PRELIMINARY NOTES

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Dissociating activity of purified colicin E_2 on the isolated membrane complex of *Escherichia coli*

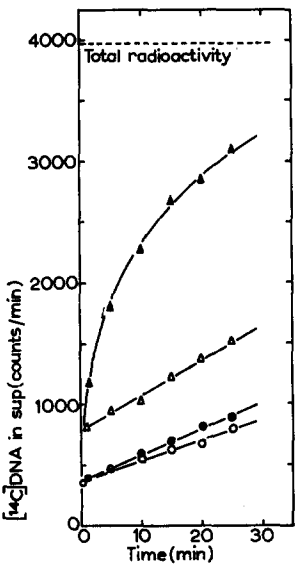
Colicins are highly specific antibiotic proteins produced by Enterobacteriaceae, and the adsorption of colicins on specific receptor sites in the cell surface of sensitive bacteria induces remarkable alterations of cellular functions and loss of viability. For instance, the adsorption of colicin E_2 induces rapid degradation of cellular DNA and cell death. Investigations on colicin action have suggested that the cytoplasmic membrane of the sensitive cell has a functional importance in the transmission of colicin action from the receptor sites to a specific and lethal intracellular target[1]. However, observation of biochemical reactions of colicins in subcellular systems has not been successful. In order to clarify the nature of presumed conformational changes of the cell membrane induced by colicin, we have tried to observe the biochemical reaction of colicin E_2 on the isolated membrane complex, *i.e.* the ghost of the sensitive *Escherichia coli* cells. Remarkable dissociation of the ghost was found to occur by the addition of colicin E_2 *in vitro*.

Colicin E_2 was extracted with 0.002 M EDTA from the cells of *E. coli* W3110 (E_2^+), treated with 0.33 μg/ml mitomycin C and purified by ammonium sulfate fractionation, DEAE-cellulose chromatography and CM-Sephadex C-50 chromatography by the method of HERSCHMAN AND HELINSKI[2]. Disc electrophoresis of the final lyophilized preparation showed a single protein band without any contaminant. *E. coli* W2252 met$^-$thy$^-$ λ^S, sensitive to colicin E_2, were grown in a synthetic medium containing [1-^{14}C]thymine and [5-^{3}H]uracil. The cells were harvested at a density of $5 \cdot 10^8$ cells/ml, washed once with 0.01 M Tris–HCl (pH 8.0) and incubated with 0.01 M Tris–HCl (pH 7.5) containing 0.01 M EDTA, 0.1 mg/ml lysozyme and 20 % sucrose for 2 min at 30°. The resulting protoplasts were harvested, washed once with 0.01 M Tris–HCl (pH 7.5) containing 1 mM magnesium acetate and 20 % sucrose, and gently lysed in 0.01 M Tris–HCl (pH 7.5) containing 1 mM magnesium acetate at 0°. The total lysate was used as the ghost suspension. In order to measure the dissociation of the membrane complex by colicin E_2, the ghost suspension was incubated with colicin E_2 in the presence of 1.3 mM ATP at 30°, and the reaction was stopped by rapid chilling in an icebath. Then the residual membrane complex was sedimented by centrifugation for 15 min at 10000 $\times$ *g*, and released amounts of protein (by the method of LOWRY *et al.*), DNA (by ^{14}C counts) and RNA (by ^{3}H counts) in the supernatant were assayed. Appropriate concentration of the ghost (about $5 \cdot 10^8$ ghosts/ml) and careful decantation were necessary for the separation of the supernatant from the centrifuged jelly-like pellet.

When the ghost suspension incubated at 30° without any addition was centrifuged, almost all DNA precipitated with the membrane material, and prolonged

incubation resulted only in slow release of DNA into the supernatant. The addition of colicin E_2 did not accelerate this release. In the presence of 1.3 mM ATP, however, the addition of colicin E_2 resulted in remarkable acceleration of the DNA release, and almost 80% of the radioactivity of DNA appeared in the supernatant within 25 min (Fig. 1). Zonal centrifugation analysis using an alkaline sucrose gradient of the incubated sample with colicin E_2 + ATP showed no detectable fragmentation of the DNA molecule which suggested that release but not extensive degradation of the membrane-bound DNA had occurred. It was also observed that the membrane-bound protein and RNA were released concomitantly into the supernatant by incubation with colicin E_2 + ATP (Fig. 2). This suggested that colicin E_2 induced rapid dissociation of the ghost and almost all DNA, RNA and protein bound to the membrane complex were rapidly released into the supernatant. Since the ghost suspension incubated with colicin E_2 + ATP or ATP alone were confirmed by microscopic observation to contain less than 3% of residual intact cells or protoplasts, the precipitable material could be assumed to be the ghost or the membrane complex itself, and the effect of colicin E_2 in the presence of ATP was due to the dissociation of the ghost but not to bursting of the residual protoplasts.

The concentration of colicin E_2 required for this dissociation reaction seemed to be extremely low. As shown in Fig. 2, about 5.0 μg/ml of colicin E_2 was sufficient for the remarkable dissociation under experimental conditions. Highly purified colicin K preparation, generously provided by MATSUHASHI AND KUNUGITA[3], Institute of



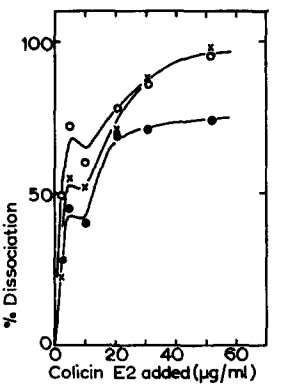


Fig. 1. Release of DNA from the membrane complex by colicin E_2 *in vitro*. Incubations were carried out at 30° in the presence of 1 mM Mg^{2+} and other supplements, *i.e.* 20 μg/ml colicin E_2 and 1.3 mM ATP, were added at 0 min. ○—○, control, no addition; ●—●, colicin E_2 alone; △—△, ATP alone; ▲—▲, colicin E_2 + ATP.

Fig. 2. Dissociation of the membrane complex by various concentrations of colicin E_2. Incubations were carried out at 30° for 23 min in the presence of 1.3 mM ATP. The complex incubated without colicin E_2 retained 59.5% of total protein in the lysate, 58.6% of RNA and 83.2% of DNA, respectively. % dissociation was calculated assuming these amounts as 100%. ×—×, protein released from the complex; ○—○, RNA; ●—●, DNA.

Applied Microbiology, University of Tokyo, which seemed to contain a single-protein component free of carbohydrates, did not show similar dissociating activity even at 50 μg/ml.

Our recent investigations have suggested that rapid dissociation of DNA from the membrane complex (measured by the modified Sarkosyl method[4]) with little DNA degradation also occurred at the early stage of colicin E_2 challenge in the intact sensitive cells. The ATP requirement for the *in vitro* reaction of colicin E_2 could possibly be related to the fact that an energy-producing system of the sensitive cells was necessary for the killing action of colicin E_2 in the intact cells[5]. Further experiments are needed to ascertain whether the *in vitro* reaction of colicin E_2 observed here is involved in the *in vivo* transmission mechanism of colicin action from receptor sites across the cell membrane. However, the fact that a very low concentration of colicin E_2 but not colicin K was sufficient for the dissociation reaction of the membrane complex seems to be the consequence of a specific interaction of colicin E_2 protein with the cytoplasmic membrane of *E. coli*.

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Sterol structure and ordering effects in spin-labelled phospholipid multibilayer structures*

Cholesterol is a common component of vertebrate cellular membranes[1]. Sterols with a similar structure (3β-OH group, hydrocarbon chain at position 17) are also found in vascular plants, algae, fungi[2], and microorganisms[2,3]. The biological function of these compounds is not completely understood. We have investigated the effects of steroid structure on the degree of order in multibilayer structures of polar membrane lipids[4,5] using a spin label technique[6–9]. The results indicate that cholesterol and structurally related sterols increase the degree of order of the spin label, and hence that of the lipids in the lamellar structure. The term "degree of order" is used to denote the extent to which the long axes of the lipids orient preferentially in a

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