583–587.

Stoffel, W., Zierenberg, O., & Tunggal, B. D. (1972) *Hoppe-Seyler's Z. Physiol. Chem. 354*, 1962–1969.

Stoffel, W., Zierenberg, O., Tunggal, B. D., & Schreiber, E. (1974) *Hoppe-Seyler's Z. Physiol. Chem. 355*, 1381–1390.

Stoffel, W., Salm, K., & Tunggal, B. D. (1979) *Hoppe-Seyler's Z. Physiol. Chem. 360*, 523–528.

van Zoelen, E. J. J., Verkleij, A. J., Zwaal, R. F. A., & van Deenen, L. L. M. (1978a) *Eur. J. Biochem. 86*, 539–546.

van Zoelen, E. J. J., van Dijck, P. W. M., de Kruijff, B., Verkleij, A. J., & van Deenen, L. L. M. (1978b) *Biochim. Biophys. Acta 514*, 9–24.

Vold, R. L., Waugh, J. S., Klein, M. P., & Phelps, D. E. (1968) *J. Chem. Phys. 48*, 3831–3832.

Weber, K., & Osborn, M. (1969) *J. Biol. Chem. 244*, 4406–4412.