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Thiogalactoside Transacetylase of the Lactose Operon as an Enzyme for Detoxification

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Thiogalactoside transacetylase, the *lacA* gene product, confers selective advantage to cells of *Escherichia coli* K-12 growing on β-galactosides in the presence of non-metabolizable analogues.

It appears that bacterial species, such as *Escherichia coli*, that employ the proton gradient-driven permease for lactose transport (15, 20, 27, 28, 31) are able to exploit a broader range of β-galactosides for growth than bacterial species, such as *Staphylococcus aureus*, that use the phosphoenolpyruvate:sugar phosphotransferase system for lactose uptake (10, 14). The latter system, however, in addition to being energetically less costly, confers a higher substrate scavenging power (1a). The ability of *E. coli* to utilize several different β-galactosides for growth cannot be a mere reflection of fortuitous properties of the permease, since coadaptation of the β-galactosidase and the repressor proteins is also necessary for this growth trait. For the common substrate lactose (α-D-galactosyl-β-1,4-D-glucose) to be utilized, three stereochemical screenings are necessary: (i) it must be accepted by the M protein (13); (ii) it must be isomerized to allolactose (α-D-galactosyl-β-1,6-D-glucose) by β-galactosidase to neutralize the repressor (3, 7, 12); and (iii) it must be cleaved by the hydrolase in the principal reaction (26). Because each of these proteins has a special function, it might be impossible to superimpose on them the same spectrum of ligand specificity. On the other hand, if the specificities of the proteins are not completely concordant, metabolic predicaments might arise. For instance, a compound that qualifies as a transport substrate and as an inducer may not be hydrolyzable. Such a compound may accumulate to the detriment of the cell. (Growth retardation of a *lacI*⁻ constitutive mutant of *E. coli* ML by isopropyl-β-thiogalactose [IPTG] or thiomethyl-β-D-galactoside [TMG] was demonstrated with succinate as the source of carbon and energy [25].) A more common situation might be the incidental uptake of non-metabolizable structural analogues while the cell is utilizing physiological β-galactosides. In such a case an analogue merely has to satisfy the steric requirements of the permease, which are rather low. (The influx K_m values for lactose and TMG are close to 0.5 mM [15, 20, 31].) By what measure can a cell protect itself against this kind of contingency? A clue is provided by the observation that acetylated IPTG and TMG formed under the influence of the transacetylase (32, 33) are discharged into the medium, and that the acetylated compound, in contrast to the free form, cannot be pumped into the cell (29). The presence or absence of the transacetylase was shown not to affect the transport of free TMG (6).

The experiments described in this report were aimed at testing whether the possession of the acetylase can confer a selective advantage to cells when they are growing on physiological β-galactosides in the presence of an analogue. For this purpose a pair of K-12 strains, differing in the *lacA* allele but otherwise isogenic, was first examined for their response to IPTG during growth on lactose or lactulose (α-D-galactosyl-β-1,4-D-fructose) as the sole source of carbon and energy. The addition of IPTG to cultures of strain 148 (*lacI*$^+$,*Z*$^+$,*Y*$^+$,*A*$^+$) growing on either of the two carbon sources had only a slight effect on the generation time (Fig. 1). A stronger growth inhibition of strain 149 (*lacI*$^+$,*Z*$^+$,*Y*$^+$,*A*$^-$) occurred after the addition of the analogue. Similar results were obtained with TMG (data not shown).

A direct demonstration of the selective advantage of *lacA*$^+$ over *lacA*$^-$ cells under conditions similar to those described above was achieved by growing the two kinds of cells in a medium containing a utilizable β-galactoside in the presence or absence of IPTG. To facilitate the population tally during an experiment, a nutritional marker was introduced into each strain by spontaneous mutations. Cells of a mutant derived from the *lacA*$^+$ strain that are able to grow on D-arabinose and those of a mutant derived from the *lacA*$^-$ strain that are able to grow on L-1,2-propanediol were inocu-

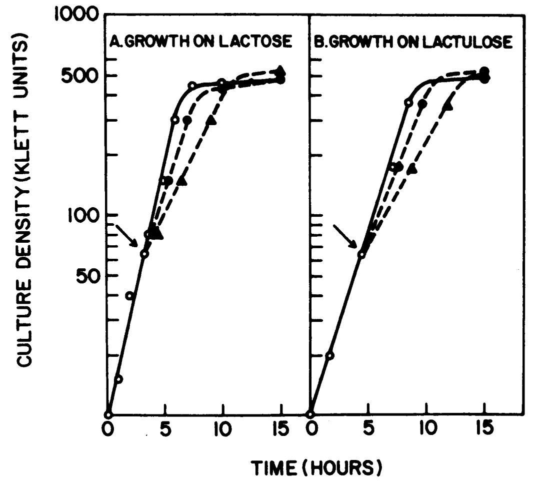

FIG. 1. *Effect of IPTG on the growth rate of E. coli strain 148 (F^- $\nabla lac\phi 80dlacI^+,Z^+,Y^+,A^+$) and strain 149 ($F^-$ $\nabla lac\phi 80dlacI^+,Z^+,Y^+,A^-$). The phenotype with respect to thiogalactoside transacetylase was verified by its activity in the cell extracts (1). The cells were grown in a β-galactoside (15 mM) mineral medium supplemented with vitamin B_1 (21) at 37°C with aeration. At the time indicated by the arrow, 5 mM IPTG was added to a culture of strain 148 (●) and to a culture of strain 149 (▲), and the growth rates were compared with control cultures without analogue addition (○).*

lated at a 1:1 ratio and grown for 50 generations. No detectable departure from the initial input ratio occurred (Fig. 2). But after the introduction of 5 mM IPTG, a 15-fold enrichment of the *lacA*$^+$ cells took place during the next 50 generations. A reciprocal growth competition experiment with *lacA*$^+$ cells marked by the ability to grow on L-1,2-propanediol and *lacA*$^-$ cells marked by the ability to grow on D-arabinose gave the same results (not shown). A 13-fold enrichment of *lacA*$^+$ cells is expected on the basis of the data presented in Fig. 1.

Although the number of utilizable and nonutilizable β-galactosides employed in this preliminary study is limited, the striking outcome of the growth competition experiments strongly suggests that the fourth gene product of the *lac* system, thiogalactoside transacetylase, serves as a backup device to avert metabolic congestion. The benefit of the removal of the interfering compounds clearly outweighs the expenditure of energy. Probably also of adaptive significance are the high K_m values of this enzyme for its acetyl accepting substrates (0.77 M for IPTG [1, 18]), which allow the cell to mobilize the energy-consuming mechanism to an extent that is commensurate with the prevailing level of an undesirable substance. Additionally, it might be of relevance to note that a number of galactosides other than lactose are found in milk (22), and the substrate specificity of the transacetylase is not limited to thio-compounds. At 2 mM, *p*-nitrophenyl β-D-galactoside is twice as active as a substrate than IPTG (1, 18). Another important property of this enzyme that should not be overlooked is its complete inactivity towards lactose (18).

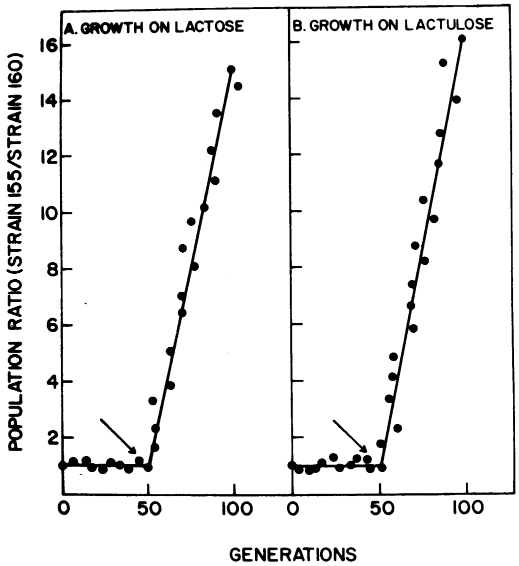

FIG. 2. *Effect of 5 mM IPTG on the growth competition between cells of strain 155 (a spontaneous mutant of strain 148 capable of growth on D-arabinose selected by repeated subculturing in minimal medium containing 30 mM novel carbon source [16]) and strain 160 (a spontaneous mutant of strain 149 capable of growth on L-1,2-propanediol selected by repeated subculturing in minimal medium containing 20 mM novel carbon source [21]). Cells of each strain grown to mid-exponential phase on the β-galactoside (15 mM) were inoculated in equal numbers into 500 ml of prewarmed fresh medium to give a total density of 2×10^4 cells/ml. The culture was incubated in a 2-liter Erlenmeyer flask on a rotary shaker at 37°C. Appropriate dilutions of the culture were periodically spread on four kinds of agar plates containing: (i) rich medium (Difco no. 2 antibiotic medium); (ii) eosin-methylene blue-lactose; (iii) D-arabinose minimal medium; and (iv) DL-1,2-propanediol minimal medium. The first two agar plates gave the total cell count, whereas media in (iii) and (iv) gave the cell numbers of strains 155 and 160, respectively. Once the culture density had reached 200 Klett units (5×10^8 cells/ml), a sample was reinoculated into 500 ml of prewarmed fresh medium to give a density of 10^4 cells/ml, and the incubation was continued. IPTG was added after about 50 generations, indicated by the arrow, and the composition of the population was sampled for another 50 generations.*

With respect to the evolutionary status of the *lac* operon, it might be cited that *Shigella dysenteriae*, which is believed to be undergoing retrogressive change in this genetic system because of the absence of an intact *lacY* gene (17), apparently also lost the *lacA* gene (1). The same may apply to *Salmonella typhimurium* LT-2 (1).

The lack of a thiogalactoside transacetylase need not be a sign of decline or primitiveness in a dissimilatory system for lactose. Transport mechanisms that are highly discriminatory, such as the phosphotransferase system for lactose, may have little use for this enzyme as an accessory. It might be rewarding to examine some of these systems in gram-positive organisms to see if this is true. The non-utilizable glucose analogue, α-methylglucoside, is taken up by the cells and trapped in the cytoplasm in phosphorylated form through the intervention of a phosphotransferase system. In this case, the detoxification process seems to be by dephosphorylation. Furthermore, the presence of glucose hastens the expulsion of the analogue (8, 30).

Finally, it is tempting to imagine an evolutionary connection between metabolic safeguards such as thiogalactoside transacetylase and certain defense mechanisms against antibiotics conferred by drug resistance factors (RTF) (2). Chloramphenicol and kanamycin can both be inactivated by RTF-specified transacetylases (19). Investigators have wondered (4) why these potentially vital enzymes (5, 9), as well as several others that inactivate antibiotics by adenylation (5, 9) or by phosphorylation (23), should be subject to catabolite-repressive control. The answer might be that genes specifying these kinds of enzymes were appropriated from catabolic systems. Homology between thiogalactoside transacetylase and kanamycin acetytransferase is additionally suggested by a similarity in substrate specificities; both enzymes attack the nucleophilic group on the carbon 6 of a hexose at the nonreducing end of a polysaccharide (11, 24).

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LITERATURE CITED

1. **Alpers, D. H., S. H. Appel, and G. M. Tomkins.** 1965. A spectrophotometric assay for thiogalactoside transacetylase. J. Biol. Chem. 240:10-13.

1a. **Andrews, K. J., and E. C. C. Lin.** 1976. Selective advantages of various bacterial carbohydrate transport mechanisms. Fed. Proc. 35:2185-2189.

2. **Benveniste, R., and R. Davies.** 1973. Mechanisms of antibiotic resistance in bacteria. Annu. Rev. Biochem. 42:471-506.

3. **Burstein, C., M. Cohn, A. Kepes, and J. Monod.** 1965. Rôle du lactose et de ses produits métaboliques dans l'induction de l'opéron lactose chez *Escherichia coli*. Biochim. Biophys. Acta 95:634-639.

4. **Davies, J. E., and R. Round.** 1972. Transmissible multiple drug resistance in *Enterobacteriaceae*. Science 176:758-768.

5. **De Crombrugghe, B., I. Pastan, W. V. Shaw, and J. L. Rosner.** 1973. Stimulation by cyclic AMP and ppGpp of chloramphenicol acetyltransferase synthesis. Nature (London) New Biol. 241:237-239.

6. **Fox, C. F., J. R. Beckwith, W. Epstein, and E. R. Signer.** 1966. Transposition of the *lac* region of *Escherichia coli*. II. On the role of thiogalactoside transacetylase in lactose metabolism. J. Mol. Biol. 19:576-579.

7. **Gilbert, W., and B. Müller-Hill.** 1970. The lactose repressor, p. 93-109. *In* J. R. Beckwith and D. Zipser (ed.), The lactose operon. Cold Spring Harbor Laboratory, Cold Spring Harbor, N. Y.

8. **Hagihira, H., T. H. Wilson, and E. C. C. Lin.** 1963. Studies on the glucose-transport system in *Escherichia coli* with α-methylglucoside as substrate. Biochim. Biophys. Acta 78:505-515.

9. **Harwood, J., and D. H. Smith.** 1971. Catabolite repression of chloramphenicol acetyltransferase synthesis in *E. coli* K_{12}. Biochem. Biophys. Res. Commun. 42:57-62.

10. **Hengstenberg, W., J. B. Egan, and M. L. Morse.** 1967. Carbohydrate transport in *Staphylococcus aureus*. V. The accumulation of phosphorylated carbohydrate derivatives, and evidence for a new enzyme-splitting lactose phosphate. Proc. Natl. Acad. Sci. U.S.A. 58:274-279.

11. **Herzenberg, L. A.** 1961. Isolation and identification of derivatives formed in the course of intracellular accumulation of thiogalactosides by *Escherichia coli*. Arch. Biochem. Biophys. 93:314-315.

12. **Jobe, A., and S. Bourgeois.** 1972. *Lac* repressor-operator interaction. VI. The natural inducer of the *lac* operon. J. Mol. Biol. 69:397-408.

13. **Kennedy, E. P.** 1970. The lactose permease system of *Escherichia coli*, p. 49-92. *In* J. R. Beckwith and D. Zipser (ed.), The lactose operon. Cold Spring Harbor Laboratory, Cold Spring Harbor, N. Y.

14. **Kennedy, E. P., and G. A. Scarborough.** 1967. Mechanism of hydrolysis of *o*-nitrophenyl-β-galactoside in *Staphylococcus aureus* and its significance for theories of sugar transport. Proc. Natl. Acad. Sci. U.S.A. 58:225-228.

15. **Kepes, A.** 1960. Études cinétiques sur la galactoside-perméase d'*Escherichia coli*. Biochim. Biophys. Acta 40:70-84.

16. **LeBlanc, D. J., and R. P. Mortlock.** 1971. Metabolism of D-arabinose: a new pathway in *Escherichia coli*. J. Bacteriol. 106:90-96.

17. **Luria, S. E.** 1965. On the evolution of the lactose utilization gene system in enteric bacteria, p. 357-358. *In* V. Bryson and H. J. Vogel (ed.), Evolving genes and proteins: a symposium. Academic Press Inc., New York.

18. **Musso, R. E., and I. Zabin.** 1973. Substrate specificity and kinetic studies on thiogalactoside transacetylase. Biochemistry 12:553-557.

19. **Okamoto, S., and Y. Suzuki.** 1965. Chloramphenicol-, dihydrostreptomycin-, and kanamycin-inactivation enzymes from multiple drug-resistant *E. coli* carrying episome 'R'. Nature (London) 208:1301-1303.

20. **Rickenberg, H. V., G. N. Cohen, G. Buttin, and J. Monod.** 1956. La galactoside-perméase d'*Escherichia*

coli. Ann. Inst. Pasteur Paris 91:829–857.

21. **Sridhara, S., T. T. Wu, T. M. Chused, and E. C. C. Lin.** 1969. Ferrous-activated nicotinamide adenine dinucleotide-linked dehydrogenase from a mutant of *Escherichia coli* capable of growth on 1,2-propanediol. J. Bacteriol. 98:87–95.
22. **Stacy, M., and S. A. Barker.** 1962. Milk oligosaccharides, p. 122–134. *In* Carbohydrates of living tissues. D. van Nostrand Co., London.
23. **Tsukada, I., M. Yagisawa, M. Umezawa, M. Hori, and H. Umezawa.** 1972. Stimulation of kanamycin phosphotransferase synthesis in *Escherichia coli* by 3′,5′-cyclic AMP. J. Antibiot. 25:144–146.
24. **Umezawa, H., M. Okanishi, R. Utahara, K. Maeda,. and S. Kondo.** 1967. Isolation and structure of kanamycin inactivated by a cell free system of kanamycin-resistant *E. coli*. J. Antibiot. Ser. A 20:136–141.
25. **von Hofsten, B.** 1960. The inhibitory effect of galactosides on the growth of *Escherichia coli*. Biochim. Biophys. Acta 48:164–171.
26. **Wallenfels, K., and R. Weil.** 1972. β-Galactosidase, p. 618–663. *In* P. D. Boyer, H. Lardy, and K. Myrback (ed.), The enzymes, vol. 7. Academic Press Inc., New York.
27. **West, I. C.** 1970. Lactose transport coupled to proton movements in *Escherichia coli*. Biochem. Biophys. Res. Commun. 41:655–661.
28. **West, I., and P. Mitchell.** 1972. Proton-coupled β-galactoside translocation in non-metabolizing *Escherichia coli*. Bioenergetics 3:445–462.
29. **Wilson, T. H., and E. R. Kashket.** 1969. Isolation and properties of thiogalactoside transacetylase-negative mutants of *Escherichia coli*. Biochim. Biophys. Acta 173:501–508.
30. **Winkler, H. H.** 1971. Efflux and the steady state in α-methylglucoside transport in *Escherichia coli*. J. Bacteriol. 106:362–368.
31. **Winkler, H. H., and T. H. Wilson.** 1966. The role of energy coupling in the transport of β-galactosides by *Escherichia coli*. J. Biol. Chem. 241:2200–2211.
32. **Zabin, I.** 1963. Galactoside transport in relation to bacterial genetics and protein synthesis. Fed. Proc. 22:27–30.
33. **Zabin, I., A. Kepes, and J. Monod.** 1962. Thiogalactoside transacetylase. J. Biol. Chem. 237:253–257.