Oxygen Regulation of L-1,2-Propanediol Oxidoreductase Activity in Escherichia coli

ELISA CABISCOL,1 ELENA HIDALGO,2 JOSEFA BADÍA,2 LAURA BALDOMÁ,2 JOAQUIM ROS,1 AND JUAN AGUILAR2*

Department of Biochemistry, School of Pharmacy, University of Barcelona, 08028 Barcelona,2 and Department of Basic Medical Sciences, School of Medicine (Lleida), University of Barcelona, 25007 Lleida,1 Spain

Received 18 January 1990/Accepted 18 June 1990

Regardless of the respiratory conditions of the culture, Escherichia coli synthesizes an active propanediol oxidoreductase. Under anaerobic conditions, the enzyme remained fully active and accomplished its physiological role, while under aerobic conditions, it was inactivated in a process that did not depend on protein synthesis or on the presence of a carbon source.

Boronat and Aguilar (2) showed that in crude extracts of cells of Escherichia coli grown anaerobically on fucose, propanediol oxidoreductase activity was five times higher than in extracts grown aerobically in this methylpentose. These changes in activity were not matched by changes in the levels of enzyme protein, measured by immunologic procedures, which were nearly equivalent under both conditions (2). Furthermore, Chen et al. (6), using a strain with the fucO promoter fused to lacZYA, showed that β-galactosidase activity was inducible by fucose both anaerobically and aerobically, indicating that the respiratory control did not affect the expression of Φ(fucO-lac). Chen and Lin (5) also showed that, in merodiploids bearing both fucO and Φ(fucO-lac), propanediol oxidoreductase activity was inducible only anaerobically, while the β-galactosidase remained inducible independent of the respiratory condition. Therefore, respiratory control of the induced propanediol oxidoreductase has to occur posttranscriptionally.

To characterize this regulatory process, cells of E. coli EC11 (HfrC phoA8 relA1 tonA22 T21 [Δ]) (10) were grown and harvested as described previously (1). Cell extracts were made and enzyme activity was determined as indicated before (2) except that since propanediol oxidoreductase was also reported to be responsible for the conversion of glycolaldehyde to ethylene glycol (3), this aldehyde was routinely used instead of lactoldehyde when the measurement was performed in the reductive sense. Determination of specific activities referred to propanediol oxidoreductase protein, measured immunologically by the procedure described by Boronat and Aguilar (2), yielded 35.2 U/mg for the anaerobic cells and 10.8 U/mg for the aerobic cells. Comparison of these data strongly suggests a process of inactivation of anaerobic enzyme in the presence of oxygen.

In vivo inactivation of propanediol oxidoreductase. When E. coli was grown on fucose under anaerobic conditions, the number of enzyme activity units in the culture increased as the cell mass increased. Eventually this level of enzyme activity was stabilized as the culture reached stationary phase. Cells were then centrifuged at 20,000 × g, washed twice in the same volume of minimal medium, and suspended in a fucoseless medium containing glucose. The cell suspension was immediately incubated aerobically in a rotary shaker, and at the indicated times, samples were taken to determine enzyme activity.

The monitoring of the total units of enzyme activity showed a 70% decrease in 2 h (Fig. 1). The same inactivation was observed when the culture was shifted to aerobic conditions by suspending the cells in minimal medium in the presence of fucose or without a carbon source (not shown). However, the presence of casein acid hydrolysate at a concentration of 0.5% in the aerobic incubation medium largely prevented inactivation (Fig. 1). The function of several amino acids as scavengers of oxygen-derived free radicals has been extensively documented (4, 7, 9) and could be the explanation for the casein acid hydrolysate effect.

When, in these inactivation experiments, cells suspended in glucose mineral medium containing chloramphenicol (10 μg/ml) were split into two portions and one of them was incubated anaerobically and the other aerobically, the aerobic culture was 65% inactivated while the anaerobic culture was only 15% inactivated (Fig. 1). The use of a nitrogen-deprived minimal medium [without (NH4)2SO4] to stop protein synthesis, avoiding the use of antibiotics, yielded the same results, which indicated that the inactivation mechanism was not dependent on protein synthesis.

Reversibility of inactivation. To analyze the possible reversibility of the inactivation process, a culture incubated aerobically was shifted back to anaerobic conditions without a carbon source. The total activity in the culture was not altered under such circumstances, even after 10 h of incubation, and the amount of enzyme protein, as indicated by the rocket height, was also stable (not shown). The presence of a carbon source such as glucose in the medium permitted the growth of the cell mass, although no change in the propanediol oxidoreductase (total units of activity or specific protein level) could be detected. In contrast, the presence of fucose as a carbon source gradually restored the propanediol oxidoreductase activity, which reached the original activity level in 8 to 10 h of cell growth (increases in additional specific protein were parallel to those of enzyme activity). No restoration of enzyme activity by fucose was apparent when the experiment was carried out in the presence of chloramphenicol (10 μg/ml) (Fig. 2).

Reactivation of inactive propanediol oxidoreductase by NAD. The activity of crude extract preparations of active and inactive propanediol oxidoreductase was measured in the oxidative catalytic sense by using increasing concentrations of NAD, ranging from 0.33 to 10 mM. The enzyme

* Corresponding author.
activity of cells grown anaerobically on fucose was virtually unchanged as the cofactor concentration was increased, displaying a constant high specific activity of about 0.25 U/mg of protein. When the same experiment was performed with an enzyme inactivated by previous shift of the cells from a fucose anaerobic to a glucose aerobic culture, specific activity increased from 0.06 to 0.13 U/mg of protein. Thus, when the concentration of NAD was raised to 10 mM in the assay mixture, the enzyme activity was restored to about half of its original level.

For other oxidoreductases inactivated by oxygen, such as glycerol dehydrogenase (8, 11), it has already been shown that high concentrations of the cofactor reactivated the enzyme. These authors assigned the inactivation to an alteration at the site for the coenzyme. Similarly, the reactivation effect on propanediol oxidoreductase oxidative activity described here could presumably be assigned to a decrease in affinity for the cofactor promoted by the oxygen-mediated modification of the active center of the enzyme.

Increasing concentrations of glycolaldehyde (0.5 to 5 mM), when the enzyme was measured in the reductive sense, or of D,L-1,2-propanediol (50 to 500 mM), when the enzyme was measured in the oxidative sense, had no reactivation effect on the inactive enzyme, nor did preincubation of the inactive enzyme at 30°C with L-lactaldehyde at 5 mM for up to 120 min prior to enzyme activity determination have an effect on oxidoreductase activity.

This research was supported by grant PB88-0215 from the Dirección General de Investigación Científica y Técnica, Spain.

---

**FIG. 1.** Time course of propanediol oxidoreductase (POR) activity in a culture shifted from anaerobiosis to different conditions. The cells were incubated after the shift (time zero) under aerobic conditions (open symbols) or kept under anaerobic conditions as a control (solid symbols). Incubation media contained glucose (□, ■), glucose plus chloramphenicol (Δ, ▲), or glucose plus casein acid hydrolysate (○).

---

**FIG. 2.** Time course of propanediol oxidoreductase (POR) activity in an aerobic culture shifted to anaerobiosis in different media. The cells were incubated after the shift (time zero) in minimal medium containing no carbon source (○), glucose (□), fucose (■), or fucose plus chloramphenicol (△).

---

**LITERATURE CITED**


