CELL ENVELOPE PROTEINS OF DIVIDING AND NON-DIVIDING CELLS OF *ESCHERICHIA COLI*

J. STÂRKA
Laboratoire de Chimie Bactérienne, CNRS, Marseille, France

Received 14 June 1971

1. Introduction

Recently, chemical changes of cytoplasmic membrane components have been reported to occur after inhibition of cell division of *Escherichia coli.* Different membrane protein alterations have been observed in thermosensitive mutants that are defective in DNA replication [1, 2]. On the other hand, phospholipid composition of membranes varies when division ceases during the transition from exponential to stationary phase [3] or when the cytokinesis is inhibited by penicillin [4]. Whether these changes are in direct or indirect correlation with altered DNA replication and/or cellular division remains still unclear.

The present paper shows that the polyacrylamide gel electrophoretograms of envelope protein fraction from exponentially dividing *E. coli* contain one distinct band (molecular weight about 15 000) which is absent in non-dividing but growing filaments and in normal stationary cells.

2. Material and methods

E. coli B (No. 39 from the collection of Department of Microbiology, Charles University, Prague) was cultivated on tryptose medium (Bacto Tryptose 0.5%, NaCl 1%) at 37° on a reciprocal shaker. Filament formation was induced by addition of penicillin (2.5 units/ml) to exponentially growing cultures when the absorbance at 450 nm reached 0.2 [5].

Envelope fraction was prepared by a method based on that of Inouye and Guthrie [6]. 500 ml samples were rapidly chilled and centrifuged for 30 min at 3 500 *g*. Collected cells were washed twice with 0.01 M Na-phosphate buffer, pH 7.1. The bacteria resuspended in this buffer were disrupted using a sonic probe (Branson Sonifier, models S 110) at the maximal output for 5 min under constant refrigeration. The homogenate was centrifuged at 3 500 *g* for 15 min to remove whole cells. The supernatant was then spun at 100 000 *g* for 1 hr and the collected cell envelope fraction was washed once with the same buffer. The pellet was homogenized in β buffer diluted 1:20 [7] and solubilized by adding sodium dodecyl sulfate to a final concentration of 1% and incubating at 60° for 20 min. Non-soluble material was removed by centrifugation at 35 000 *g* for 30 min, 1 ml of solubilized envelope fraction was added to 2 ml of pheno–acetic acid–water mixture (2:1:0.5, w/v/v). Aliquots of the soluble fraction so treated were electrophoresed on 7.5% polyacrylamide gels containing 35% acetic acid and 5 M urea. The gels were run in 10% acetic acid at 2 mA/tube for 4 hr [8]. The lower electrode served as a cathode. The proteins in the gel were stained with Amido Black 10 B or with Coomassie Brilliant Blue. Densitometer tracings of the stained gels were made in a Unicam spectrophotometer equipped with a scanner.

3. Results and discussion

Samples of envelope fractions solubilized in the highly dissociating mixture of phenol–acetic acid were prepared (1) from exponentially growing and dividing bacteria, (2) from stationary cells (18 hr culture), (3) from penicillin-induced filaments (exponentially cells growing for 90 min in the presence of 2.5 units of penicillin/ml) and (4) from dividing cells ob-

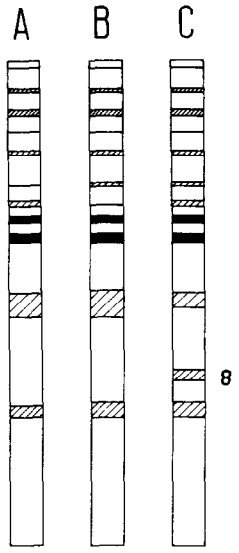

Fig. 1. Gel electrophoretograms of envelope proteins of *E. coli* B. (A) Stationary cells. (B) Penicillin-induced filaments. (C) Exponentially dividing cells. Reverting filaments gave a pattern similar to that of exponential cells. Band 8 corresponds to peak 8 on fig. 2.

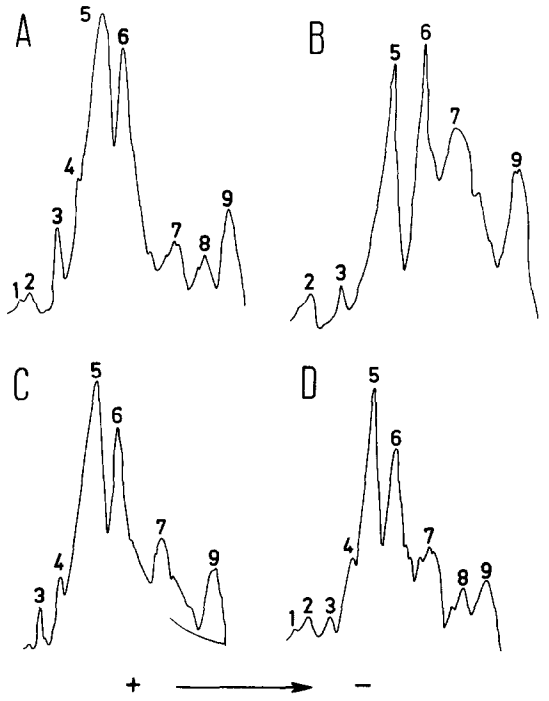

Fig. 2. Densitometric tracings of gel electrophoretograms. (A) Exponentially dividing cells. (B) Stationary cells. (C) Penicillin-induced filaments. (D) Reverting filaments.

tained from filaments treated with penicillinase (samples withdrawn 60 min after addition of the enzyme).

Fig. 1 shows schematic drawings of the bands of separated proteins observed on stained gels. Optical density scans of the same gels are shown on fig. 2. All samples contain two major protein bands. The essential difference between dividing cells (exponential cells and reverting filaments) and non-dividing cells (stationary cells and filaments) is represented by the presence in dividing cells of band 8, which is absent in non-dividing cells. Quantitative differences of some bands (e.g., stronger bands 6 and 7 in stationary cells) have been repeatedly observed, but their significance was not tested.

To determine approximately the molecular weight of the protein present only in membrane fraction of dividing cells, the technique of Shapiro, Viñuela and Maizel [9] was applied to urea–acetic acid polyacrylamide gels. Proteins of known molecular weight were dissolved in phenol–acetic acid mixture and added directly to the samples of envelope protein fraction. Samples without added protein standard were run in parallel and served as control. The results of this experiment indicate that the band of the protein present in envelope fraction of dividing cells (band 8) is located in the proximity of the lysozyme band (egg white lysozyme, molecular weight 14,388). Thus the molecular weight of this envelope protein can be estimated to be about 15 000. This protein is probably not identical to the altered membrane proteins found in thermosensitive mutants affected in the synthesis of DNA [2, 6], since their molecular weights are different. However, it should be emphasized that different methods of isolation, solubilization and dissociation of membrane proteins were used in all of these three reports.

Our results bring further indications concerning the correlation between changes on the membrane protein level and the mechanism of cell division. In our earlier paper [5] it was observed that the reversion of penicillin-induced filaments of *E. coli* to normally dividing cells depends on the *de novo* synthesis of proteins immediately after addition of penicillinase. Although the filaments synthesize proteins normally,

the renewal of 'division potential' after penicillin destruction is suppressed by chloramphenicol added simultaneously with the penicillinase. When chloramphenicol was added 10 min later, some division was subsequently observed in the presence of the antibiotic, indicating that the accumulation of some protein in the filaments was necessary for the division. It would be interesting to see if there is any correlation between these results and the present observation of reappearance of band 8 in reverting filaments. Importance of protein synthesis for the 'division potential' has been investigated in some detail recently in thermosensitive mutants of *E. coli*, defective in DNA synthesis and in division [10,11]. Since DNA is synthesized normally in our filamentous cells in the presence of penicillin, the observed changes of envelope protein composition might be correlated in some way with the process of cell division.

References

[1] M. Inouye and A.B. Pardee, J. Biol. Chem. 245 (1970) 5813.
[2] B.M. Shapiro, A.G. Siccardi, Y. Hirota and F. Jacob, J. Mol. Biol. 52 (1970) 75.
[3] J.E. Cronan, J. Bacteriol. 95 (1968) 2054.
[4] J. Stárka and J. Moravová, J. Gen. Microbiol. 60 (1970) 251.
[5] J. Stárka and J. Moravová, Folia Microbiol. 12 (1967) 240.
[6] M. Inouye and J.P. Guthrie, Proc. Natl. Acad. Sci. U.S. 64 (1969) 957.
[7] S. Razin, H.J. Morowitz and T.M. Terry, Proc. Natl. Acad. Sci. U.S. 54 (1965) 219.
[8] S. Rottem and S. Razin, J. Bacteriol. 94 (1967) 359.
[9] A.L. Shapiro, E. Viñuela and J.V. Maizel, Biochem. Biophys. Res. Commun. 28 (1967) 815.
[11] J.N. Reeve, D.J. Groves and D.J. Clark, J. Bacteriol. 104 (1970) 1052.
[11] K. Nagai, H. Kaneko and G. Tamura, Biochim. Biophys. Res. Commun. 42 (1971) 669.