CHANGES OF THE MEMBRANE PROTEIN COMPOSITION IN THE MUTANTS ON REGULATORY GENES OF *ESCHERICHIA COLI* ALKALINE PHOSPHATASE

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1. Introduction

The alkaline phosphatase secreted by *Escherichia coli* is synthesized on membrane-bound polysomes [1–3] and crosses the cytoplasmic membrane as a growing polypeptide chain [4], thus entering complex hydrophobic interactions with membrane proteins and lipids. The increase in the lipid fluidity was shown at the level of enzyme biosynthesis and secretion was shown in [5–7]. The specific interrelation may be between the enzyme during its biosynthesis and secretion, and individual phospholipids, i.e., phosphatidylglycerol [8–9]. Little is known about the participation of membrane proteins in the biosynthesis and translocation of alkaline phosphatase.

Insight may be gained from the correlation between biosynthesis of alkaline phosphatase and changes in the membrane protein composition, as well as the dependence of the latter on the state of the regulatory system of enzyme biosynthesis.

The coregulation of pore protein '*e*' of the outer membrane of *E. coli* with alkaline phosphatase was described in [10–12]. The analysis of the envelope protein composition of the wild strain *E. coli* K10 and derivative mutants on the regulatory genes [13, 14] also showed that the biosynthesis of some membrane proteins was coregulated with that of alkaline phosphatase by exogenous orthophosphate. However, a number of membrane proteins was lost in the mutants of regulatory genes of this enzyme.

This work deals with the localization of *E. coli* envelope proteins, the content of which changes depending on the presence of orthophosphate in the medium and the integrity of the regulatory genes. One protein, coregulated with alkaline phosphatase, is the cytoplasmic membrane protein. The loss of two outer membrane proteins *a* and *tsx* due to mutations in the regulatory genes is also shown [15].

2. Experimental

2.1. *Strain and growth conditions*

Wild strain *E. coli* K10 and its derivative mutants on regulatory genes of alkaline phosphatase were used: *E. coli* LEP1 (*phoB*$^-$) with a defect in *phoB* gene responsible for the expression of alkaline phosphatase structural *phoA* gene and *E. coli* C61 (*phoS*$^-$) with constitutive synthesis of enzyme due to the defect in the phosphate-binding protein, also participating in the formation of the alkaline phosphatase repressor. The strains were generously provided by Professor A. Torriani. *E. coli* were grown in synthetic medium with 1 mM orthophosphate to mid-log (the repressed cells). For derepression of the alkaline phosphatase, cells were harvested, washed twice with 0.14 M NaCl at 4°C and transferred into medium lacking orthophosphate. After 2 h incubation under phosphate starvation, derepressed cells were harvested, washed and were disintegrated by ultrasonic treatment in the 0.01 M Tris–HCl buffer (pH 7.8) with 0.001 M EDTA as were the repressed cells.

2.2. *Isolation of membrane fractions*

Cell envelopes were sedimented after ultrasonic disintegration by centrifugation at 105 000 × *g* for 60 min, washed twice with 0.01 M Tris–HCl buffer (pH 7.4) containing 0.001 M $MgSO_4$. Cytoplasmic membrane and outer membrane proteins were separated by treatment of envelopes with 2% Triton X-100 in the presence of 0.01 M $MgSO_4$ according to [16].

Protein–peptidoglycan complexes were isolated

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by extraction of cell envelopes with 2% SDS at 60°C following [17] as modified [18].

2.3. *Analytical methods*

The gel system with 10% acrylamide and convex exponential gradient gels (10–16% acrylamide) in the presence of SDS were used for the analysis of the protein composition of the membrane fraction according to [19–21]. Protein was determined by Lowry method [22].

3. Results and discussion

3.1. *Coregulation of cytoplasmic membrane protein 28 000* M_r *with alkaline phosphatase*

The content of two envelope membrane proteins of M_r 28 000 (I) and 17 000 (X) increased under phosphate starvation under conditions of alkaline phosphatase derepression (fig.1). However, the biosynthesis of only protein I was determined by the genes, common with the alkaline phosphatase regulatory genes. This protein was not synthesized in the *phoB*⁻strain, in which the expression of the alkaline phosphatase was absent (fig.1). It was synthesized independently of orthophosphate in the *phoS*⁻ strain with constitutive, non-repressible biosynthesis of enzyme, and its content

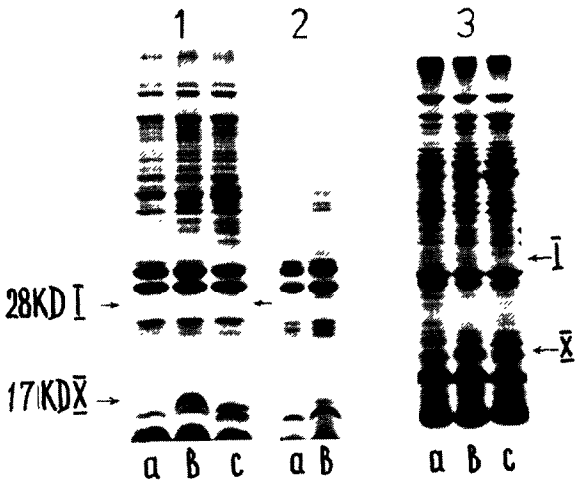

Fig.1. SDS–polyacrylamide gel electrophoresis patterns of cell envelope proteins (1), outer membrane proteins (2) and cytoplasmic membrane proteins (3) of *E. coli* K10, grown in the medium with orthophosphate (a), without orthophosphate (b) and *E. coli* LEPI (*phoB*⁻) in the medium without orthophosphate (c).

corresponded to the level of this protein in the wild strain under derepression condition [11]. The same character of the change in this protein content was observed by the analysis of the cytoplasmic membrane fraction (fig.1). We found no protein in the outer membrane fraction which corresponded to the protein I motility. So, protein I is the cytoplasmic membrane protein, the synthesis of which is controlled by the same regulatory mechanism as the synthesis of alkaline phosphatase. Protein X is also a cytoplasmic membrane protein, but its dependence on the genes *phoS* and *phoB* differs from alkaline phosphatase and protein I. It is found in the *phoB*⁻ mutant and its content decreases in the *phoS*⁻ mutant (fig.1). So the biosynthesis of this protein, apparently, is not coregulated with alkaline phosphatase.

Coregulation of a number of the periplasmic proteins with alkaline phosphatase was shown in [23]. Among them the binding proteins for P_i [24] and glycerol-3-phosphate [12], as well as enzymes polyphosphatase [25,26] and acid phosphatase [27]. The nature of the cytoplasmic membrane protein I is unknown, but similar to the periplasmic proteins, its function is probably connected with the phosphorous metabolism of *E. coli*.

3.2. *The loss of two outer membrane proteins (*a *and* tsx*) in the mutants on the regulatory genes of alkaline phosphatase*

As seen from fig.1,2, there are no outer membrane proteins the content of which depends on orthophosphate in the medium. The expression of outer membrane protein '*e*' under phosphate starvation [12] and its determination by *phoR* and *phoS* gene common with alkaline phosphatase [13,14] were shown. In our *E. coli* K10 strain and under our conditions, this protein is in trace quantities if present, as seen from the comparison of electrophoregrams of outer membranes and purified pore protein *e* (fig.2).

We found, however, that two outer membrane proteins were lost in the *phoB*⁻ and *phoS*⁻ strains with the defect in the regulation of the alkaline phosphatase biosynthesis (fig.2,3). The 37 000 M_r protein corresponds, by its electrophoretic motility in two systems, to protein *a* (classification [18]) (fig.2, (1,2)). This protein was lost in the membrane preparation after its extraction with 2% SDS for 60 min and therefore it is not bonded with peptidoglican, in agreement with [18]. The 25 000 M_r protein, which is lost in these strains, is the minor protein, bonded with pep-

Fig.2. SDS–polyacrylamide gel electrophoresis patterns of the outer membrane proteins of *E. coli* K10 in the medium with orthophosphate (+P) (a), without orthophosphate (–P) (b), *E. coli* LEPI (*phoB*⁻) on –P (c), *E. coli* C61 (*phoS*⁻) on +P (d) and –P (e), the purified outer membrane protein *e* (f), and peptidoglican bound proteins of *E. coli* K10 on +P (h) and –P (j); 9% gel was used according to [21] I, and the system D [16] II.

tidoglican and corresponds apparently to *tsx* protein by its electrophoretic motility [28,29] (fig.3).

According to the chromosome map (Taylor) of *E. coli* genes involved in alkaline phosphatase formation [23], the *tsx* gene (map position near 10 min) is localized near the *phoR* and *phoB* regulatory genes of this enzyme. In [30,31] the biosynthesis of the outer membrane protein 'a' was determined by the *capR* (*lon*) gene responsible for the capsular polysaccharide biosynthesis. This gene is also localized in the same locus (near 10 min) not far from the *phoB* gene.

The loss of the outer membrane proteins designated as *a* and *tsx* proteins in the *phoB*⁻ mutant, including an extensive deletion [32] allows us to suggest that these proteins are determined by the *lon* and *tsx* genes and that the loss of these proteins in *phoB*⁻ mutant may result from the defect of the corresponding genes. On the other hand, *phoB* gene, probably, is required not only for the expression of alkaline phosphatase structural genes *phoA* but also for the expression of the *tsx* and *lon* genes, encoding two outer membrane proteins.

Some other data confirm the interconnection of

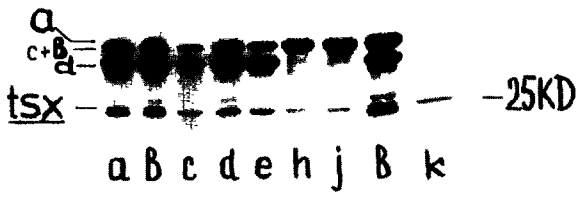

Fig.3. SDS–polyacrylamide gel electrophoresis pattern of the outer membrane proteins of *E. coli* K10 and the derivative mutants. Designations are as in fig.2; K, chymotrypsinogen with M_r 25 000, 11–16% gradient gel was used.

all these genes, e.g., the *phoB*⁻ mutant requires adenine. This is probably the result of the defect of *tsx* gene, which is as known, responsible not only to the reception of T_6 phage and colycine K, but also for the nucleoside uptake [29]. However, it was shown that thymidine starvation of corresponding auxotrophs (probably with defect in the *tsx* gene) resulted in the decrease in the content of '*y*' outer membrane protein [33] corresponding to '*a*' protein [34].

It is worthy of note that the mutation of *phoB* gene is accompanied by the increase in the content of the cytoplasmic membrane protein with M_r ~54 000 (fig.1). Probably this protein is the precursor of some periplasmic proteins, e.g., alkaline or acid phosphatases, practically absent in this mutant, and *phoB* mutation results in the defect in the post-translational modification of these enzymes. This is the result of the defect in *lon* gene, which determines the degradation (processing) of a number of proteins [35].

As seen from fig.2,3, the presence of the two outer membrane proteins *a* and *tsx* depends on the integrity of the *phoS* gene also and this dependence differs from that of alkaline phosphatase. The intact *phoS* gene is required for the repression of alkaline phosphatase in the presence of orthophosphate in the medium [36,37] and the defect in it results in the constitutive synthesis of this enzyme. Our results show, that the *phoS* gene is required on the contrary for the biosynthesis of an '*a*' and '*tsx*' proteins and the *phoS* mutation as well as *phoB* mutation results in the loss of these proteins. So, two genes, participating in the regulation of alkaline phosphatase biosynthesis determine the biosynthesis of two outer membrane proteins.

Some analogy exists between the behaviour of these outer membrane proteins and acid phosphatase.

We observed in [27], that the activity of this enzyme in the cells of the *phoS*$^-$, *phoB*$^-$ and *phoT*$^-$ mutants was drastically low as compared to that in the wild strain, and *phoS* gene was required for the induction of polyphosphatase and acid phosphatase under phosphatase starvation [26,27].

These results allow us to suggest the existence of some interconnection between the biosynthesis of outer membrane proteins and acid phosphatase in *E. coli*, and the necessity of the *phoS* gene for their biosynthesis.

However, there may be another reason for the loss of the outer membrane proteins in the *phoS*$^-$ mutant. We have shown the weakening of the bond between a number of proteins and membranes in *phoS*$^-$ mutant. Among them proteins I, V, II [14] and ATPase [38], which were released from the membrane and revealed in the periplasm of *phoS*$^-$ strain C61, as compared to the wild strain. It might result from the destabilization of membrane in this strain. The fact is that the orthophosphate and its binding factors stabilize the mitochondrial membrane [39]. The same situation may exist in bacteria. The *phoS* gene encodes the phosphate-binding protein, which is absent both in the *phoS*$^-$ mutant periplasm and in membranes with which the minor part of this protein is probably connected [13,14]. The weakening of the bond between proteins and membranes may result in the loss of outer membrane proteins in *phoS*$^-$ strain.

From the above we can see that one cytoplasmic membrane protein is co-regulated with alkaline phosphatase and two outer membrane proteins are absent due to mutations in the regulatory genes of this enzyme. It is important, that changes in the regulation of the alkaline phosphatase biosynthesis correlate with changes both in cytoplasmic membrane and outer membrane proteins thus allowing us to suggest the participation of membrane proteins in the regulatory system of alkaline phosphatase and probably membrane localization of products of regulatory genes of this enzyme.

The existence of the *E. coli* membrane proteins participating in the biosynthesis and regulation of alkaline phosphatase is possible, because the biosynthesis of the enzyme, processing and its secretion take place on the membrane level and at least 3 types of membrane proteins may participate in the regulation of the alkaline phosphatase biosynthesis: proteins forming the transport site (if present) for the enzyme; proteins modifying the enzyme molecule during secretion, e.g., cleavage proteases; and proteins binding and transporting orthophosphate and participating indirectly in the regulation of the alkaline phosphatase biosynthesis.

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