TABLE II
^{31}P SPIN LATTICE RELAXATION TIMES AT 81 MHz.

System	T_1 (±10%)	No. of preparations
Unsonicated egg phosphatidylcholine	1.3s	2
rod outer segment disks	1.4 s	2

disk membrane. As shown in Fig. 3, just above 30°C the immobilization of phospholipids by proteolyzed rhodopsin undergoes a dramatic reduction, while no temperature effect is observed for native, intact disks in the temperature range studied. This result indicates that the protein-phospholipid complex of the proteolyzed rhodopsin is not as thermally stable as the original complex.

Both ^{31}P NMR spectral width and T_1 data indicate that the phospholipids which were observed exhibited behavior similar to phospholipids in a pure phospholipid bilayer, shown in Fig. 1 and Table II. This is significant in that it suggests the loss of spectral intensity is not due to an average property of the membrane bilayer, but rather that a component has been removed from an otherwise typical phospholipid bilayer.

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MEMBRANE LIPID FLUIDITY AND PHYSICAL STATE AND THE ACTIVITY OF THE Na^+,Mg^{2+}-ATPASE OF *ACHOLEPLASMA LAIDLAWII* B

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Nonlinear Arrhenius plots are often obtained when the temperature dependence of membrane enzymes and transport systems is investigated. Such plots are conventionally analyzed as sets of two (or sometimes more) straight-line segments, the slopes of which are assumed to represent reaction activation enthalpies of different states of the enzyme or transport system. Apparently sharp changes in the slopes of these Arrhenius plots are usually ascribed to thermotropic membrane structural alterations, especially lipid phase transitions. We have recently studied in a quantitative and systematic way the temperature dependence of the membrane Na^+,Mg^{2+}-ATPase of the simple, cell wall-less prokaryote *Acholeplasma laidlawii* B (1). By utilizing *A. laidlawii* B membranes enriched or made homogeneous in a variety of exogenous fatty acids, both the temperature and the cooperativity of the gel to liquid-crystalline lipid phase transition can be markedly altered (2, 3), permitting a rigorous investigation of the influence of lipid fluidity and physical state on the temperature dependence of the activity of this enzyme in intact membranes. The results thus obtained were analyzed using the theoretical rate-temperature relationships derived for a variety of physically plausible membrane rate processes described in detail elsewhere (4).

METHODS

The organism used in this study, the growth medium and conditions employed, the determination of ATPase activity, and the preparation and differential thermal analysis of isolated membranes, have all been previously described (1–3, 5).

RESULTS

The temperature dependence of the Na^+,Mg^{2+}-ATPase in two *A. laidlawii* B membranes whose lipids exist exclusively in the liquid-crystalline state at physiological temperatures are shown in Fig. 1. These Arrhenius plots are clearly smooth curves and their slopes decrease as a nearly linear function of temperature. Similar behavior is observed for membranes enriched in other fatty acids exhibiting lipid phase transitions below 0°C, or at temperatures above the phase transition midpoint temperature in membranes exhibiting phase changes above 0°C. Thus the temperature dependence of this ATPase activity is not

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BIOPHYS. J. © Biophysical Society · 0006–3495/82/01/36/03 $1.00
Volume 37 January 1982 36–38

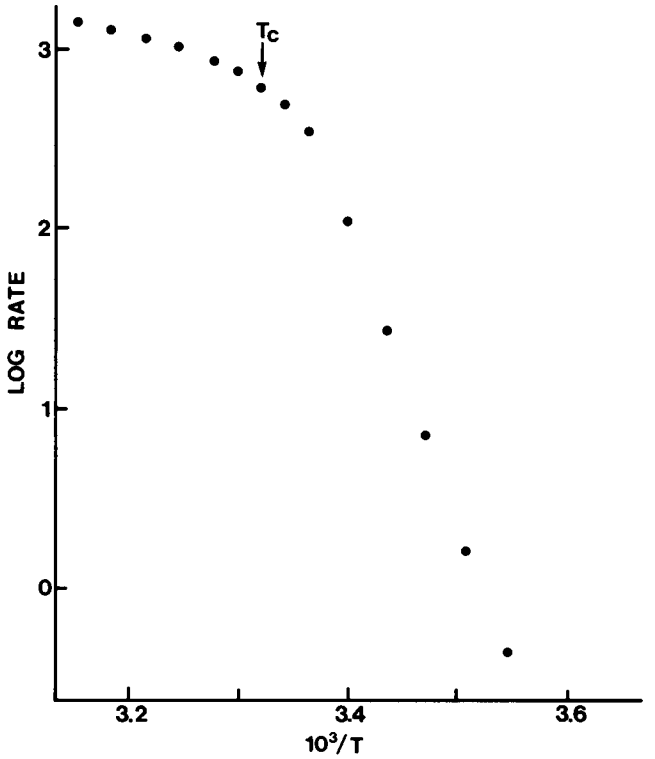

FIGURE 1 Arrhenius plots of Na^+,Mg^{2+}-ATPase activity of *A. laidlawii* B membranes made homogeneous in anteisopentadecanoic acid (●) or *cis*-vaccenic acid (O). The actual measured activities have been scaled to facilitate comparison.

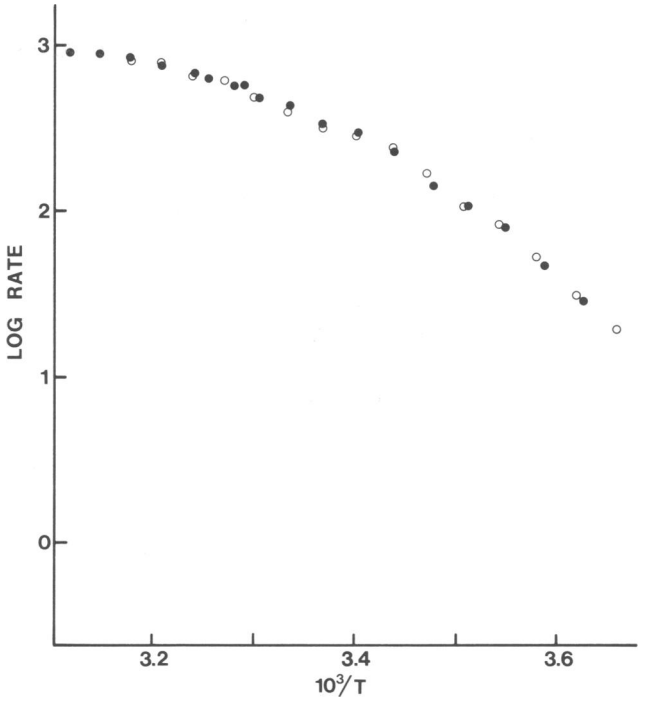

FIGURE 2 Arrhenius plot of Na^+,Mg^{2+}-ATPase activity in *A. laidlawii* B membranes made homogeneous in isoheptadecanoic acid. The lipid phase transition midpoint (T_c) of 28.8°C is indicated.

affected by lipid fatty acyl chain structure as long as the enzyme is surrounded by fluid lipids. These Arrhenius plots seem most satisfactorily described by a model postulating a finite heat capacity of activation for the enzyme-catalyzed reaction rather than a model postulating a smooth and continuous change in enzyme conformation, or rate-limiting step, over the physiological temperature range. In contrast, the apparent absolute activity of this enzyme is dependent on fatty acyl chain structure, but there is no correlation between ATPase activity and membrane lipid phase transition temperature, suggesting that lipid "fluidity" per se does not markedly influence enzyme activity. It is possible, however, that the observed variations in absolute activity arise from differences in the number of functional ATPase molecules present in membranes of different fatty acid composition, rather than from variations in true specific activity.

The temperature dependence of ATPase activity in a membrane exhibiting a lipid phase transition in the physiological temperature range is shown in Fig. 2. This Arrhenius plot is biphasic, exhibiting a gently sloping region at higher temperatures and a steeply sloping region at lower temperature. The "break" in slope is not sharp but gradual and lies near the midpoint temperature of the lipid phase transition. As illustrated in Table I, more than half of the ATPase molecules may remain active when >90% of the membrane lipid is in the gel state, and the enzyme is typically not "fully" (>99%) inactivated until temperatures some 10–20°C below the detectable lower boundary of the bulk lipid phase transition are reached.

The temperature and cooperativity of the bulk lipid phase transition and the "transition" in enzyme activity parallel one another in membranes enriched in a variety of different fatty acids, but the steep change in enzyme

TABLE I
COMPARISON OF THE TEMPERATURE DEPENDENCE OF Na^+, Mg^{2+}-ATPase ACTIVITY AND LIPID-PHASE STATE IN *ACHOLEPLASMA LAIDLAWII* B MEMBRANES ENRICHED IN TWO DIFFERENT FATTY ACIDS

Parameter measured*	Fatty acid enrichment utilized					
	Isoheptadecanoate			*cis*-15-Octadecenoate		
	Lipids	ATPase	[Difference]	Lipids	ATPase	[Difference]
T_{50}	28.8°C	25.3°C	3.5°C	27.6°C	20.6°C	7.0°C
$(T_{90} - T_{10})$	4.8	14.1	9.3	8.9	22.0	13.1
T_{10}	25.4	18.2	7.2	22.6	9.6	13.0

*The temperature parameter T_x represents the temperature at which the ATPase or lipid phase transition from the low- to the high-temperature state is x% complete. T_{50} thus corresponds to the midpoint of the transition of interest; T_{10} represents the temperature at which the system is 90% in the low-temperature state; and $(T_{90} - T_{10})$ is an index of the broadness of the transition. T_x values for the ATPase transition were calculated by quantitative analyses of the Arrhenius plots (4, 5), and for the lipid transition by differential thermal analysis (3, 5).

activity always occurs at lower temperatures and over a broader temperature range. These results suggest that this enzyme is active only in association with fluid lipid and is progressively inactivated when its "boundary lipid" undergoes a "phase transition" to a gel-like state, the boundary lipid transition being driven by the bulk lipid transition but being less cooperative and taking place at a lower temperature than the latter. The lateral aggregation of intramembranous protein particles, which normally occurs as the bulk lipid phase enters the gel state, is not responsible for the accompanying loss of ATPase activity.

This research was supported by Operating Grant MT-4261 from the Medical Research Council of Canada.

John R. Silvius was supported by a Medical Research Council of Canada Studentship.

Received for publication 29 April 1981.

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