Effects of Ca^{2+} and K^{+} on the Physical State of Membrane Lipids in *Thermoplasma acidophila*

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(Received 13 September 1976; revised 4 November 1976)

SUMMARY

Thermoplasma acidophila, a mycoplasma-like organism, was grown at 56 °C and pH 2. The microfluidity of membrane vesicles was determined for the temperature range -2 to 35 °C using spin labels. The temperature at which the low-temperature phase transition occurred in membrane lipids increased with increasing Ca^{2+} concentration. However, there was no evidence of a low-temperature lipid phase transition for membrane vesicles suspended in a medium containing monovalent instead of bivalent cations.

INTRODUCTION

Temperature-induced phase transitions of lipids in biological membranes have been extensively investigated (Chapman, 1975; Lee, 1975). Various enzyme activities depend on the physical state of the membrane lipids. The physical state is a function of physicochemical parameters such as temperature or the nature and concentration of metal ions (Esfahani *et al.*, 1971; Kimelberg & Papahadjopoulos, 1974; Bruni, Van Dijck & De Gier, 1975; Davis, Inesi & Gulik-Krzywicki, 1976; Linden *et al.*, 1973).

Divalent ions help to maintain the mechanical stability of Gram-negative envelopes in a psychrophilic *Achromobacter* (Ledebø, 1976), whilst the thermophilic *Bacillus stearothermophilus* requires Ca^{2+} for thermal stability during vegetative growth and takes up Ca^{2+} at a rate which increases with temperature (Stahl & Ljunger, 1976). *Thermoplasma acidophila* is a thermophilic acidophilic mycoplasma which grows optimally at pH 2 and 59 °C (Darland *et al.*, 1970). Membranes from this organism, when labelled with fatty-acid spin probes, show two thermally-induced lipid phase transitions, at about 15 °C and about 45 °C (Smith, Ruwart & Haug, 1974). These transitions probably represent respectively gel → gel + gel/liquid/crystalline and gel + liquid/crystalline → liquid/crystalline lipid regions (Chapman, 1975; Lee, 1975; Shimshick & McConnell, 1973).

Our aim was to determine whether membranes from *T. acidophila* respond to changes in Ca^{2+} and K^{+} concentrations. This was investigated by observing alterations in the temperature of the low-temperature phase transition. The membranes were labelled with fatty-acid spin probes and changes in the degree of order in membrane lipids were followed by electron spin resonance measurements.

METHODS

Culture of organism. *Thermoplasma acidophila* was grown in aerated 18 l vessels at 56 °C in a medium, pH 2, containing (mM): $(\text{NH}_4)_2\text{SO}_4$, 1.5; MgSO_4 , 2.0; CaCl_2 , 1.7; KH_2PO_4 , 22; and D-glucose, 55. Bacteria were harvested in the late-exponential phase (about 2×10^8

Fig. 1. Electron spin resonance spectrum of *T. acidophila* membranes spin-labelled with 5NS, suspended in 0.01 M-CaCl₂ at pH 6.4 and 25 °C.

cells ml⁻¹), and lysed at pH 9.3. Membranes were isolated and washed three times with deionized water after removal from sucrose density gradients (Ruwart & Haug, 1975).

Electron spin resonance (e.s.r.) experiments. The nitroxide fatty-acid spin label 2-(3-carboxydecyl)-4,4-dimethyl-2-tridecyl-3-oxazolidinyloxy (5NS) was purchased from Synvar Corp., Palo Alto, California, U.S.A. Membranes were suspended in known concentrations of K⁺ or Ca²⁺ at pH 6.4 and spin-labelled as described previously (Smith *et al.*, 1974). The protein concentration of the spin-labelled membrane vesicles was between 15 and 30 mg ml⁻¹ as determined by the Lowry method (Lowry *et al.*, 1951). The spin label concentration was about 0.1 % of the lipid content. All e.s.r. measurements were made in a Varian model 4502-15 spectrometer equipped with a variable temperature controller. The temperature of the membrane sample was measured with a copper-constantan thermocouple and a Keithley digital multimeter, model 160. Each transition temperature was determined from the intersection of line segments fitted by linear regression.

RESULTS AND DISCUSSION

A typical e.s.r. spectrum of 5NS spin-labelled membrane vesicles for the temperature range -2 to 35 °C is shown in Fig. 1. The hyperfine splitting parameter, $2T_{\parallel}$, is related to the degree of order of the labelled membrane lipids (Griffith & Jost, 1976). Increasing values of $2T_{\parallel}$ imply an increasing degree of order. The temperature dependence of $2T_{\parallel}$ was examined in the presence of 100, 10, 1 and 0.1 mM KCl or CaCl₂.

The temperature dependence of $2T_{\parallel}$ for membrane vesicles suspended in KCl at pH 6.4 was linear and the rate of decrease of $2T_{\parallel}$ with increasing temperature was approximately the same for all concentrations tested. The plots of $2T_{\parallel}$ against temperature for membranes suspended in 10, 1 and 0.1 mM-KCl (not shown) were nearly coincident, reflecting similar degrees of order at the various temperatures. However, at each temperature the $2T_{\parallel}$ values for membranes suspended in 100 mM-KCl were about 0.45 G (0.45×10^{-4} T) lower than for membranes at the other KCl concentrations, indicating a lower degree of order in 100 mM-KCl at all temperatures. Thus, there was no evidence of a low-temperature transition in

Fig. 2

Fig. 3

Fig. 2. Temperature dependence of the hyperfine splitting parameter $2T_{||}$ for *T. acidophila* membranes in 100 mM- CaCl_2 (●) and 0.1 mM- CaCl_2 (▲). Membranes were spin-labelled with 5NS at pH 6.4.

Fig. 3. The temperature of the low-temperature transition, T_L , as a function of Ca^{2+} concentration for *T. acidophila* membranes at pH 6.4.

KCl. Preliminary results indicated that lipids in *T. acidophila* membranes suspended in KCl at pH 2 were generally more ordered than membranes at pH 6.4.

Plots for membranes in 100 mM and 0.1 mM- CaCl_2 at pH 6.4 are shown in Fig. 2. At these and the other Ca^{2+} concentrations examined, a low-temperature membrane-lipid phase transition occurred. The transition temperature, T_L , increased with increasing Ca^{2+} concentration (Fig. 3). The plots of $2T_{||}$ against temperature for membranes suspended in 10, 1 and 0.1 mM- CaCl_2 at pH 6.4 were nearly coincident for temperatures below the respective transition temperatures. This suggests that in the gel state the degree of order of the lipids is the same irrespective of the Ca^{2+} concentration. Below 32 °C, the $2T_{||}$ values for membranes suspended in 100 mM- CaCl_2 were greater than those at other concentrations. Below the transition temperature, the rate of decrease of $2T_{||}$ with increasing temperature was approximately the same regardless of the Ca^{2+} concentration (Fig. 2). Preliminary results indicate that *T. acidophila* lipids in membranes suspended in CaCl_2 at pH 2 were generally less ordered and exhibited low-temperature transitions at lower temperatures than the membrane lipids of vesicles suspended in CaCl_2 at pH 6.4. The lower degree of membrane lipid order at pH 2 indicates that fewer negatively-charged lipid groups were exposed to the aqueous phase. Thus, there would be fewer Ca^{2+} ions bound per unit area of exposed membrane.

These results are consistent with recent findings for aqueous dispersions of synthetic negatively-charged lipids. In *T. acidophila* membranes, lipid order and the temperature of the low-temperature transition increased concomitantly with Ca^{2+} concentration. In dispersions of phosphatidic acid bilayers at pH 6 to 10.5, an increase in Mg^{2+} or Ca^{2+} concentration resulted in increased lipid order and a higher order $\rightarrow$ disorder transition temperature (Träuble & Eibl, 1975). These effects may be explained by a reduction in adsorption of divalent cations at the negatively-charged surface. Moreover, addition of Ca^{2+} to sonicated dispersions of dipalmitoylphosphatidylglycerol or phosphatidylserine at pH 7.4 caused increases in the temperature of the gel $\rightarrow$ liquid/crystalline phase transition to an extent dependent on the cation concentration (Jacobson & Papahadjopoulos, 1975).

The presence of monovalent and divalent cations at the polar lipid groups of *T. acidophila* membranes probably induces alterations of alkyl chain conformation, as indicated by the 5NS spin probes. Raman spectroscopic experiments on aqueous phosphatidylcholine dispersions have demonstrated that Ca^{2+} ions decrease the proportion of gauche character in the hydrocarbon chains (Lis, Kauffman & Shriver, 1975). In contrast, K^+ caused no modifications.

In summary, *T. acidophila*, which grows in a rather harsh environment, has membrane lipids whose physical state changes in response to alterations in the external concentrations of Ca^{2+} ions. The significance of this in the physiology of the organism awaits elucidation.

This work was supported by United States-ERDA contract no. E(11-1)-1338. H. Weller was supported by funds from the National Institute of Health Training Grant no. GM-01422-11 and the College of Osteopathic Medicine of Michigan State University.

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