LIPID PHASE TRANSITIONS IN MEMBRANE VESICLES FROM *THERMOPLASMA ACIDOPHILA*

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1. Introduction

Thermoplasma acidophila has been classified as a *Mycoplasma*. The organism grows optimally at pH 2 and 59°C [1]. Although it has no cell wall, the cell is extremely resistant to osmotic shock and mechanical rupture [2–6]. Highly purified membranes are prepared by lysing the cells at alkaline pH [6]. The lipid content of the membrane is low (19%, w/w). More than 50% of the lipid by weight is composed of two long-chain alkyl ethers [2,6], a small amount are fatty acid esters [6]. The proteins in the membranes are hydrophobic and are apparently destabilized at high pH values due to ionization of carboxyl groups [3,6].

In the present work, we report about investigations of the physical properties of the lipid phase in membrane vesicles isolated from *T. acidophila*. For that purpose fatty acid spin labels were incorporated in the membranes lipids. The electron paramagnetic resonance (EPR) spectra of such spin labels provide information about the lipid phase of the membrane [7,8].

2. Materials and methods

T. acidophila was grown in aerated cultures at pH 2 and 56°C. Membranes were purified after cell lysis at pH 9.3 [6], and washed two times with 40 vol of distilled water after removal from sucrose density gradients.

The nitroxide stearate spin labels, 5NS and 12NS, were purchased from Synvar Corp. (Palo Alto, California). Aliquots from a stock solution of spin label in hexane were measured into small glass test tubes; hexane was removed by evaporation. Membrane vesicles were suspended in water at pH 6 or T-buffer consisting of 0.02% $(NH_4)_2SO_4$, 0.05% $MgSO_4$, 0.025% $CaCl_2 \cdot 2H_2O$, and 0.3% KH_2PO_4, adjusted to pH 2. This suspension was added to the spin label. The mixture was then sonicated for 10 min. The protein concentration of the membrane vesicles was between 10 and 20 mg/ml, as determined by the Lowry method [9]. The spin label concentration approximated about 0.1% of the lipid weight of the vesicles. Assuming an average molecular weight of 1500 for the lipid (based on the composition of the major diether phospholipid [2]), the lipid to stearate spin label molar ratio was about 250 : 1.

All EPR experiments were carried out with a Varian spectrometer, model 4502-15.

3. Results

A typical EPR spectrum of spin labelled *Thermoplasma* vesicles is shown in fig. 1. Since ascorbic acid does not reduce the signal height [10], the spin label

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Abbreviations: 5NS = 2-(3-carboxydecyl)-4,4-dimethyl-2-tridecyl-3-oxazolidinyloxyl; 12NS = 2-(10-carboxydecyl)-2-hexyl-4,4-dimethyl-3-oxazolidinyloxyl.

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is not exposed at the membrane surface. Immobilization of the spin label is evident at all temperatures tested, but is especially pronounced at low temperatures (table 1). This immobilization is indicative of a rigid membrane structure. At temperatures lower than 40°C, the 5NS spin label reflects greater or equivalent membrane rigidity as compared to that reported by the 12NS label (table 1). However, above 40°C, the 12NS label apparently experiences an environment with greater rigidity than that of the 5NS label (table 1).

The temperature dependence of *Thermoplasma* membrane fluidity is illustrated in fig. 1. The hyperfine splitting parameter 2 $T_{\parallel}$ is related to the rotational mobility of the spin label and therefore reports the local fluidity of membrane lipids [10]. A high value of 2 $T_{\parallel}$ reflects a low fluidity and vice versa. When the membranes were labelled with 5NS at pH 6.0, lipid phase transitions were observed at 15°C and 45°C. However, if labelling was performed at pH 2.0, the 45°C transition was absent and a new transition occurred at about 60°C. Membranes labelled at pH 6.0 and then resuspended in pH 2.0 buffer, maintained their transition temperature at 45°C. This suggests that the pH at the time of labelling determines the membrane domain to which the label migrates. The 12NS spin label consistently indicated a transition temperature at 60°C, regardless of the pH at the time of labelling. For the 12NS label a transition also appeared at 15°C.

Tabel 1

Hyperfine splitting parameter 2 $T_{\parallel}$ and rotational correlation time τ [7] of fatty acid spin labels incorporated into membranes isolated from *T. acidophila*

Spin label	Temperature °C	$2T_{\parallel}$ gauss	τ nsec
5NS	37	57.2	---
12NS	37	57.2	---
5NS	55	55.5	5.6
12NS	55	---	11.7
5NS	80	47.9	2.9
12NS	80	---	5.6

The membranes were labelled and run at pH 6.0. Where no values are given, the spectral shape was such that a proper calculation was impossible.

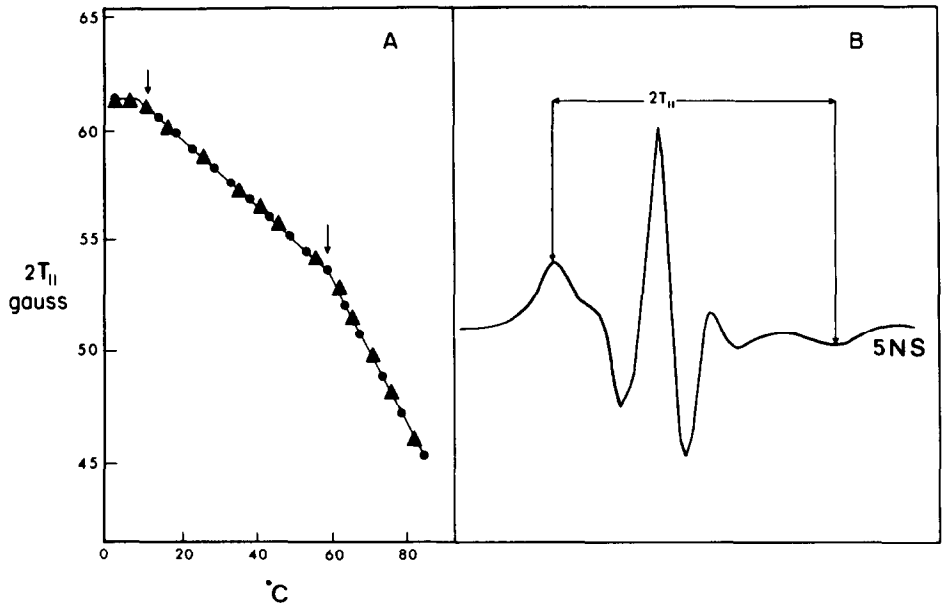

Fig. 1. (A) Hyperfine splitting parameter 2 $T_{\parallel}$ as a function of temperature for *T. acidophila* membranes labelled with 12NS. The pH at the time of labelling was either pH 6 (●–●–●–●) or pH 2 (▲–▲–▲–▲). (B) Electron paramagnetic resonance spectrum of *T. acidophila* membranes at pH 2 and 56°C, labelled with 5NS. The spin label was introduced into the membrane at room temperature.

4. Discussion

The lipid regions of *T. acidophila* may best be described as 'highly rigid', since both spin labels indicate less fluidity than has been reported for *Halobacterium cutirubrum* [11], which had the most rigid membrane hitherto known. Obviously, the accurate location of the spin probes within the membrane is uncertain. However, the fatty acid spin labels seem to report the most fluid regions of a heterogeneous phase of the membrane lipids [12].

T. acidophila and *H. cutirubrum* have several properties in common [5,6,11]. Both organisms have a low lipid content ($< 20\%$ of the membrane weight) and a high occurrence of branched alkyl ethers. Both factors probably contribute to the high rigidity found. Moreover, *Halobacterium* requires a high salt concentration (greater than 3 M) for growth and lyses readily when the salt concentration is lowered (below 1 M). Similarly, *Thermoplasma* has a high proton requirement (greater than 10^{-4} M) and lyses when the hydrogen ion concentration is decreased (less than 10^{-5} M) [6].

Sharp lipid phase transitions were detected in *Thermoplasma* membranes with both nitroxide stearate spin labels employed. This observation is consistent with the relative lipid homogeneity where over 50% of the lipid weight is composed of two branched alkyl ethers [6]. Depending upon the spin label used, two lipid phase transitions were recorded at higher temperature (45°C, 60°C). These two temperatures also define approximately the range over which *T. acidophila* cells grow at pH 2 [1]. Further experiments are necessary to clarify whether there exists a causal relationship between membrane lipid phase separation and physiological growth range. Within that temperature interval the hyperfine splitting parameter $2T_{\|}$, which represents a measure for the lipid fluidity, varies by about one gauss approximately. This might indicate that the membrane lipid fluidity is rather constant within the growth range [13,14].

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