HETEROGENEITY IN THE PHYSICAL STATE OF THE EXTERIOR AND INTERIOR REGIONS OF MYCOPLASMA MEMBRANE LIPIDS

Shlomo Rottem

Institute of Microbiology, The Hebrew University-Hadassah Medical School, Jerusalem, Israel

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SUMMARY

The electron paramagnetic resonance spectra of spin-labeled fatty acid in intact mycoplasma cells and isolated membrane preparations have been compared. With *Mycoplasma hominis* and *Acholeplasma laidlawii* preparations, the freedom of motion of the spin-label was higher in labeled intact cells than in labeled isolated membranes but no differences could be detected between the labeled intact cells and membranes isolated from the labeled intact cells. It is proposed that the higher freedom of motion of the spin-label in the intact cells is due to a higher fluidity of the outer half of the lipid bilayer of mycoplasma membranes rather than to alterations in the structure of the membrane upon isolation.

INTRODUCTION

Electron paramagnetic resonance (EPR) spectrometry has been widely used to obtain information on the structure and dynamics of biomembranes (1, 2). The EPR spectra of a spin-labeled fatty acid in the membrane gives an insight into the physical state of mambrane lipids. The spin-label is preferentially oriented perpendicular to the plane of the membrane (3), and from its spectrum an estimate of the freedom of motion of the hydrocarbon chains of adjacent membrane lipids can be obtained (4). It has been previously shown that the freedom of motion of spin-labeled fatty acids in different membranes is influenced by the specific lipid composition of the membrane (5) and by membrane proteins (6). The recent observations that the disposition of lipids and proteins in some biomembranes is asymmetrical (7) raise the question regarding the similarity of the physical state of the outer and inner half of the lipid bilayer. In this communication a comparison between the freedom of motion of spin-labeled fatty acids in intact mycoplasma cells and isolated

membranes was performed. In view of the finding that the rate of "flip-flop" of amphipatic lipid components in the membrane might be very low (8), it was assumed that when intact cells are spin-labeled, the probe is incorporated into the exterior lipid region, while when isolated membranes are spin-labeled, the probe will be incorporated into both interior and exterior lipid regions. Thus, comparing the freedom of motion of the probe in isolated membranes and intact cells will provide useful information on the fluidity of membrane lipids in the outer and inner half of the lipid bilayer.

MATERIALS AND METHODS

Acholeplasma laidlawii and *Mycoplasma hominis* were grown in a modified Edward medium (9) supplemented with 2% PPLO serum fraction (Difco). After 16-20 hr of incubation at 37 C, the cells were harvested by centrifugation at 12,000 x *g* for 15 min. and washed once with 0.25 M NaCl. Cell membranes were obtained by osmotic lysis of the organisms (10). The membranes were collected by centrifugation at 34,000 x *g* for 30 min., washed twice and resuspended in 0.25 M NaCl. Intact cells or isolated membrane preparations in 0.25 M NaCl were spin-labeled with N-oxyl-4',4'-dimethyloxazolidine derivatives of 5-ketostearic (5NS) acid and 12-ketostearic (12NS) acid (Syva, Palo Alto, Calif.) by exchange from bovine serum albumin at 4 C as previously described (5). Part of the spin-labeled intact cells were lysed by osmotic shock and the membranes were collected and washed as described above. Electron paramagnetic resonance spectra of the spin-labeled membranes was performed with a Varian E-4 spectrometer. The molecular motion is reported as $2T_{\parallel}$, the hyperfine splitting, and as τ_o, an empirical motion parameter calculated from the expression used by Henry and Keith (11).

$$\tau_o = 6.5 \cdot 10^{-10} \cdot W_o \left(\sqrt{h_{o/h_{-1}}} - 1\right)$$

where W_o is the line width of the mid field line and h_o and h_{-1} are the heights of the mid and high field lines on a first derivative absorption spectrum.

RESULTS AND DISCUSSION

Fig. 1 shows the temperature dependence of the hyperfine splitting

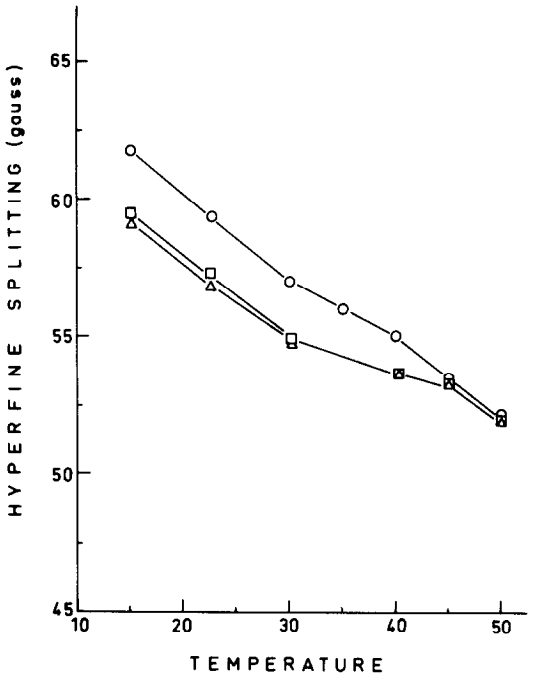

Fig. 1. Temperature dependence of the hyperfine splitting of N-oxyl-4',4'-dimethyloxazolidine derivative of 5-ketostearic acid in intact *M. hominis* cells (□), isolated membranes (o), and membranes isolated after spin-labeling of intact cells (Δ).

($2T_{\parallel}$) of N-oxyl-4',4'-dimethyloxazolidine derivatives of 5-ketostearic (5NS) acid in intact *M. hominis* cells and isolated membrane preparations. The hyperfine splitting is related to the motion of the nitroxide radical in the membranes (4) in a way that increased freedom of motion is associated with smaller values of $2T_{\parallel}$. Thus, it is apparent from the figure that at the temperature range of 15-40 C the freedom of motion of the

nitroxide radical in spin-labeled intact cells was higher than in membranes that were first isolated and then spin-labeled, but it was the same as that in membranes isolated from spin-labeled intact cells. Hence, no measurable alterations in the physical state of membrane lipids occur during the procedure used to isolate the membranes and the lower mobility of the nitroxide radical in the spin-labeled isolated membranes may be due to the heterogeneity in the physical state of the outer and inner halves of the lipid bilayer of *M. hominis* membranes, the outer half being more fluid. The greater molecular motion of spin-labeled fatty acid in intact mycoplasma cells than in isolated membranes was further demonstrated by measuring the empirical motion parameter (τ_o) from spectra of 12NS incorporated into cell and membrane preparations (Table 1), where lower τ_o values are associated with increased

Table 1. Motion parameters for *M. hominis* and *A. laidlawii* cells and isolated membranes labeled by 12NS *

Preparation	Motion parameter ($\tau_o \cdot 10^{10}$ sec)	
	M. hominis	*A. laidlawii*
Intact cells	62.0	42.5
Isolated membranes	71.0	45.5
Membranes isolated from labeled intact cells	63.5	43.0

* The τ_o values were calculated according to Henry & Keith (11) from EPR spectra obtained at 37 C. The results are the mean values of 3-5 experiments.

freedom of motion of the spin-label. However, whereas the motion parameter (τ_o) of *M. hominis* spin-labeled isolated membranes was markedly higher than that of spin-labeled intact cells, the motion parameter of *Acholeplasma laidlawii* spin-labeled isolated membranes was only slightly higher than that of the intact cells. Several

explanations may be brought up to explain this difference. One such mechanism may be a different rate of 'flip-flop' of lipids in membranes of *M. hominis* and *A. laidlawii*. A high rate of 'flip-flop' of the spin-labeled fatty acid in *A. laidlawii* would cause a rapid transfer of the spin-labeled fatty acid from the outer half to the inner half of the lipid bilayer of the intact cells. Thus, only small differences would be found between motion parameters of isolated membranes and intact cells. A second possible mechanism may be the major difference in the lipid composition of the two organisms, mainly the high cholesterol content of *M. hominis* cells (up to 40% of the total membrane lipids, ref. 12) and the low cholesterol content of *A. laidlawii* cells (2-8% of total membrane lipids). Cholesterol is known to affect the physical state of membrane lipids by increasing the packing of their hydrocarbon chains (13). Thus, an asymmetric distribution of cholesterol in *M. hominis* membranes would result in a decrease in the molecular motion of spin-labeled fatty acid incorporated in the cholesterol-rich lipid region. However, in view of the fact that cholesterol is not synthesized by *M. hominis* but is incorporated from the growth medium (12), one would expect to find an enrichment in cholesterol in the outer half of the the lipid bilayer rather than in the inner half as is implicated from the more restricted motion of spin-labeled fatty acids in the isolated membrane preparations. One cannot exclude, however, the possibility that membrane proteins are responsible for the difference in the freedom of motion of the nitroxide radical in spin-labeled isolated membranes of intact cells. *M. hominis* membranes differ from *A. laidlawii* membranes in their protein composition and disposition (14) and, as was previously demonstrated (6), membrane proteins may markedly affect the freedom of motion of the nitroxide radical in the membrane.

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