ON THE ENZYMIC ACETYLATION OF ISOPROPYL-β-D-THIOGALACTOSIDE AND ITS ASSOCIATION WITH GALACTOSIDE-PERMEASE

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The studies of H. V. RICKENBERG et al[1], which revealed the presence in E. coli of a specific inducible mechanism for the accumulation of galactosides and thiogalactosides (galactoside-permease), showed that the major fraction of the substance accumulated by the cells was found unchanged after extraction. A minor component, usually less than 5 per cent, was found to be present under certain conditions when thiogalactosides were used as substrates. This derivative has been identified by HERZENBERG[2] as a 6-O-acetyl-thiogalactoside. Acetyl-thiogalactoside does not appear to represent an intermediate in the permeation process because it is not formed in the absence of an external carbon source while the permease reaction still occurs. Moreover, acetyl-thiogalactosides are not converted to the original galactoside by whole cells. Nevertheless, it was of interest to study this reaction.

We have succeeded in observing the formation of acetyl-isopropylthiogalactoside (AcIPTG) from isopropyl-thiogalactoside (IPTG) in extracts of E. coli. Bacteria from an exponential phase culture of ML 308, a strain which is constitutive for β-galactosidase and galactoside-permease[1], were treated for 5 minutes in the Raytheon sonic oscillator at 10 KC and the resultant extract centrifuged for 20 minutes at 30,000 g. The supernatant solution was dialyzed overnite against 0.05 M potassium phosphate buffer, pH 7.2 and 0.005 M β-mercaptoethanol. After incubation with the additions shown in Table I,

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an aliquot was chromatographed in n-propanol:H_2O, 7:3, exposed to film and the radioactive spots eluted and counted. As shown in Table I, the complete system including radioactive IPTG required the addition of acetate, ATP and coenzyme A ; omission of any one of these substances reduced the formation of AcIPTG to negligible values. When acetate, ATP and CoA were replaced by acetyl CoA, the amount of AcIPTG formed was almost twice. Addition of ATP to acetyl CoA resulted in a slight increase in product, no doubt due to the maintenance of acetyl CoA concentration by regeneration with ATP during the incubation. The data suggest a direct acetylation of the thiogalactoside by acetyl CoA.

TABLE I

FORMATION OF AcIPTG BY EXTRACTS OF E. COLI ML 308

Additions or omissions	μ Moles AcIPTG
Complete system	0.47
No acetate	0.06
No ATP	0.07
No CoA	0.05
Complete + Acetyl CoA, minus CoA and acetate	0.96
Complete + Acetyl CoA, minus CoA, acetate and ATP	0.80
Complete + Acetyl CoA, minus CoA and acetate, heated enzyme	0

To each tube was added dialyzed enzyme solution containing 1.0 mg protein, 50 μ moles of potassium phosphate buffer, pH 7.2, and 20 μ moles of S-35 labeled isopropyl-β-D-thiogalactoside, 3.5×10^5 c.p.m. per μ mole. The "complete system" consisted in addition of 10 μ moles of ATP, 20 μ moles of sodium acetate, and 0.5 mole of CoA. Acetyl coenzyme A (7.4 μ moles) was added as indicated. Final volume 1.0 ml. Incubation 1 hour at 35°C; reaction stopped with trichloroacetic acid.

Studies with whole cells had shown that under conditions suitable for acetyl-galactoside formation, significant quantities of this material were formed only by those strains of E. coli containing galactoside-permease.

Since the permease-negative strains do not concentrate galactosides, it could not be decided whether the acetyl-forming activity was present in all cells or was present only in those cells which have permease activity. The development of a cell-free system which carries out the acetylation allowed a direct test.

TABLE II

FORMATION OF AcIPTG BY EXTRACTS OF DIFFERENT BACTERIAL STRAINS

Strains	Genotype	Non induced (1)		Induced (1)	
		µMoles AcIPTG formed in vitro	Permease activity in vivo	µMoles AcIPTG formed in vitro	Permease activity in vivo
ML 308	$i^-z^+y^+$	0.25	present	-	present
ML 3	$i^+z^+y^-$	0.02	absent	0.05	absent
ML 30	$i^+z^+y^+$	0.01	absent	0.22	present
ML 35	$i^-z^+y^-$	0.03	absent	-	absent

(1) - Growth medium mineral salts, succinate or maltose, with (induced) or without (uninduced) IPTG 2×10^{-3}M.

Extracts of a number of bacterial strains were prepared and incubated with labeled IPTG and acetate, ATP and CoA under conditions similar to those of Table I. As shown in Table II, strains which do not have permease activity have little or no AcIPTG activity. Those strains which are inducible for permease, are also inducible for the acetylating system, and the strain which is constitutive for permease is also constitutive for the acetylating system. Since the strain to strain differences involved here are known to be quite specific for galactoside-permease, the observed correlations constitute strong evidence that the acetylation reaction is carried out by a system closely connected with, or part of, the permease system.

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BIBLIOGRAPHY.

1. H.V. RICKENBERG, G.N. COHEN, G. BUTTIN, and J. MONOD, Ann. Inst. Pasteur, 91 (1956), p. 829.

2. L. HERZENBERG, unpublished.