A SPIN LABEL STUDY OF *E. COLI* MEMBRANE VESICLES

Joseph J. Baldassare, Alice Gerke McAfee, and Chien Ho

Department of Biophysics and Microbiology,
University of Pittsburgh, Pittsburgh, Pennsylvania 15260

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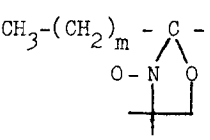
Summary

The phase transition in *E. coli* membrane vesicles has been investigated by the spin labeling technique. N-oxyl-4',4'-dimethyloxazolidine derivatives of stearic acid were incorporated into the vesicles. The results suggest that there are two phase transitions in these bacterial membrane vesicles (one at $\sim 20^{\circ}C$ and the other at $\sim 30^{\circ}C$). These two phase transitions may be related to some of the functional properties of the membranes.

Considerable effort has been devoted to an understanding of the molecular basis of the functions of biological membranes. As a model system, Kaback and coworkers have chosen bacterial cytoplasmic membrane vesicles to investigate certain aspects of the mechanisms of transport of substrates across cell membranes (1). These vesicles which have a closed or continuous surface can transport various sugars and amino acids as do the natural membranes (1).

Spin labeling has recently been used as a method of obtaining structural and dynamic information about model and biological membranes (2-11). McConnell and coworkers (6,8), Seelig (12), and Griffith and coworkers (13) have presented quantatitive theories relating parameters of the electron paramagnetic resonance (EPR) spectra of amphiphilic spin labels to molecular motion, and orientation of these labels in biological membranes and phospholipid vesicles. Spin labeled fatty acids of the general formula



I [m,n]

have been found to provide information about the conformation of the hydrocarbon chains and the effects of membrane proteins on the conformations of

phospholipids in membranes. Spin label investigations have been performed with sarcoplasmic reticulum (10,14), with Mycoplasma membrane (4), with a nerve fiber of Homarus americanus (6), and with a reconstituted photoreceptor membrane (11). It appears that one effect of protein-lipid interactions is to increase the stiffness of the hydrocarbon chains of the lipid moieties.

The purpose of this communication is to report our preliminary results on a spin label study of the phase transitions in the membrane vesicles obtained from *E. coli* ML 308-225 strain. We have observed two phase transitions (one at $\sim 20^{\circ}C$ and the other at $\sim 30^{\circ}C$) in these vesicles and would like to suggest that these two phase transitions may be related to the functional properties of the membrane.

EXPERIMENTAL

Materials:

E. coli ML 308-225, provided by Dr. H. R. Kaback, was grown at $37^{\circ}C$ on minimal medium A (15) containing 1% glucose while vigorously aerated in five gallon carboys. Mid-log cells were centrifuged at $4^{\circ}C$ on a Sorvall continuous-flow SZ-14 rotor at 19,500 r.p.m. with a flow rate of 6 liters/hour. An optical density (OD) of 2.0 at 600 nm on a Zeiss PMQ II (with 1 cm path length cuvette) yielded an average wet pellet of 3 g per liter of medium.

Membrane vesicles were prepared by the lysozyme-EDTA method of Kaback (16) and stored as a 45,000 x g pellet for up to seven days. Vesicles were dispersed in buffer as needed using as a standard an OD of 1.5 at 600 nm for 1% vesicles (17). The vesicles were routinely examined by the electron microscope and by checking steady state uptake of proline (16) with both normal and spin labeled vesicles.

Experiments utilized N-oxyl-4',4'-dimethyloxazolidine derivatives of spin labeled stearic acids, I [1,14] and [12,3] which were purchased from Synvar. The labels were dissolved in ethanol and were evaporated to dryness with a

circulating stream of dry N_2. Membranes in 0.1M potassium phosphate pH 6.6 with an OD (at 600 nm) range of 7.8-11.5 corresponding to 5-8% vesicles were then added to the desired label in a ratio of 100:1. Each sample was vortexed and drawn into a melting point capillary tube for EPR measurements.

Method:

EPR spectra of spin labeled *E. coli* vesicles were recorded on a Varian E-4 X-band spectrometer utilizing a temperature range of 10°-45°C maintained by a Varian variable temperature control unit with an accuracy of $\pm 1.5^{\circ}$C.

RESULTS AND DISCUSSION

Figure 1 contains representative EPR spectra of stearic acid spin labels I [12,3] and I [1,14] incorporated into *E. coli* vesicles at 20°C. Figure 1A is characteristic of rapid anisotropic motion of the label I [12,3] about the molecular axis of the hydrocarbon chain from which an order parameter, S, can be obtained (6,12). It is a measure of chain flexibility, or the amplitude of anisotropic motion in lipid bilayer. Figure 1B is characteristic of an isotropically tumbling molecule of the label I [1,14] from which a correlation time, τ_c, can be calculated (18). These spectra are very similar to published spectra of model and biological membranes.

Figure 2 gives plots of $(T_{\parallel}-T_{\perp})$ or τ_c vs. 1/T for labels I [12,3] and I [1,14] incorporated into *E. coli* vesicles over the temperature range of 10°-45°C. For spin label I [12,3], the values of $(T_{\parallel}-T_{\perp})$ were plotted against 1/T. The value of $(T_{\parallel}-T_{\perp})$ is directly related to the order parameter, S. For label I [1,14], we have plotted τ_c as a function of 1/T. Discontinuities in the above plots are interpreted in terms of phase changes in the membrane lipids. The arrows indicate the temperatures at which phase transitions occur. Table I summarizes the results on several membrane preparations. It is evident that there are two transition temperatures at 20°C $\pm$ 2°C and 30°C $\pm$ 2°C. The

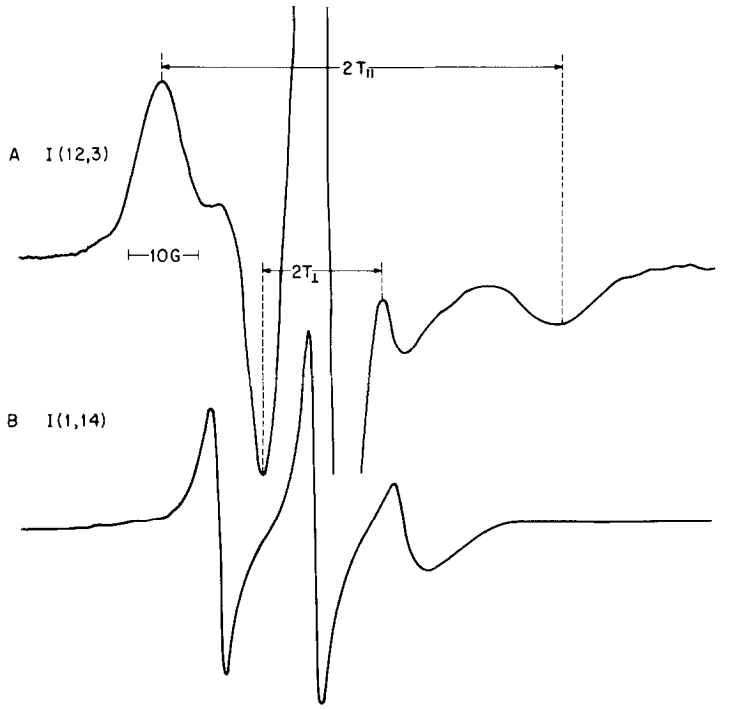


Fig. 1. Electron paramagnetic resonance spectra at 20°C of N-oxyl-4',4'-dimethyloxazolidine derivatives of spin labeled stearic acid incorporated in *E. coli* vesicles in 0.1M potassium phosphate pH 6.6: A, spin label I [12,3]; and B, spin label I [1,14].

occurrence of two phase transitions is contrary to what has commonly been found in a wide variety of membranes, including the membrane of yeast (19), the plasma membrane of Mycoplasma laidlawii (3), and the mitochrondrial membranes of sweet potato and of rat liver (20), all of which have only one phase transition as detected by the spin labeling technique.

At the present time the factors giving rise to the two phase transitions are not known. A possible explanation could be the selective melting of different classes of phospholipids or of lipids with varied fatty acid composition. Bacterial vesicles of *E. coli* are approximately 60-70% protein, 30-40% phospholipid, and 1% carbohydrate (1). The major phospholipids are phosphatidylethanolamine, diphosphatidylglycerol, and phosphatidylglycerol. If these were not uniformly distributed throughout the membrane, we might expect to find several melting temperatures. Moreover, lipids with varied fatty acid com-

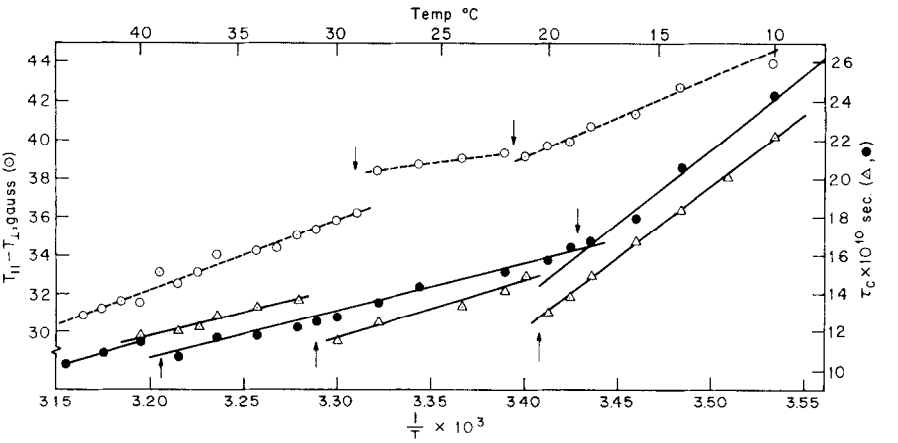


Fig. 2. The temperature dependence of $(T_{\parallel}-T_{\perp})$ and τ_c for spin labeled stearic acids I [12,3] and I [1,14] incorporated into *E. coli* vesicles; ○, I [12,3]; △, I [1,14]; and ●, I [1,14] incorporated into vesicles first heated to 60°C for 15 minutes.

positions might also be distributed nonuniformly. Since it has been shown that membranes with varying amounts of saturated and unsaturated fatty acids have different transition temperatures (21-23), this would result in "patches" of membrane with different transition temperatures. The two phase transitions that we have observed could be due to the melting of "patches" of the membrane containing a particular lipid composition.

A second possibility is that the protein which makes up 60-70% of the membrane has an effect on at least one of the transition temperatures. Figure 2 shows the temperature dependence of label I [1,14] incorporated into vesicles which had been heated for 15 minutes at 60°C. Again two phase transitions are present but the transition which had appeared at 30°C in unheated vesicles has increased significantly to 39°C. This change in only one of the transition temperatures could be due to altered lipid-protein interactions. The transition at 20°C could then be due to one type of structural change in the phospholipids which is not affected by heating while the one at 30°C would be dependent on both lipids and proteins. In support of this idea, heating the vesicles appears to affect only the proteins as shown by preliminary proton magnetic resonance results (unpublished results). This is consistent with the supposition that one of the transition temperatures depends on the structural integrity of the membrane proteins. An underlying assumption in the interpretation of our

Table I Phase Transitions in *E. Coli* Membrane Vesicles

Spin Label	Transition Temperatures	
I [12,3]	20°C	30°C
I [12,3]	20°C	29°C
I [1,14]	20°C	31°C
I [1,14]	21°C	31°C
I [1,14]	19°C	31°C
I [1,14]	21°C	31°C
I [1,14]*	19°C	39°C

*The vesicles were first heated to 60°C for 15 minutes before EPR measurements were made.

results is that when the spin labeled stearic acids are incorporated into membranes they align themselves along the lipid long axis with the carboxyl group anchored to the polar region of the phospholipids. This assumption is supported by our recent proton magnetic resonance results, which show a selective line broadening of the protons assigned to the terminal methyls in the vesicles labeled with I [1,14] (unpublished results).

The two phase transitions in *E. coli* vesicles indicate the possibility that the membrane is structurally heterogeneous. Previous studies (19-20) of membranes which show only one phase transition suggest a uniform distribution of lipids. The finding of two phase transitions in *E. coli* vesicles suggest a nonuniform distribution of lipids. This correlates functionally with protein activity by supporting the findings of Mavis and Vagelos (24) who found that glycerol-3-phosphate acyltransferase is not dependent on the physical properties of the bulk lipids. Nonuniformity would allow for the association of enzymes and their activities with lipids of appropriate composition.

The demonstration of the existence of two phase transitions in the membrane vesicles from *E. coli* suggests that the nonuniformity of the membrane

should be taken into consideration in any correlation of the structure-function relationships in biological membranes. Further studies are being carried out to relate these two phase transitions to the mechanisms of transport of sugars and amino acids in these bacterial membrane vesicles.

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