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Publication Date

1971-03-01

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March 1971

AEC Contract No. W-7405-eng-48

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Inducible Synthesis of β -Galactosidase in a Membrane
Preparation from Escherichia coli: A Critical Appraisal

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The ability of a disrupted spheroplast preparation (3) from Escherichia coli to synthesize β -galactosidase was shown to be correlated with the number of viable cells.

In a recent publication (3), Maruo et al. described a disrupted spheroplast preparation from Escherichia coli which was able to synthesize β -galactosidase under inducible control. Evidence was presented against the synthetic ability residing in undisrupted spheroplasts. A cell-free system of such simplicity, capable of enzyme synthesis, has obvious experimental attractions, and we have attempted to verify their observations.

Using two inducible strains of E. coli (the wild-type 3000, and MS 1054, which carries a deletion in galactoside permease), we followed exactly the method of Maruo et al. (3). The method calls for 25 μ g/ml of lysozyme during the preparation of spheroplasts; an earlier paper (4) specified 100 μ g/ml of this enzyme. We have used both concentrations in different experiments; in some of our studies the lysozyme was the crystalline product from Sigma Chemical Company, St. Louis,

Mo., as used by Maruo et al., while in others the source was Calbiochem, Los Angeles, Calif. In all experiments, spheroplast formation in the prescribed 30 min at 30C was poor; prolongation of this period to 60-80 min greatly increased the yield. Homogenization in a Teflon homogenizer at 600 rev/min for 5 min (3) was quite inadequate for spheroplast disruption. We therefore used a Virtis homogenizer at 13,000 rev/min for 5 min at 0C, and even then disruption was incomplete. Disruption of spheroplasts from E. coli K-12 is reported to be difficult (A.L. Koch, personal communication); both we and Maruo et al. used cells derived from this strain.

Maruo et al. (3) compared the synthetic activity of their membrane fraction with that of "intact cells", but the latter were nowhere defined. In our experiments we have used for comparison both cells which were actively growing in F-medium (3) before and during exposure to inducer, and cells treated in the same way as the membrane fraction except for omission of lysozyme in the spheroplast preparation stage, and the omission of homogenization in the disruption stage. Viable counts were made on all preparations (3). Table 1 shows that the synthetic ability of the membrane fraction was different from that of actively growing cells, and similar to that of EDTA-treated, but undisrupted cells. Thus, growing cells were resistant to actinomycin C, while EDTA-treated cells (1,2) as well as membranes were sensitive. All were repressed to the same extent by glucose. The synthetic capacities of the EDTA-treated cells and membranes were similar on a viable

cell basis, and both were lower than growing cells. We have been unable to confirm the observation (3) that β -galactosidase synthesized by membranes is released to the medium, while that of "intact cells" is not. It is perhaps pertinent to note that Maruo et al. (3) claim no detectable sedimentable activity in their membrane fraction which seems inconsistent with their recorded viable cell count. In our experiments, enzyme from both membranes and EDTA-cells was present to about 45% in the supernatant and 55% in the pellet.

We conclude that the β -galactosidase synthesizing abilities of the membrane preparation closely resembles that of EDTA-shocked cells as described by Leive (2). Such cells are sensitive to glucose repression (1) and to actinomycin (2); they show a reduced rate of enzyme synthesis (2), probably as a result of damage sustained during treatment with EDTA. Membrane damage in these cells may be responsible for the leakage of β -galactosidase to the medium.

This work was sponsored by the U.S. Atomic Energy Commission.

LITERATURE CITED

1. Jacquet, M., and Kepes, A. 1969. The step sensitive to catabolite repression and its reversion by 3'-5'-cyclic AMP during induced synthesis of β -galactosidase in E. coli. *Biochem. Biophys. Res. Commun.* 36: 84-92.
2. Leive, L. 1965. Actinomycin sensitivity in Escherichia coli. *Biochem. Biophys. Res. Commun.* 18: 13-17.
3. Maruo, B., Seto, H., and Nagata, Y. 1969. Inducible synthesis of β -galactosidase in disrupted spheroplast of Escherichia coli. *J. Bacteriol.* 100: 209-214.
4. Nagata, Y., Mizuno, S., and Maruo, B. 1966. Preparation and properties of active membrane systems from various species of bacteria. *J. Biochem., Tokyo.* 59: 404-410.

Table 1. β -Galactosidase synthesized by membranes, EDTA-shocked cells and growing cells

β -Galactosidase activity ^a	Cell preparation		
	Growing cells	EDTA-shocked cells	Membranes
on cell number basis ^b			
control	942	206	94
plus actinomycin	921	0	28
plus glucose	124	32	10
on cell mass basis ^c			
control	-	5.1	7.6
plus actinomycin	-	0.0	2.3
plus glucose	-	0.8	0.9

^a after 120 min incubation at 30C in F-medium containing 1 mM isopropylthiogalactoside.

Conditions for incubation and enzyme assay were as described by Maruo et al. (3).

^b enzyme units (3)/10⁸ viable cells.

^c enzyme units (3)/25 mg of original wet wt of cells.

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