THE CELL-FREE SYNTHESIS OF β-GALACTOSIDASE BY ESCHERICHIA COLI

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During an investigation of the inhibition of the induced formation of β-galactosidase in cells of E. coli by ultraviolet irradiation and the reversal of inhibition by photoreactivation, the presence of a particle-bound β-galactosidase was revealed by electrophoresis of cell extracts on Geon. Further analysis of the behavior of the particle-bound enzyme during induction and after removal of the inducer gave results suggesting that the particle might be the site of synthesis of this enzyme (Kameyama and Novelli, 1960). Experiments were therefore undertaken to determine whether the particle was capable of carrying out the synthesis of β-galactosidase in a cell-free preparation. The results of this investigation are briefly reported here and will be published in detail elsewhere.

Fifty liters of log phase cells of E. coli B growing in a minimal salts medium with glycerol as carbon source were induced to make β-galactosidase with thiomethyl β-d-galactopyranoside (TMG) (0.5 μM/ml) as inducer. Cells were harvested, washed and resuspended in 500 ml .01 M Tris buffer pH 7.2 with 0.01 M Mg^{++}. The suspension was passed through a French Pressure cell two times. Cells and cellular debris were removed by centrifugation at 8,000 X g for 60 min followed by another centrifugation at 30,000 X g for 90 min. The supernatant was now centrifuged at 105,000 X g for 30 min to yield a particle fraction P_{30}. The supernatant was again centrifuged at 105,000 X g for 90 min to yield a second particle fraction P_{90} and a supernatant S_{90}. P_{30} and P_{90} were washed in 0.01 M Tris pH 7.2, 0.005 M Mg^{++} and recovered by centrifugation at 105,000 X g for 90 min. S_{90} was dialyzed for 7 hours against the same buffer.

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When P_{30} was incubated with S_{90} in the presence of ATP, an ATP generator and amino acids, an inducer-dependent increase in β-galactosidase activity was observed (Table 1). The increase in enzyme activity represents about a 2 fold increase over the initial amount of β-galactosidase present. P_{90} is also capable of synthesizing β-galactosidas and incorporating radioactive amino acids, but with less efficiency than P_{30}.

Table 1

Synthesis of β-Galactosidase by Cell-Free Preparation

		Δ Enzyme units/60 min
Complete system		71
"	minus S_{90}	41
"	" TMG	0
"	" ATP and generator	0
"	" amino acids	25
"	" XTP, XDP	31
"	plus RNase (20 μg/ml)	-39
"	" chloramphenicol (80 μg/ml)	0
"	" DNase (4.2 μg/ml)	-17.5

Complete system consisted of the following in μmoles/ml: Tris buffer pH 7.2, 100; Mg acetate 4; $MnCl_2$ 2; ATP 10; phosphocreatine 4.8; UTP, GTP, CTP, UDP, GDP, CDP 0.03 each; TMG 2; L-amino acids 50 μg (a balanced mixture representative of the amino acid composition of β-galactosidase, Wallenfels and Arens, 1960) 40 μg creatine kinase, unwashed P_{30} 1.67 mg protein, and S_{90} 0.84 mg protein. Initial β-galactosidase 48.9 units. Incubation, 37° C, 60 min.

It is clear that the system is absolutely dependent upon the presence of the inducer and a source of energy. The reaction is markedly stimulated by the dialyzed supernatant, a full complement of amino acids, the nucleoside di- and triphosphates and is inhibited by chloramphenicol, RNase and DNase. While these data indicate that this system is synthesizing β-galactosidase, they do not exclude the possible activation of a preformed protein. To rule out the latter possibility and to establish that the observed increase in enzyme activity actually represents synthesis of new protein molecules the experiment was repeated with the inclusion of C^{14} leucine in the amino acid mixture. After the incubation period an aliquot was removed and the radioactivity of the protein was measured.

In another aliquot, β-galactosidase was specifically precipitated with rabbit antiserum prepared against highly purified enzyme. The radioactivity in the antibody precipitate was measured. The results are shown in Table 2.

Table 2

Synthesis of β-Galactoside and Incorporation of C^{14} Leucine

	Δ Enzyme units/ml	cpm/ml Total protein	cpm/ml Ab ppt. protein
Complete system	60.6	2560	1428
" minus TMG	- 0.8	1580	384
" " S_{90}	- 0.2	960	600
" " ATP and generator	5.2	182	128
" " amino acids	36.0	1276	436

Complete system: Same as for Table 1 except C^{14} leucine added to amino acid mixture and washed P_{30} was used.

Although there seems to be some variable nonspecific precipitation of radioactive protein by the anti-β-galactosidase serum the data show quite clearly that the newly formed β-galactosidase contains radioactive leucine and was therefore derived from the amino acid pool. This is most evident when the effect of removing the inducer is studied. It can be seen that the presence of inducer (complete system) led to an increase of about 1000 cpm in the total protein together with an increase of 60 units of enzyme activity. All of the inducer dependent increase in radioactive protein is precipitable by the specific antiserum. An even clearer picture is obtained when the reaction mixture is precipitated with saturated ammonium sulfate, dialyzed to remove radioactive leucine, separated by electrophoresis and the eluted fractions analyzed for radioactive protein, β-galactosidase activity and radioactive protein precipitable by the specific antiserum. In this analysis radioactivity appeared over the peaks of β-galactoside activity as well as over the peak of cellular protein. Precipitation with antiserum and carrier β-galactosidase (to insure precipitation in those portions of the electropherogram not containing enzyme activity) yielded radioactive precipitates only in those regions of the electropherogram originally containing β-galactosidase activity. Whereas in the experiment in Table 2 treatment of the reaction mixture directly with antiserum results in a precipitation of over 50% of the radioactive protein, similar treat-

ment of the eluates from Geon electrophoresis results in about 35% precipitation of radioactive protein. This observation suggests that electrophoresis or some other step in the procedure results in the removal of substances causing nonspecific precipitation. The β-galactosidase that contains the highest radiospecific activity is the fraction that is associated with the particle and which does not migrate during electrophoresis. This particle fraction contains radioactive protein not associated with β-galactosidase, since precipitation with specific anti-β-galactosidase serum removes all of the β-galactosidase activity and only about 35% of the radioactive protein. When these experiments were repeated using particles from noninduced cells supplemented with S_{90} from induced or noninduced cells no synthesis of β-galactosidase was observed. Thus it is clear that the synthesis of β-galactosidase occurs on the particle and the enzyme is then released to the soluble fraction. Some small fraction of the enzyme remains attached to the particle and these particles can be removed with antiserum. This observation suggests the possibility that certain particles may be separated on a functional basis with specific antiserum. This possibility is being investigated with several different antisera to specific proteins.

References

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