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 ability (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-1,3-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-thiogalactoside [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-
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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 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 acetyltransferase is additionally suggested by a similarity in substrate specificities; both enzymes attack the nucleophilic group on the carbon 6 of a hexose at the non-reducing end of a polysaccharide (11, 24).

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LITERATURE CITED


