LIPID–PROTEIN INTERACTIONS IN MEMBRANES: ARRHENIUS PLOTS AND HILL PLOTS IN MEMBRANE-BOUND (Ca^{2+})-ATPASE OF *ESCHERICHIA COLI*

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1. Introduction

Correlations between different physiological parameters and the fatty acid composition of the membranes of unsaturated fatty acid auxotrophs of *Escherichia coli* have been investigated in recent years [1]. Different thermal phase transitions were found in phospholipids from membranes obtained by growing cells in oleic and linolenic acid supplemented media by studies in phospholipid monolayers and by X-ray diffraction techniques [2, 3]. This last method confirmed the existence of different transition points for the phase changes of the lipids in intact membranes of cells grown with oleic and linolenic acids as supplements [3]. The range of temperature during which transition occurs was 19–29° and 36–46° for oleic acid and linolenic acid, respectively. With membranes of these same cells, differences were found in the Arrhenius plots of growth rates [2] and of several membrane-associated functions: i) transport systems [2–4] and ii) respiratory systems [2, 3]. On the other hand, recent studies by Mavis et al. [5] reported no differences in the Arrhenius plots of three enzymes bound to membranes from cells grown with oleic and linolenic acid, suggesting in this case a lack of influence of the lipid phase transitions on the enzyme. We studied the temperature dependence of the (Ca^{2+})-ATPase (EC 3.6.1.3) of the *E. coli* auxotroph L010 grown with oleic and linolenic acid. From the point of view of temperature dependence, the enzyme falls into the last case shown above. However, the presence of lipid–protein interaction had been detected by the study of the allosteric transitions [6]. In the present report, we provide direct evidence of the methodological importance that acquires the study of allosteric transitions of membrane-bound enzymes in the evaluation of protein–lipid interactions.

2. Materials and methods

Strain L010, an unsaturated fatty acid auxotroph of *E. coli* [7] was grown aerobically at 37° in a defatted L broth [8] containing 0.05% Triton X-100. The unsaturated fatty acid supplements were added as potassium salts to give a concentration of 0.02% in the medium. Membranes were prepared essentially according to the procedure of Evans [9].

(Ca^{2+})-ATPase activity of the membrane was measured as described previously [8]. Inhibition by sodium was recorded in the range from 0 to 130 mM of NaCl. Changes of activity with temperature were measured between 10° and 46°. The values of n (from the Hill plots) and activation energies (Ea) of

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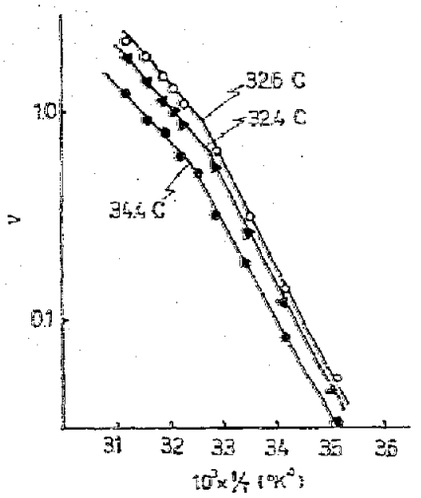

Fig. 1. Arrhenius plots of the (Ca^{2+})-ATPase. Membrane-bound enzyme: Medium supplemented with oleic (●—●—●), and linolenic acid (○—○—○). Soluble enzyme: Medium supplemented with linolenic acid (▲—▲—▲). v is initial velocity expressed as mole P_i/hr.

the Arrhenius plots were calculated by the least square method with the use of a computer. Intercepts (Ti) were also found by computation.

Solubilization of the (Ca^{2+})-ATPase was carried out by washing twice the membranes with 10 mM Tris-HCl buffer, pH 7.4 and once more with the same buffer 1 mM. After each washing step, membranes were spun at 30,000 g for 20 min. The three washings were pooled and referred to as soluble enzyme. The activity remained in the supernatant after centrifuging at 100,000 g for 1 hr. No differences in the specific activities of the soluble and particulate enzyme were noticeable.

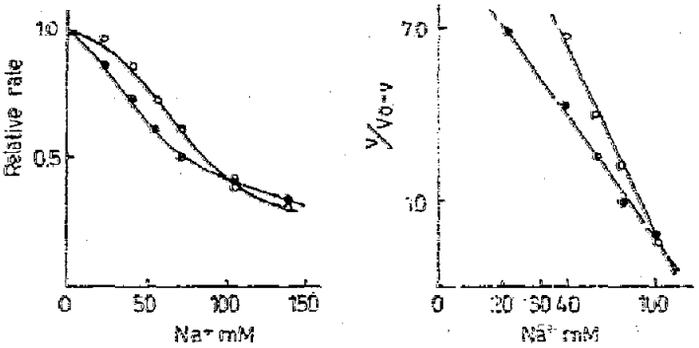

Fig. 2. Inhibition by Na^+ of the (Ca^{2+})-ATPase at 36°. Membranes from cells grown with oleic (●—●—●) and linolenic acids (○—○—○).

3. Results

The Arrhenius plots of the (Ca^{2+})-ATPase activity from membranes of cells grown in a medium supplemented with oleic and linolenic acid are shown in fig. 1. Lipid phase transitions do not alter the position of the break and the values of the activation energies under and below the transitions. An additional proof that the conformational change induced by the temperature is not influenced by the lipid phase is obtained by the study of the soluble form of the enzyme. No difference in the Arrhenius plots is observed between the membrane-bound and the soluble enzyme. These facts indicate that the breaks and slopes are inherent to conformational changes in the protein itself and that they are not influenced by the fatty acid composition of the membrane. The effect of Na^+ inhibition on the (Ca^{2+})-ATPase from oleic and linolenic membranes is shown in fig. 2. Hill plots with slopes of −1.6 and −2.3 are obtained. The dependence on the membrane of the allosteric behavior is lost when the enzyme is solubilized [6, 10].

Table 1
Values of the activation energies, transition points and Hill coefficients of the (Ca^{2+})-ATPase.

Supplement to medium	Ea I Kcal/mole	Ea II Kcal/mole	Ti (°C)	n
Oleic (4)*	11.7 ± 1.5**	23.4 ± 2.5	31.2 ± 1.2	1.60 ± 0.06
Linolenic (4)	10.8 ± 1.0	21.4 ± 0.9	31.7 ± 1.1	2.22 ± 0.06
	n.s.	n.s.	n.s.	$p < 0.001$

* In parentheses is the number of preparations.
** Values are expressed as the mean ± S.E.M.

Results of several determinations of activation energies, transition points and Hill coefficients are presented in table 1.

Differences were significant only for the values of n.

4. Discussion

Several features of the changes of the Arrhenius plots distinguish them from the changes in the slopes of the Hill plots.

The former reflect influences on the catalytic site while the latter refer to regulatory sites.

All the papers dealing with physicochemical methods for the study of lipid–protein interactions in intact membrane stress the fact that these methods are not sensitive enough for specific cases because their results are statistic, that is, the measurements reflect the overall contributions of all the proteins and/or of the lipid bilayer as a whole [11–13]. The study of Arrhenius and Hill plots of enzymes imbedded in the membrane might reflect interactions with the surrounding lipid environment at a more specific level. In the case of the (Ca^{2+})-ATPase from *E. coli*, the influence of the lipid composition is put in evidence only by means of the Hill plots. This different response to both methods could be due to the fact that the ATPase from *E. coli* behaves, according to the model of Singer et al. [14], as a "peripheral" protein since it can be easily removed from the membrane. In this case a feeble enzyme–membrane interaction would arise. This interaction is detected by means of the regulatory site for Na^+ while the study of temperature dependence fails in this purpose. This fact indicates that the study of the allosteric behavior of membrane-bound enzyme might be a very sensitive probe to evaluate lipid–protein interactions.

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