Lowered Levels of Colicin Ia Membrane Receptors in an *Escherichia coli* Mutant Defective in Heme Biosynthesis

M. J. R. GILCHRIST AND JORDAN KONISKY*

Department of Microbiology, University of Illinois, Urbana, Illinois 61801

Received for publication 26 September 1975

Cells of an *Escherichia coli* mutant defective in heme biosynthesis (hemA) grown under conditions for respiration deficiency (grown in the absence of aminolevulinic acid) have 20% of the number of specific colicin Ia receptors found in cells of the same strain grown under conditions for respiration competency (grown in the presence of aminolevulinic acid).

Colicin Ia has been under investigation to determine its mechanism of bactericidal action. In general, the effect of colicin Ia is an interference with energy metabolism of *Escherichia coli* (3, 9). Colicin Ia treatment lowers adenosine 5'-triphosphate levels, inhibits active transport, and stimulates respiration but does not induce obvious proton leakage across the cytoplasmic membrane (J. Konisky, M. J. R. Gilchrist, D. Nieva Gomez, and R. B. Gennis, *In H. R. Kaback, H. Neurath, G. K. Radda, R. Schwyzser, and W. R. Wiley [eds.], Molecular Aspects of Membrane Phenomena*, in press).

We have examined the effects of colicin Ia on a respiration-deficient mutant (hemA). This mutant is unable to synthesize heme in the absence of exogenously supplied δ-aminolevulinic acid (ALA), a precursor of heme biosynthesis. Thus, only when grown in the presence of ALA do such cells contain functioning cytochromes and the respiratory capacity of the wild-type cells (1, 4, 11). We have found that at least 10-fold higher levels of colicin Ia are required to effectively inhibit proline uptake in the respiration-deficient cells when compared with cells grown under conditions for respiration competency.

The reduced colicin Ia sensitivity of cells grown without ALA might be due to an alteration in the target of colicin Ia action or to a decreased ability of the cells to bind the colicin.

I-labeled colicin Ia was employed to study the binding of colicin to cells grown with and without ALA (Fig. 1A). Cells grown in the presence of ALA bind approximately fivefold higher levels of colicin Ia. A colicin I-resistant derivative bound no colicin, whether grown with or without ALA. Since it was possible that some metabolic property of the respiration-deficient cells might account for their diminished binding capacity, the binding of [125I]Ia to isolated cell envelopes was also examined. As can be seen in Fig. 1B, a similar fivefold difference in binding capacity was observed.

Triton X-100 extraction of envelopes from colicin-sensitive strains solubilizes a component that forms a complex with colicin Ia (8). Since this component is not detectable in extracts of colicin Ia-resistant strains, it is assumed to be the colicin I receptor. We considered the possibility that the I receptor might be cryptic in the respiration-deficient cells due to an alteration in the envelope, which reduced access to the receptor or affected the conformation of the receptor. However, attempts to detect such cryptic receptors have been unsuccessful in that extracts of envelopes derived from respiration-deficient cells were found to contain 20% of the receptor activity found in cells grown in the presence of ALA. Furthermore, we have not been able to detect elevated receptor activity in either the cytoplasmic contents or the growth medium of the respiration-deficient cells. These findings do not preclude the possibility that receptors are synthesized yet lack biological activity.

The diminished receptor levels could be due to a regulatory effect on the synthesis of specific proteins. Alternatively, a functioning cytochrome chain might be required to activate the colicin receptor. We have reconstituted functioning cytochromes in the presence and absence of protein synthesis (Fig. 2). As can be seen, colicin receptor activity increases only in the presence of protein synthesis. This would be consistent with the notion that some property of the respiration-deficient phenotype exerts a regulatory effect on colicin Ia receptor synthesis. Alternatively, it is possible that the receptor itself or some other component required for colicin binding depends on heme for its function. We cannot be certain of the possibility that such a hemoprotein would be reconstituted under the conditions used here.
We have previously shown that a specific colicin Ia receptor resides in the outer membrane of E. coli [7]. It therefore appears that in E. coli there is a coupling of inner membrane function (electron transport) and the elaboration of an outer membrane protein component (the Ia receptor). It is now known that certain

**Fig. 1.** Binding of $^{125}\text{I}}$Ia to cells (A) and envelopes (B) of respiration-competent and respiration-deficient hemA mutants. JK143 (obtained from P. D. Bragg, SASX76) and JK169 (a colicin Ia-resistant derivative of JK143) were grown in the absence and presence of ALA (50 μg/ml) in M63 medium (10) containing 10 mM glucose and 0.15% Casamino Acids. Binding experiments were conducted as described previously (6, 7). Cells ($1 \times 10^8$) were treated with various amounts of $^{125}\text{I}}$Ia at $3.26 \times 10^4$ counts/min per μg. Envelopes (38 μg) were incubated with various amounts of $^{125}\text{I}}$Ia at $1.64 \times 10^5$ counts/min per μg. The amount of $^{125}\text{I}}$Ia bound was determined by filtration. Each point represents the average of two determinations. JK143 envelopes from cells grown with ALA had a reduced nicotinamide adenine dinucleotide (NADH) oxidase activity (determined with a YSI oxygen electrode, using NADH at a concentra-

**Fig. 2.** Binding of $^{125}\text{I}}$Ia to cell envelopes after in vivo reconstitution of cytochrome activity. JK143 cells were grown in the absence of ALA. They were harvested and resuspended in medium containing ALA (50 μg/ml) and incubated at 37 C. Chloramphenicol (50 μg/ml) was added to one set of cells. The cells without chloramphenicol were harvested after five, two, three, four, and five mass doublings in ALA. The cells with chloramphenicol were harvested at the same time as the cells allowed to undergo five mass doublings. Envelope preparations from both sets of cells were utilized to measure NADH oxidase activity and colicin binding capacity. A 0.119-μg portion of $^{125}\text{I}}$Ia (3.32 × 10^5 counts/min per μg) was added to each tube containing various amounts of envelope. The NADH oxidase activity of the chloramphenicol-treated cells was restored to 2,312 ng-atoms/min per mg. The inset shows NADH oxidase activities in cells reconstituted in the absence of chloramphenicol. Symbols: □, five mass doublings; △, three mass doublings; ○, two mass doublings; ●, zero mass doublings; □, chloramphenicol-treated cells.
colicin receptors have functional roles in *E. coli* cellular metabolism. For example, the colicin E3 receptor is known to be involved in the transport of vitamin B$_{12}$ (2), whereas the receptor for colicin M has been implicated as a component of iron transport systems (5). In view of these results, a heretofore undiscovered functional role for colicin Ia receptors in other cellular processes initiated at the outer membrane might explain the apparent regulation of Ia receptor synthesis by heme.

We thank A. Miguel for technical assistance.

This investigation was supported by Public Health Service research grant AI 10106 from the National Institute of Allergy and Infectious Diseases. JK is the recipient of Public Health Service career development award K04 Al 00049 from the National Institute of Allergy and Infectious Diseases.

LITERATURE CITED