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Regulation by Membrane Fluidity of the Allosteric Behavior of the (Ca^{2+})-Adenosine Triphosphatase from *Escherichia coli*

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The allosteric properties of the membrane-bound (Ca^{2+})-adenosine triphosphatase of an unsaturated fatty acid auxotroph of *Escherichia coli* were studied in membranes with different fatty acid compositions. The Hill coefficient of the inhibition by Na^+ ranged from 1.4, in the case where the auxotroph was grown with *cis*-vaccenic acid as supplement, to 2.8 when grown on linolenic acid. The results indicate that no fatty acid is particularly involved in the allosteric phenomena. A correlation between the values of the Hill coefficient and the double bond index or the ratio of the double bond index saturated to the fatty acids of the membrane was found. These facts are interpreted as a modulation by the membrane fluidity of the allosteric behavior of the membrane-bound enzyme. The general biological character of this phenomenon is discussed in this paper.

The allosteric transitions of several membrane-bound enzymes have been found to change in erythrocytes and heart and kidney microsomes from rats fed fat-deficient diets (6, 7, 10-12).

The allosteric inhibition by Na^+ of the (Ca^{2+})-adenosine triphosphatase (EC 3.6.1.3) from the unsaturated fatty acid auxotroph of *Escherichia coli* L010 (14) was different when the auxotroph was grown with oleic acid or linoleic acid as supplements (8). In addition, we communicated that the Hill coefficient, "*n*," changed with other unsaturated fatty acid supplements too (Siñeriz, Farías, and Trucco, Abstr. Ann. Meet. Amer. Soc. Microb., 1972, p. 220). This study now is an approach to the understanding of the molecular mechanisms involved in the regulatory properties of the bacterial membrane on the allosteric behavior of membrane-bound (Ca^{2+})-adenosine triphosphatase.

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MATERIALS AND METHODS

Bacterial strain, growth conditions, and membrane preparation. Strain L010, an unsaturated fatty acid auxotroph of *E. coli* K_{12} (14) was grown at 37 C in a defatted L broth (8) containing 0.05% Triton X-100. In some experiments, the same medium but without glucose was used to grow cells. The fatty acids employed as supplement were palmitoleic (cis-Δ^9 hexadecenoic), oleic (cis-Δ^9 octadecenoic), vaccenic (cis-Δ^{11} octadecenoic), linoleic (cis,cis-$\Delta^{9,12}$ octadecadienoic), and linolenic acid (cis,cis,cis-$\Delta^{9,12,15}$ octadecatrienoic). They were added as the potassium salts at 0.02%.

Membranes were prepared according to the procedure of Evans (5).

(Ca^{2+})-adenosine triphosphatase activity. The enzymatic activity was measured by the method previously described (8).

Fatty acid analysis. Lipids from whole cells were extracted according to Folch et al. (9). Phospholipids were isolated by thin-layer chromatography on Silica Gel G, 0.5 mm thick, in petroleum ether-ethylether-acetic acid, 70:40:3. The origin areas of silica gel were scraped into tubes, and 1 ml of 0.5 N Na methoxide in methanol was added. After 30 min at room temperature the mixture was neutralized with methanol-hydrochloride. The methyl esters were extracted into petroleum ether and analyzed by gas-liquid chromatography employing an F & M model 700 instrument equipped with a hydrogen flame detector. The glass column (1.8 m by 4 mm) was packed with 10% EGSS-X on Chromsorb W (Applied Science Laboratories, State College, Pa.). The analyses were performed at 180 C with a carrier gas (nitrogen) flow rate

TABLE 1. *Effect of exogenous fatty acid on the fatty acid composition (mol %) and n values for the inhibition by Na^+ of the (Ca^{2+})-adenosine triphosphatase from an auxotroph of Escherichia coli*

Unsaturated fatty acid suppl.	Fatty acid (mol %)											Unsaturated/ saturated	DBI	DBI/ saturated	n^b
	14:0	16:0	Total saturated	16:1	16:2	16:3	17:0[a]	18:1	18:2	18:3	Total unsaturated	Unknown				
Palmitoleic	12.0	50.5	62.5	33.8			2.1				35.9	1.6	0.57	0.36	0.57	1.91 ± 0.04[c] (15)[h]
Vaccenic	26.4	50.0	76.4	5.7			1.0	15.0			21.7	1.9	0.28	0.23	0.30	1.34 ± 0.03[d] (6)
Oleic	17.2	46.0	63.2	4.5			1.4	29.2			35.1	1.7	0.55	0.35	0.55	1.60 ± 0.06[e] (12)
Linoleic	15.7	49.2	64.9		1.0				28.3		29.3	5.8	0.45	0.59	0.87	2.08 ± 0.07[c, f] (6)
Linolenic	24.7	45.9	70.6			0.5				22.7	23.2	6.2	0.33	0.70	0.99	2.22 ± 0.04[f] (8)
Vaccenic[g]	18.4	41.1	59.5	7.0			2.1	28.5			37.6	2.9	0.63	0.38	0.63	2.1
Linolenic[g]	7.3	50.5	57.8			2.1				31.7	33.8	8.4	0.58	1.02	1.75	2.9

[a] Cyclopropane derivative of 16:1.
[b] Results are given as mean ± standard error of the mean; n is the Hill coefficient.
[c, d, e, f] Pairs of values followed by different letters are significantly different ($P < 0.005$).
[g] Cells grown on complex medium without glucose.
[h] Number of the membrane preparations.

of 30 ml per min. Fatty acids were identified by comparing the retention times with standard fatty acid methyl esters, and peak areas were calculated as the product of the peak height by the width at half height.

Fluidity parameters. The double bond index (DBI) equals the sum of the products of the mole fraction and the number of double bonds for each fatty acid. Double bond index/saturated fatty acids is the ratio between DBI and the sum of the mole fractions of the saturated fatty acids.

RESULTS AND DISCUSSION

Table 1 shows the Hill coefficient for the inhibition by Na^+ of the membrane-bound (Ca^{2+})-adenosine triphosphatase of cells grown with the different unsaturated fatty acids as supplements. Significant differences are present in the values of *n*.

The mutant, isolated by Silbert and Vagelos (14), incorporates into the lipids of the membrane the unsaturated fatty acid supplemented to the culture medium. The unsaturated fatty acid employed as supplement is the most abundant of the unsaturated fatty acids present in the phospholipids of the membranes (Table 1). The fatty acid composition of duplicate cultures with the same fatty acid supplement were indistinguishable. The lipids of *E. coli* are more than 90% glycerophosphatides constituting phosphatidylethanolamine 70 to 90% of the total phospholipids fraction (1). Studies on monolayers of phosphatidylethanolamine indicate that the more unsaturated the fatty acid, the bigger the area per molecule occupied by the phospholipid (3, 13). The membrane with more unsaturated fatty acids would be more fluid. An appropriate parameter for the fluidity is the ratio of double bond index to saturated fatty acids (2). This ratio varies with the supplement as can be seen in Table 1, apparently being a parallel increase of the fluidity parameters and the Hill coefficient. Furthermore, the absence of glucose in the complex medium causes an increase in the content of the total unsaturated fatty acids in the phospholipids (21.7 to 37.6 for vaccenic acid and 23.2 to 33.8 for linolenic acid) with a concomitant increase of the fluidity parameters and the Hill coefficient (lower part of Table 1).

When the overall correlation coefficient was calculated between the values of n and the ratio double bond index/saturated fatty acids, a value of r = 0.94, which is highly significant ($P < 0.001$), was obtained as can be seen in Fig. 1. Correlation was also found between the values of *n* and the double bond index, which has the same physico-chemical significance. In this case r = 0.93 ($P < 0.005$) was found. These correlations demonstrated the active role of the fluidity of the fatty acids of the membrane-phospholipids in controlling the allosteric behavior of the enzyme. On the other hand, no correlation was found between the values of *n* and total unsaturated fatty acid content, the ratio of unsaturated to saturated fatty acids, nor with any particular fatty acid. The non-involvement of any particular fatty acid is confirmed by the experiments in the absence of glucose. The changes of the fatty acid composition observed in membranes of cells grown in media supplemented with linolenic or vaccenic acid in the presence or absence of glucose allowed us in each case to have the same fatty acids in the phospholipids but in different quantities. It is observed here that an increase in the unsaturated fatty acid (vaccenic or linolenic) is accompanied by a decrease in myristic acid content. Esfahani et al. (4) interpreted this fact as if myristic acid could counterbalance the absence of unsaturated fatty acid in order to maintain the overall physical properties of the membrane within narrow limits. This hypothesis would not apply here, because the values of *n* as well as the fluidity parameters vary. This fact casts doubt on the physicochemical significance of the compensation between the quantities of medium chain-saturated and of unsaturated fatty acids in membranes.

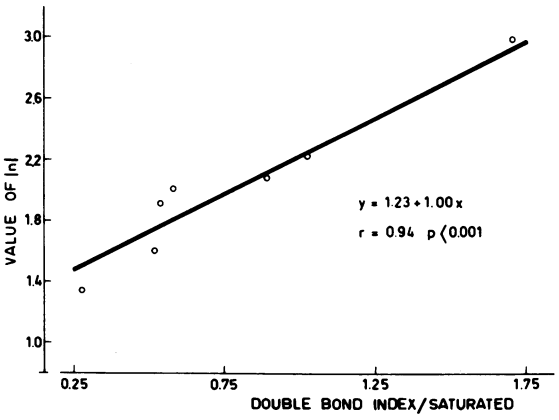

FIG. 1. *Scattergram of the correlation between the values of n (Hill coefficient) and the ratio double bond index per saturated fatty acids. The equation of the regression line and the overral correlation coefficient r with its significance are included.*

The correlation observed between the values of *n* and the fluidity parameters is clear evidence of the regulatory properties of the bacterial membrane on the conformational changes of the membrane-bound (Ca^{2+})-adenosine triphosphatase. The general biological

character of this phenomenon is supported by the fact that the allosteric transition of the membrane-bound acetylcholinesterase and $(Na^+ + K^+)$-adenosine triphosphatase from rat erythrocytes are subject to a comparable modulation by fluidity. These results were obtained from studies with animals fed with different fat-supplemented diets (2).

The physiological significance must be explored before we can understand the implications of these results. However, these studies suggest that the evaluation of the conformational changes of membrane-bound enzymes (by mean of the Hill coefficient) could be a useful tool for recording changes in situ of the membrane conformation.

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