ALTERATION OF MEMBRANE PROTEINS IN A CHLORATE-RESISTANT MUTANT OF ESCHERICHIA COLI

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Summary

A chlorate-resistant mutant of E. coli was found to be lacking the enzymes nitrate reductase and formate dehydrogenase. This mutant was also lacking a major membrane protein of a molecular weight of 20,000 which did not appear to be identical with either of these enzymes.

The addition of chlorate to anaerobic cultures of E. coli permits the selection of pleotropic mutants lacking several membrane bound enzymes (1,2) including nitrate reductase and formate hydrogenlyase. These mutants, designated chlorate-resistant mutants, have been found to map at three distinct loci on the bacterial chromosome (3). Membranes isolated from chlorate-resistant mutants have been shown to have an altered sedimentation profile in sucrose density gradients (4). In more recent studies a particulate fraction containing reconstituted nitrate reductase activity was obtained after incubation of a mixture of soluble extracts from two mutant strains mapping at different loci (5).

This system is ideal as a model for the study of the organization of complex membrane-bound enzyme systems. For this reason, a particulate fraction from a chlorate-resistant mutant was examined by a new gel electrophoresis system (6) designed for the resolution of membrane proteins. This mutant was found to be lacking one major membrane protein species.

Materials and Methods

Escherichia coli $O111B_4$ (obtained from Dr. E. Heath, Dept. of Physiological

Chemistry, the Johns Hopkins School of Medicine) and a chlorate-resistant mutant mutant obtained from this strain by the procedure of Piechaud et al. (1) were grown with vigorous aeration at 37° on Trypticase Soy Broth or minimal salts containing either 1% glucose or 2% casamino acids, and as indicated, 10 mM KNO_3. Cells were washed once with 20 mM Tris buffer, pH 7.8, containing 5 mM KCl and 10 mM EDTA, resuspended in the same medium, and sonicated. After removal of whole cells by low-speed centrifugation the particulate fraction was obtained by centrifugation at 144,000 g for 1 hr. The pellet was suspended in distilled water and treated with 0.2 mg Lubrol WX (6) to remove soluble protein. This suspension was again centrifuged at 144,000 g for 1 hr, and the pellet was resuspended in distilled water and dissolved in acidified dimethyl formamide and chromatographed on Sephadex LH-20 for the removal of lipids exactly as previously described (6). Dialysis, concentration, and electrophoresis of the protein in SDS-containing gels were carried out as previously described (6). Molecular weights were estimated by the procedure of Shapiro et al. (7).

The particulate fraction was prepared for enzyme assays by sonication of cells in 50 mM Tris buffer, pH 8.0, followed by removal of whole cells and centrifugation at 144,000 g for 1 hr. Nitrate reductase was measured by the method of Itagaki and Taniguchi (8) with reduced benzyl viologen as the electron donor. Formate dehydrogenase was measured by following the uptake of oxygen polarographically in an assay medium containing 0.25 M phosphate buffer, pH 6.5, 1 mM EDTA, 0.6 mM KCN, and 25 mM sodium formate. After 1 min the reaction was initiated by the addition of phenazine methosulfate at a final concentration of 1 mM. The use of artificial electron donors and acceptors in these assays rules out the possibility that low activity could be due to the lack of a cytochrome or other electron carrier.

Results and Discussion

The results shown in Table 1 indicate that the chlorate-resistant mutant is lacking the enzymes nitrate reductase and formate dehydrogenase. In the wild-type strain these enzymes appear to be regulated independently. Nitrate

TABLE 1

Enzymatic Activity of the Particulate Fraction
From Mutant and Wild-type Strains

Culture and Growth Medium	Formate Dehydrogenase	Nitrate Reductase
Wild Type:		
Casamino acid + NO_3	1080	438
Casamino acid - NO_3	790	6
Glucose + NO_3	0	176
Glucose - NO_3	0	2
Chlorate resistant Mutant:		
Casamino acid + NO_3	0	1
Glucose + NO_3	0	0

All activities are expressed in mμmoles/min/mg protein.

reductase is formed only when nitrate is included in the growth medium. Formate dehydrogenase is formed in large amounts in cells grown on casamino acids but is not formed in cells grown aerobically on glucose. The presence of nitrate in the growth medium has little effect on the level of formate dehydrogenase formed. The levels of the cytochrome b-linked enzymes succinate and lactate dehydrogenase were also examined and were found to be the same in both the mutant and the wild type, indicating that the mutant does not exhibit a general loss of cytochrome b-linked enzymes.

Figure 1 shows scans of the electrophoretic patterns of mutant and wild-type particulate proteins observed in two different concentrations of gel. Although there appear to be some quantitative changes in the higher molecular weight proteins near the top of the gels (seen particularly in the 7.5% gel) the most striking difference is the lack of one major protein band in the mutant as indicated by the arrows. A more exacting comparison obtained by co-electrophoresis of the particulate protein from both the mutant and the wild type on

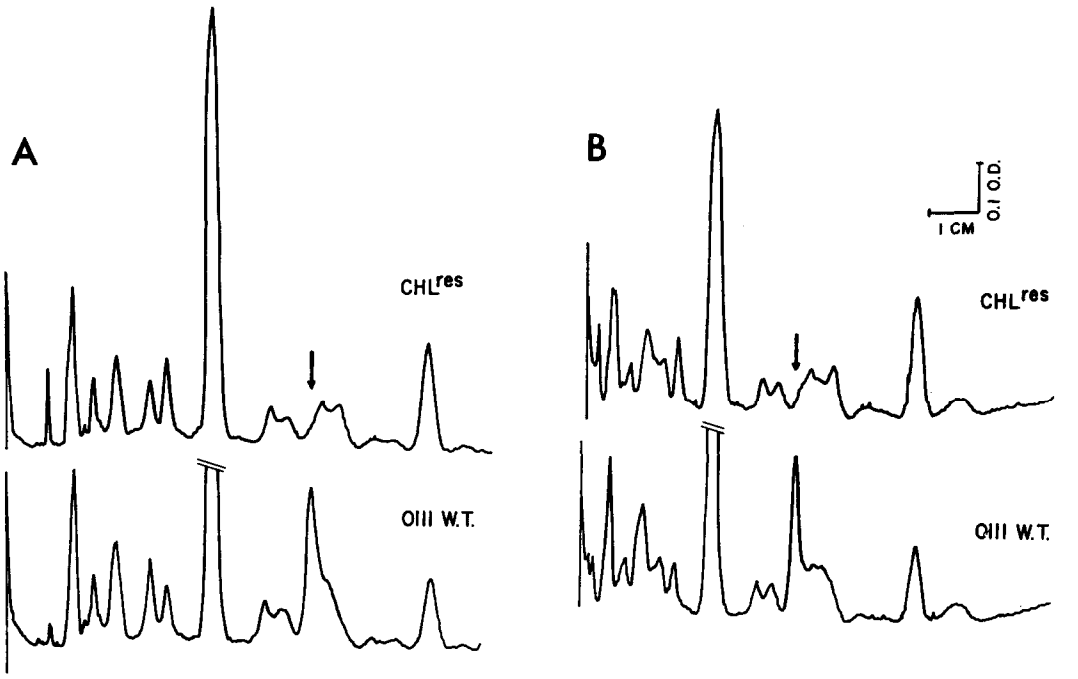

Figure 1. Gel scanner tracings of lipid-free particulate protein from chlorate-resistant and wild-type strains run in 7.5% (A) and 10% (B) acrylamide gels. The top of the gels are to the left, and the gels were stained with Coomassie blue. The protein missing in the chlorate-resistant strain is indicated by the arrows. The major protein component (indicated by a broken line in the lower tracings) of the particulate fraction was identical in both strains under all growth conditions. The cultures used in this experiment were grown on TSB and harvested in the late log phase of growth.

split gels indicated that this protein does not correspond to any of the bands remaining in the mutant.

The molecular weight of the protein missing in the mutant is 20,000. In late log phase cultures on TSB this protein may amount to as much as 10% of the total protein on the gel. However, in early log phase cultures on TSB or in cultures grown on glucose or casamino acids this protein is formed in much smaller amounts. In these cultures, the amount of this protein is not increased by the addition of nitrate to the medium, indicating that it is not identical with nitrate reductase. The level of this protein is also not correlated with the amount of formate dehydrogenase, which is highest in cultures grown on casamino acids. It is possible that this protein is the cytochrome

b_{555} reported by Ruiz-Herrera et al. (9), but this appears unlikely in view of the large amounts of this protein present in the particulate fraction and the fact that the formation of this protein is not stimulated by nitrate in the growth medium.

It has been suggested by Ruiz-Herrera et al. (9) that the pleotropic nature of mutations affecting the nitrate reductase system of E. coli are due either to nonsense mutations in a system produced on a multicistronic messenger, or to an alteration of protein components which affects the structure or assembly of this organized system. The first of these hypotheses is difficult to reconcile with the data presented in Table 1 on the independent regulation of nitrate reductase and formate dehydrogenase and with the data of Puig et al. (3) on the separate loci for chlorate resistance. The second hypothesis appears more plausible, and it is tempting to speculate that the protein which is missing in this chlorate-resistant mutant plays a structural role in the binding or organization of the components of this multi-enzyme system.

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