STRUCTURAL CHANGES IN THE CELL MEMBRANE OF λ-LYSOGENIC ESCHERICHIA COLI INDUCED BY COLICIN E_2

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SUMMARY: The structural changes in the cell membrane of λ-lysogenic Escherichia coli induced by colicin E_2 were examined. The addition of colicin E_2 made the cells susceptible to various detergents and the transport rate of o-nitrophenyl-β-D-galactoside into the colicin-treated cells was stimulated markedly by adding a low concentration of sodium dodecyl sulfate. The fluorescence intensity of 8-anilino-1-naphthalenesulfonate bound to the cells was markedly increased by adding colicin E_2. Colicin E_2 stimulated the incorporation of ^{32}P from prelabeled phosphatidylglycerol to cardiolipin. All these changes probably suggesting the structural alteration of the cell membrane were dependent on the presence of the rex gene of λ prophage in the cells.

INTRODUCTION

Colicin E_2 is a bactericidal protein which causes extensive degradation of DNA in the sensitive cells of Escherichia coli(1). Highly specific nucleolytic activity of colicin E_2 protein against λ phage DNA was observed in vitro(2), however, effects of the colicin on the cell membrane of E. coli had not been determined. Recently, we have shown that colicin E_2 inhibits the rate of active transport in λ-lysogenic E. coli cells under the condition to prevent the prophage induction(3). Colicin E_2 inhibited the active transport of ONPG and proline, and induced the leakage of intracellular K^+ ion and thiomethylgalactoside. It was also observed that the inhibition of the membrane transport systems by

Abbreviations used are: ONPG, o-nitrophenyl-β-D-galactoside; SDS, sodium dodecyl sulfate; ANS, 8-anilino-1-naphthalenesulfonate

colicin E_2 was markedly stimulated by the presence of the rex gene product of λ prophage in E. coli cells. In this paper, we examined whether colicin E_2 promoted the structural changes of cell membrane of E. coli corresponding to the inhibition of transport systems as described previously.

METHODS AND MATERIALS

E. coli 353λ^s and its lysogenic derivatives for wild λ, λrex$^-$5a or λind$^-$(3) were used. For almost all experiments, cells were grown aerobically at 37°C in Tris-glycerol(TG) medium(4). Isopropyl-β-D-galactoside(2.5×10^{-4}M) was added to the medium to induce β-galactosidase and lactose permease. To measure the turnover rate of phosphate in phospholipids, TG-medium with 1 mM phosphate was used. Colicin E_2 was purified and its multiplicity (m) was determined as described previously(3)

The ONPG-transport rate was measured according to the previous report(3). In order to measure fluorescence change of the cell bound ANS, the cells grown to 5×10^8 cells/ml were harvested and suspended at a concentration of 10^9 cells/ml in TG-medium containing chloramphenicol(80 μg/ml) and 1.8×10^{-5}M ANS was added. Colicin E_2(m = 90) was added at 15 min after the addition of the dye. The emission spectra of the fluorescence of the cell suspension excited with 360 nm light were measured in a Hitachi MPF-3 fluorospectrophotometer with stirring at 37°C. Measurements of turnover rate of phosphate in phospholipids were carried out using the cells previously labelled with ^{32}Pi. After washing, the cells were suspended in the same volume of the medium containing chloramphenicol without ^{32}P, and colicin E_2(m = 90) was added at 15 min after the resuspention. Phospholipids were extracted by the method of Bligh and Dyer(5), fractionated by thinlayer chromatography(6) and radioactivity of each spot was counted by liquid scintilation counter.

RESULTS AND DISCUSSION

Increased susceptibility to detergents induced by colicin E_2

As reported previously(3), the addition of colicin E_2 suppressed the ONPG transport rate of E. coli 353λ^s(λ) cells at first, but the subsequent addition of SDS(100 μg/ml) at 15 min after the colicin challenge was found to induce the instant and marked increase of the transport rate(Fig. 1A). Very slight or no accerelation of the transport by SDS occurred in the colicin-untreated cells. No leakage of β-galactosidase from cells into

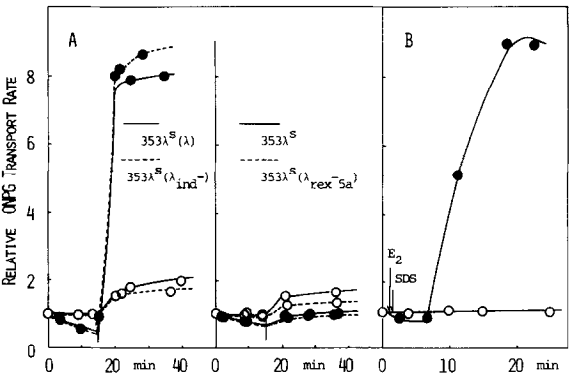

Fig. 1 SDS-induced increase of ONPG-transport rate in colicin E_2 treated cells. A, Effect of λ-lysogenization: colicin E_2(0.072 μg/ml, 93 killing units/cell) was added at 0 min. SDS (100 μg/ml) was added at 15 min after adding the colicin. Symbols: O , control without colicin; ● , colicin treated cell. B, Time course of SDS effect on $353\lambda^s(\lambda)$. Colicin(93 killing units/cell) was added at 1 min and SDS(60 μg/ml) at 1.5 min. Symbols: O , SDS without colicin; ● , SDS with colicin.

the medium took place along with this accerelation suggesting that lysis of cells did not occur in this case. The similar accerelation of the transport in the colicin-treated cells was observed when SDS was replaced by Triton X-100(20 mg/ml) or sodium deoxycholate(400 μg/ml). When SDS was added immediately after the colicin challenge, it took about 5 or 6 min to cause the distinct increase of the transport rate and about 20 min to reach the maximum accerelation(Fig. 1 B). It had been observed that the action of colicin E_2 on the membrane transport systems proceeded in two phases, and the marked inhibition along with the rapid leakage of intracellular metabolites started after 5 min of the first weak inhibition phase(3). The susceptibility of the membrane to the detergents induced by colicin E_2 might correspond to this second stage of the transport inhibition.

The marked stimulation of the ONPG-transport rate in the colicin-treated cells by SDS was observed in cells of E. coli

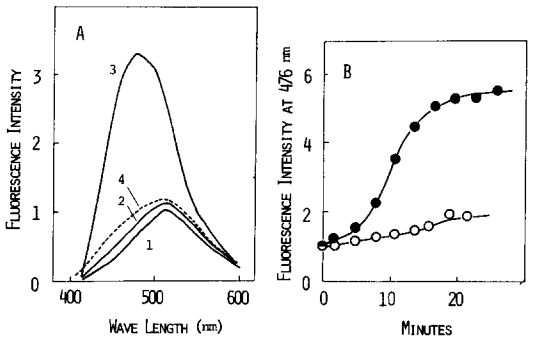

Fig. 2 Colicin E_2-induced increase of ANS fluorescence in cell suspension. A, Emission spectra of ANS bound to cells of $353\lambda^s(\lambda)$ (——) and $353\lambda^s(\lambda_{rex\text{-}5a})$ (----). 1, 0 min; 2, 3 min after adding colicin E_2(0.14 μg/ml, 90 killing units/cell) 3, 18 min; 4, 21 min. B, Time course of E_2-induced increase of ANS fluorescence. Colicin was added at 0 min. Symbols: O , $353\lambda^s(\lambda_{rex\text{-}5a})$; ● , $353\lambda^s(\lambda)$.

$353\lambda^s(\lambda)$ and $353\lambda^s(\lambda_{ind}\text{-})$ but not in cells of E. coli $353\lambda^s$ and $353\lambda^s(\lambda_{rex}\text{-}5a)$ (Fig. 1A). Therefore, the susceptibility to detergents induced by colicin E_2 is dependent on the presence of the λ-rex gene product in the cells.

Increased fluorescence of the cell bound ANS induced by colicin E_2. When E. coli $353\lambda^s(\lambda)$ cells were treated with colicin E_2 in the presence of fluorescent dye ANS, the fluorescence intensity increased markedly and blue shift of the emission spectrum occurred(Fig. 2A). As shown in Fig. 2B, the increase of fluorescence intensity was slight for the first 5 to 6 min after the colicin challenge and then increased rapidly for the following 10 to 15 min exceeding to the maximum increase by about 5 folds. During the incubation, the concentration of free ANS in solution remained almost constant and the amounts of ANS bound to cells increased by only about 30%. Therefore the greater part of the colicin-induced fluorescence increase may be explained by the alteration of circumstance surrounding the cell-bound ANS, or the

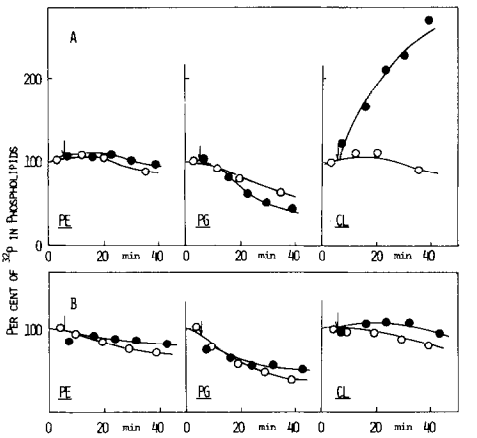

Fig. 3 Changes of turnover rates of phospholipids induced by colicin E_2 in (A) $353\lambda^s(\lambda)$ and (B) $353\lambda^s(\lambda_{rex-5a})$. Colicin E_2 was added at 5 min. PE, phosphatidylethanolamine; PG, phosphatidylglycerol; CL, cardiolipin. Symbols: O , Control without colicin; ● , colicin treated cell.

structural changes of cell membrane. Only slight increase of fluorescence intensity was observed in cells of E. coli $353\lambda^s$ (λ_{rex}^-5a) (Fig. 2B).

It has been observed that colicin E_1 also induced fluorescence increase of ANS bound to the sensitive cells(7). However, the E_2-induced fluorescence change observed here is distinguished based on the following two points. (i) The fluorescence change by colicin E_2 occurrs after a distinct lag period, while that by colicin E_1 occurrs immediately. (ii) The fluorescence change of ANS bound to cells induced by colicin E_2 is dependent on the rex gene product.

Alteration of phospholipid metabolism induced by colicin E_2

Effect of colicin E_2 on the turnover rate of phosphate moiety of the individual phospholipids were determined using E. coli $353\lambda^s$ (λ) cells previously labelled with ^{32}P. As shown in Fig. 3A, the turnover rate of phosphatidylethanolamine in the colicin-treated

cells was almost the same as that of the untreated cells, whereas the radioactivity of phosphatidylglycerol was decreased and that of cardiolipin increased markedly in the colicin treated cells. This stimulation of phospholipid turnover was not observed in *E. coli* $353\lambda^{s}(\lambda_{rex}^{-}5a)$.

Similar stimulation of the phospholipid turnover was observed when colicin E_1 was added to the sensitive cells which was supposed to be the secondary effect of the decreased level of ATP(8). The same situation is also possible in the case of colicin E_2, since colicin E_2 could decrease the ATP level in the λ-lysogenic cells (3). It is however noticeable that the stimulation of the phospholipid turnover due to colicin E_2 occurred precededly to the marked increases of the detergent sensitivity and the fluorescence intensity of ANS. The effects of colicin E_2 on the phospholipids metabolism with colaboration of the *rex* gene product might reflect some as yet unknown alterations of the cell membrane which could correspond to the first stage of the transport inhibition by the colicin.

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