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THE EFFECT OF Al^{3+} ON THE PHYSICAL PROPERTIES OF MEMBRANE LIPIDS IN THERMOPLASMA ACIDOPHILUM[+]

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SUMMARY

Using Electron Paramagnetic Resonance Spectroscopy, Al^{3+} was shown to produce a dramatic decrease of membrane lipid fluidity on the microorganism Thermoplasma acidophilum at a pH > 2. The ability of Al^{3+} to alter lipid fluidity was enhanced with increasing pH (from 3 to 5). At pH 4, 10^{-2} M Al^{3+} increased the lower lipid phase transition by 39°C, and a detectable change was observed with $AlCl_3$ concentrations as low as 10^{-5} M. The ability of Al^{3+} to increase the lower lipid phase transition temperature of T. acidophilum is the largest of any cation/lipid interaction yet reported.

INTRODUCTION

The physical state of biological membranes plays an important role in many physiological processes (1,2). This state is determined by various physico-chemical parameters including temperature, lipid and protein composition, and metal cation concentrations in the membrane's environment (3,4,5).

Divalent cations, Ca^{2+} and Mg^{2+}, have been reported to be conspicuously involved in stabilizing membranes (5) and recently trivalent ions (e.g., La^{3+}) have been shown to exert a similar effect (6). These cations induce membrane rigidity through electrostatic interactions with the anionic phosphate groups of the lipid bilayer. As far as we are aware, studies concerning the effects of Al^{3+} on membrane stability have not been

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performed. Soluble aluminum has long been recognized as a potent inhibitor of plant growth (7) and its toxicity suggested to us the involvement of membranes.

Using electron paramagnetic resonance spectroscopy (EPR), we have investigated the interactions of Al^{3+} ions with the membrane of Thermoplasma acidophilum. This thermophilic microorganism grows optimally at 59°C and pH 2 (8) and offers several advantages for our studies: 1) a procedure for isolating purified membranes is available (9), 2) cells and isolated membranes are stable at hydrogen ion concentrations where Al^{3+} is soluble (pH < 5.5), and 3) information is available concerning the interactions of membrane vesicles with mono- and divalent cations (10).

METHODS

Thermoplasma acidophilum was grown at 56°C and pH 2 as previously described (8), and harvested in the late exponential phase of growth (around 24 hrs). If intact cells were required, the cells were washed with 10 mM EDTA and rinsed twice with deionized water. Membranes were isolated after lysis with 1M glycine buffer (pH 9.3) (9), washed with 10 mM EDTA, and rinsed twice with deionized water.

Membrane or cell aliquots were resuspended in known concentrations of $AlCl_3$, $CaCl_2$, or KCl, and adjusted to the desired pH with KOH or H_2SO_4. The nitroxide fatty acid spin label 2-(3-carboxy-decyl)-4, 4-dimethyl-2-tridecyl-3-oxazolidinyloxyl (5NS) from Syva Corp. (Palo Alto, Calif.) was added to the membrane or cell preparations 20 minutes before use. Spin label concentrations were adjusted to about 0.1% of lipid content in each sample. Concentrations of intact cells were around 2×10^{10} cells/ml. Protein concentrations of membrane samples were between 10 and 20 mg/ml as determined by a modified Lowry procedure using Triton X-100 for solubilizing the membrane proteins (11).

EPR spectra were measured with a Varian EPR Spectrometer, Model E-112, equipped with a variable temperature controller. Sample temperatures were determined by an Omega Eng., Model 250, copper/constantan thermocouple. To monitor EPR spectra as a function of temperature, spectra of the spin labelled membranes or cells were recorded every 4°C from 0 to 65°C (65°C is the upper physiological limit for this organism (8)). Concentration curves for Al^{3+} at each pH represent aliquots taken from the same membrane or cell preparation. Each experiment was duplicated a minimum of three times, giving consistent results.

RESULTS AND DISCUSSION

First derivative EPR spectra of the 5NS spin label for T. acidophilum membrane vesicles are illustrated in Figure 1. From such spectra, the

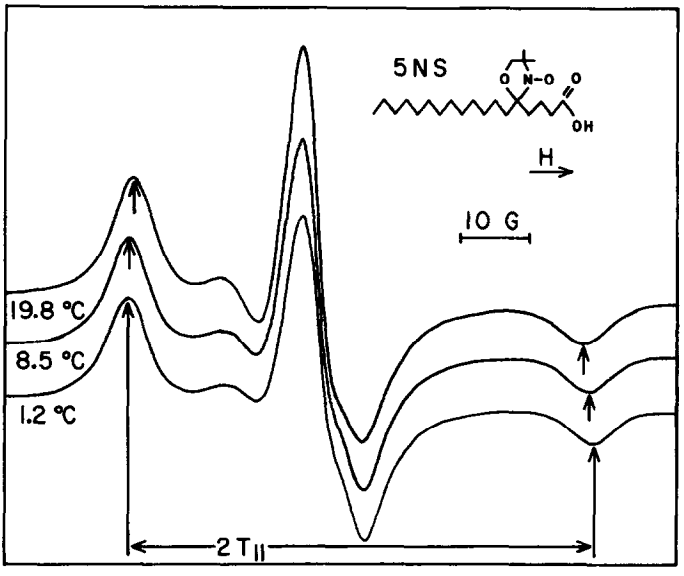
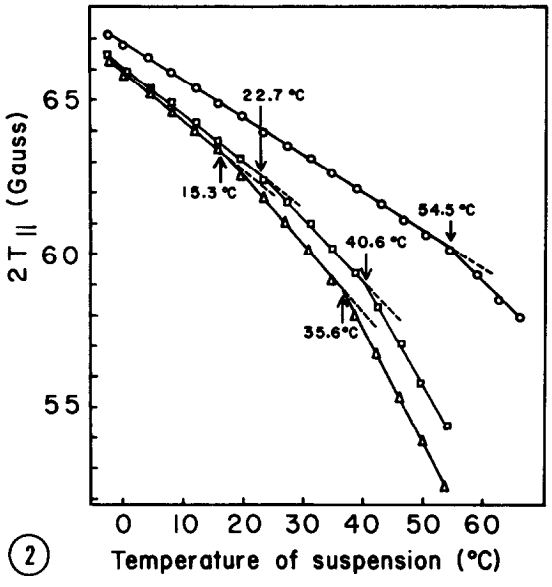


Fig. 1. First derivative electron spin resonance spectra of T. acidophilum membrane vesicles spin labelled with 5NS, suspended in 10 mM $AlCl_3$ at pH 4.

hyperfine splitting parameter ($2T_{||}$), indicative of lipid order and membrane fluidity (12), was determined. A larger value (gauss) of $2T_{||}$ implies a more rigid lipid matrix. The plot of $2T_{||}$ versus temperature (Fig. 2) exhibits discontinuities related to membrane lipid phase transitions. Lower and upper temperature discontinuities represent gel to gel + gel/liquid/crystalline and gel + gel/liquid/crystalline to liquid/crystalline lipid phase transitions, respectively (2). For each sample, such discontinuities were determined by linear regression analysis of the $2T_{||}$ values versus temperature plots.

When T. acidophilum membranes were exposed to various Al^{3+} concentrations at a pH > 2, a substantial increase in the low-temperature membrane-lipid phase transition (T_L) concomitant with an increase in $2T_{||}$ was detected (Figs. 3,4). Simultaneous shifts in the high-temperature membrane-lipid phase transition also occurred in the presence of Al^{3+} (Fig. 2). The results at pH 4 represent the largest cation induced change in phase transition



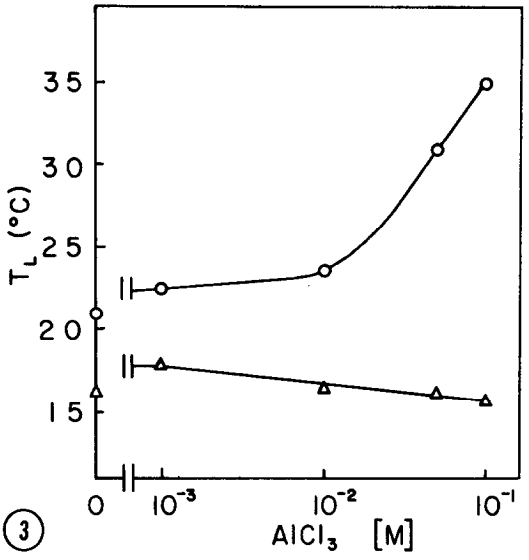


Fig. 2. Temperature dependence of the hyperfine splitting parameter $2T_{||}$ for T. acidophilum membrane vesicles suspended in 10 mM $AlCl_3$ (O), 1 mM $AlCl_3$ (□) and deionized water (Δ) at pH 4.

Fig. 3. Dependence of the low-temperature lipid phase transition, T_L, on Al^{3+} concentration for T. acidophilum membrane vesicles at pH 2 (Δ) and at pH 3 (O).

temperature yet reported. Addition of 10 mM $AlCl_3$ increased T_L by 39°C relative to the T_L of membrane vesicles resuspended in deionized water (Fig. 2). Noticeable alterations in transition temperature were observed with Al^{3+} concentrations as low as 10^{-5} M (3°C increase in T_L). If intact cells were used, the effect was equally dramatic, although higher $AlCl_3$ concentrations were required (Fig. 4). When $CaCl_2$ (10 mM) was added with increasing concentrations of $AlCl_3$ (up to 10 mM) to cells or membranes at pH 4, the Al^{3+} effect on transition temperature was still observed, the Ca^{2+} serving only to displace upwards the curve of T_L versus Al^{3+} concentration. Preliminary results indicated the membrane vesicles resuspended at pH 5 were even more sensitive to Al^{3+}, but at this pH the solubility of Al^{3+} and the stability of the membranes were a problem.

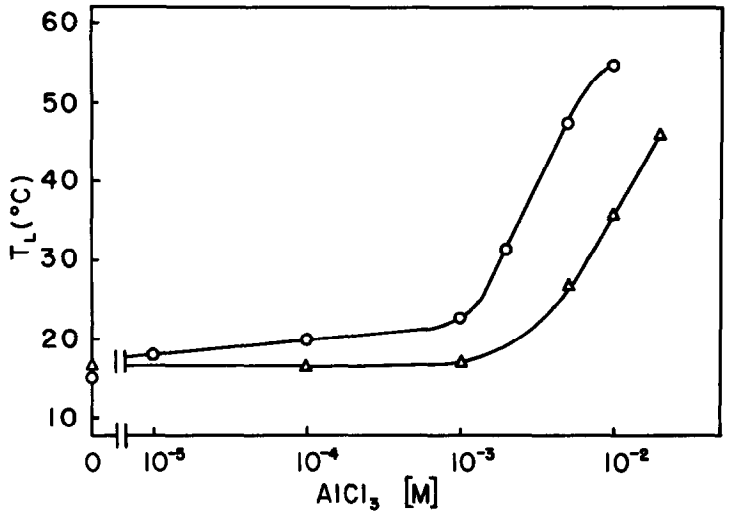


Fig. 4. Dependence of the low temperature lipid phase transition, T_L, on Al^{3+} concentration for T. acidophilum membrane vesicles (O) and cells (Δ) at pH 4.

At pH 2, however, Al^{3+} ion concentrations up to 100 mM induced no pronounced change in T_L, although fluidity increased slightly (lower $2T_{||}$ values) with increasing amounts of $AlCl_3$ (Fig. 3). This increase in fluidity appeared to be related to the ionic strength since such increases induced by 100 mM $AlCl_3$ were comparable to that of 200 mM KCl (KCl has been reported to have little effect on the phase transition temperatures of this organism (10)). The absence of interactions of the Al^{3+} with membrane lipids at pH 2 probably results from the fact that phospholipids are not appreciably anionic at this acidity.

In summary, we have found that Al^{3+} ions cause a dramatic decrease in the membrane fluidity of T. acidophilum at a pH > 2 in the presence or absence of Ca^{2+}. It is conceivable that similar cation induced changes in membrane viscosity might represent a model for the deleterious effects soluble Al^{3+} exerts on certain aspects of plant growth (13,14).

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