Phospholipids and ATPase Activity of Wild-Type and ATPase Deficient and Uncoupled Mutants of *E. coli*

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The ATPase activities and the amounts of individual phospholipids in *E. coli* wild types B 163 and ML-308-225 as well as in the mutants AN 120, DL 54 and etc-15 have been examined. The ATPase activities and the amount of cardiolipin are higher in the stationary phase than in the log phase, whereas the amounts of phosphatidylglycerol and phosphatidylserine are lower in the stationary phase. The decreased ATPase activity of the mutants is not due to an altered phospholipid composition.

Introduction

Phospholipids are believed to have at least two distinct roles in biological membranes. The first role is structural and the second is their contribution to the function of certain membrane-bound enzymes (reviewed by Cronan and Vagelos [1]). Recently it has been shown that the ATPase from *E. coli* is dependent on the membrane, although only loosely bound to it [2]. Removal of phospholipids from the ATPase preparation leads to a loss of hydrolytic activity. For full activity, at least 100 molecules of phospholipids – mainly phosphatidylethanolamine – per molecule ATPase are needed [2]. Some of these phospholipid molecules are strongly bound to the ATPase. Kobayashi and Anraku [3] reported that highly purified *E. coli* ATPase contained 1 – 2 mol of phospholipid per mol ATPase. Solubilization of the membrane-bound *E. coli* ATPase also causes a loss of activity [4]. The lost activity can be restored by addition of the original membrane fragments [4]. Addition of soy-bean phospholipids to an *E. coli* ATPase preparation ($CF_0 \cdot F_1$) caused extensive stimulation of ATPase activity [5]. Thus phospholipid requirement is indicated. It has been reported, that cardiolipin is essential for the activity of mitochondrial ATPase, which is very similar to the *E. coli* ATPase, since destruction of cardiolipin is accompanied by a reduction of ATPase activity [6]. Since the ATPase from *E. coli* depends on phospholipids, we intended to investigate whether changes in phospholipid composition during growth have an influence on ATPase activity. In addition, we wanted to see if the highly reduced ATPase activity of several *E. coli* mutants could be due to changes in the phospholipid composition.

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Materials and Methods

The conditions for the growth of bacteria and the preparation of the membrane-bound ATPase has been described recently [2]. Proteins were determined by the method of Lowry *et al.* [7]. ATPase activity was determined as described by Ahlers *et al.* [8]. The determination of the individual phospholipids was performed by quantitative thin-layer chromatography according to the method of Peter and Wolf [9] modified by Peter and Ahlers [2].

For the determination of the phospholipid composition the whole bacteria or membrane vesicles were freeze dried and the phospholipids extracted with chloroform/methanol 1:1 (v/v).

Phospholipids for references were purchased from Supelco, Inc., Bellefonte, USA, from Koch-Light, Lab. Ltd., Colnbrook, England, and from Serva, Heidelberg, Germany. All other chemicals were of reagent grade and obtained from Merck, Darmstadt, Germany.

Results and Discussion

The growth rates of *E. coli* wild-types B 163 and ML-308-225 and the mutants AN 120, DL 54 and etc-15 are to be seen in Fig. 1. During the log-phase, the phospholipid composition remains con-

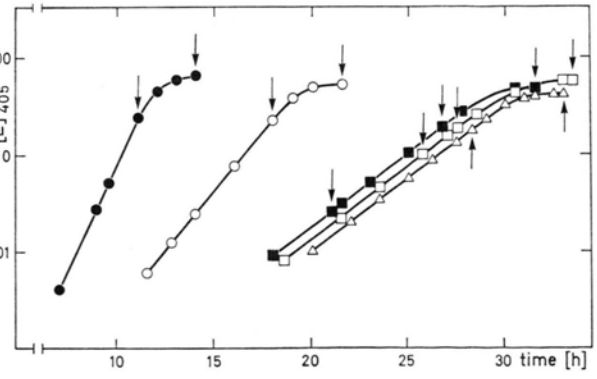

Fig. 1. Growth of *E. coli* B 163 (●), *E. coli* K 12 AN 120 (○), *E. coli* ML 308-225 (■), etc-15 (□) and DL 54 (△). The extinction was measured at 405 nm. At the times indicated by arrows, probes were withdrawn for the determination of phospholipid composition and ATPase activity.

Table I. Phospholipid composition of membrane vesicles (membrane-bound ATPase) of various *E. coli* strains from log and stationary growth phase in % of total lipids. Mean ± S.D. pe, phosphatidylethanolamine; ps, phosphatidylserine; pg, phosphatidylglycerol; cl, cardiolipin.

E. coli	Growth phase	*n*	pe	pg	cl	ps
B 163	log	3	71.8±2.1	14.9±2.8	5.2±1.5	3.0±1.0
	stat	2	73.0±2.0	10.7±3.0	9.0±3.2	0.5±0.3
ML 308-225	log	3	71.0±6.0	12.5±4.1	9.9±5.2	6.9±1.9
	stat	3	70.3±3.0	11.0±4.0	12.6±2.5	4.4±1.3
etc-15	log	5	63.7±4.5	14.5±1.2	12.5±1.0	7.9±0.4
	stat	3	63.0±5.1	10.7±2.0	19.3±2.1	4.5±0.7
DL 54	log	3	74.5±5.3	19.5±1.3	3.5±0.5	1.0±0.1
	stat	2	76.5±5.0	13.2±3.2	12.2±0.5	0.5±0.3
AN 120	log	2	69.9±2.5	20.5±3.5	5.7±1.2	0.8±0.3
	stat	4	70.5±5.7	13.8±3.0	12.2±3.3	0.6±0.3

stant. There are no differences in the phospholipid composition of the whole cells and the membrane vesicles, which are the source of the membrane-bound ATPase (not shown).

Table I shows the phospholipid composition of the bacteria investigated during the log-phase and the stationary phase. There are some differences to be seen between the strains tested: etc-15 has a lower phosphatidylethanolamine content and a higher cardiolipin content than the others, while DL 54 and AN 120 have a higher phosphatidylglycerol content, especially in the log-phase. During the transition from log to stationary phase in all strains, phosphatidylethanolamine remains nearly constant, phosphatidylglycerol and phosphatidylserine decrease, and cardiolipin increases. However, the total and relative differences between the log and stationary phases are not the same for the various strains. The changes in phospholipid composition in the log-phase and the stationary phase found here, are in accordance with the results reported by others [1]. The finding of a decrease in phosphatidylglycerol parallel to an increase in cardiolipin (Table I) supports the hypothesis that there is a pathway for biosynthesis of cardiolipin *via* phosphatidylglycerol as precursor [10, 11].

The ATPase activities of the membrane preparations are to be seen in Table II. In accordance with the results of Bragg *et al.* [12], the ATPase activities in the stationary phases are somewhat higher than those in the log-phases. As described by Bragg *et al.*, the extent of this increase in specific activity depends on growth conditions [12]. In all strains there seems to be a correlation between increasing ATPase activity and increasing cardiolipin and decreasing phosphatidylglycerol content during transition from log to stationary phase.

Table II. Activity of membrane-bound ATPase from various *E. coli* wild-types and ATPase mutants, harvested during log and stationary growth phase. Incubation at 37 °C in 0.1 M Tris-Cl buffer, pH 7.5; 0.5 mM $MgCl_2$ and 1 mM ATP were added as substrate. The data are averages of two experiments.

E. coli	μmol $P_i \cdot 10^2$ / mg protein · min log	stat
B 163	4.9	12.1
ML 308-225	3.0	6.5
etc-15	1.5	2.9
DL 54	0.43	0.88
AN 120	0.56	1.00

However, when wild types and mutants are compared, no correlation between ATPase activity and phosphatidylglycerol or cardiolipin content can be observed. The highest ATPase activity is exhibited by the B 163 membrane preparation from stationary bacteria. The preparation from DL-54 in the log phase exhibits the lowest ATPase activity. However, these extreme differences in ATPase activities are not due to an altered phospholipid composition. Obviously in all strains and phases tested, the ATPase is embedded in the membrane and is always surrounded by phospholipids in amounts which are sufficient for optimal activity. This conclusion seems to be in accordance with the findings that very small amounts of phospholipids are needed for *E. coli* ATPase activity [2, 3].

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