A GROUP OF COMPOUNDS EXHIBITING PARADOXICAL ACTIVITY IN THE REGULATION OF THE LAC OPERON[1]

Beverly Williams[2] and Kenneth Paigen

Department of Experimental Biology
Roswell Park Memorial Institute, Buffalo, New York

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We report the existence of a class of compounds that are active in the regulation of the lac operon of E. coli and that do not exhibit the properties expected of a repressor, inducer, or anti-inducer. Under some circumstances these paradoxical compounds inhibit enzyme formation; under other circumstances they induce it. The induced β-galactosidase synthesis by wild-type and o^c[3] mutant cells and the constitutive synthesis of this enzyme by i^- and $i^s i^-$ mutants are repressed by these compounds, while the basal synthesis by i^+ cells and particularly the constitutive enzyme synthesis by o^c cells are induced by them.

MATERIALS AND METHODS

All strains originate from the collection of Drs. F. Jacob and J. Monod and are derived from E. coli 3000 (HfrH). The strains 3300 lac i^- and 2000 lac o^c are described in Franklin and Luria (1961); the non-inducible strain

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[2] Pre-doctoral fellow in the Roswell Park Division of the Graduate School, State University of New York at Buffalo under Public Health Service Training Grant (CA-5016) from the National Cancer Institute.

[3] Abbreviations used are: o, operator region; i, lac regulator gene; IPTG, isopropyl-β-D-thiogalactoside.

lac i^s_{-18} arg$^-$ his$^-$ is described in Willson et al. (1964); and the constitutive double mutant lac $i^s i^-$ was obtained in this laboratory by selecting lactose fermenters from the i^s strain as described by Willson et al. (1964).

Cells were grown in M9 (Anderson, 1946) with 1 gm/l glycerol as the carbon source. The medium was supplemented with 0.5 μg/ml thiamine, and 20 μg/ml each of threonine, leucine, isoleucine and valine for strains 3000, 3300, and 2000 or arginine and histidine for the i^s and $i^s i^-$ mutants. Cultures were grown at 37° C under active aeration. The differential rate of β-galactosidase synthesis was calculated from the relative increases in enzyme content and in cell density of a culture growing for 2 - 3 generations in log phase.

IPTG was purchased from Calbiochem, D(+) fucose from General Biochemicals, and aminophenyl-β-D-galactoside from Pierce Chemical Company. o-Nitrophenyl-fucoside was kindly supplied by Dr. H. V. Rickenberg. All other compounds were the generous gift of Dr. S. Spiegelman.

The assay of β-galactosidase was adapted from the procedure of Lederberg (1950) as previously described (Paigen, 1963). The cellular concentration of enzyme is expressed as μ moles of o-nitrophenol liberated per minute at 30° C per unit absorbance of the culture. One unit of absorbance of cells measured at 550 mμ in a Zeiss PMQII spectrophotometer is approximately equal to 0.24 mg protein/ml.

RESULTS AND DISCUSSION

The class of compounds which show paradoxical behavior includes D(+) fucose, phenyl-β-D-galactoside, p-aminophenyl-β-D-galactoside and

TABLE 1

THE EFFECTS OF PARADOXICAL COMPOUNDS AND ANTI-INDUCERS ON LAC REGULATORY MUTANTS

Compound	Conc. (M)	Rate of β-galactosidase synthesis				
		i^-	$i^s i^-$	o^c	o^c + 10^{-6} IPTG	o^c + 10^{-5} IPTG
no addition		4.3	1.9	0.22	1.3	3.6
Paradoxical						
fucose	10^{-1}	2.0	1.2	1.9	1.9	2.4
phenyl-β-D-galactoside	10^{-3}	2.1	1.4	2.2	-	2.2
p-aminophenyl-β-D-galactoside	10^{-3}	1.6	1.0	1.4	-	1.6
p-aminophenyl-β-D-thiogalactoside	3×10^{-3}	1.2	0.6	1.8	1.7	1.9
Anti-inducers						
o-nitrophenyl-β-D-fucoside	10^{-3}	3.5	1.9	0.13	0.17	3.4
phenyl-β-D-thiogalactoside	3×10^{-3}	3.4	1.9	0.18	0.11	3.5

p-aminophenyl-β-D-thiogalactoside.[4] The effects of the paradoxical compounds and the two anti-inducers, phenyl-thiogalactoside (Monod, et al., 1951) and o-nitrophenyl-fucoside (Müller-Hill et al., 1964), on several lac regulatory mutants are summarized in Table 1. The constitutive enzyme synthesis by i^- or the double mutant $i^s i^-$ is not affected by the presence of anti-inducers while paradoxical compounds repress synthesis in both mutants. Although the constitutive synthesis by the o^c mutant is slightly inhibited by anti-inducers, it is, surprisingly enough, induced by paradoxical compounds. The super-induction of o^c cells by IPTG is competitively inhibited by anti-inducers, and limited by paradoxical compounds to the level of induction achieved by the paradoxical compounds alone. (When the inducers, IPTG or TMG, are present at concentrations of 10^{-2} M or above, they reduce enzyme formation to about 80% of the maximal value in all strains used in these experiments.)

Fucose also represses the induced synthesis of β-galactosidase by lac^+ cells. This repression is compared with the repression of constitutive synthesis by the i^- mutant in Figure 1. Both wild-type cells that were maximally induced for β-galactosidase by 5×10^{-5} M IPTG and i^- cells showed a progressive repression of enzyme synthesis with increasing concentrations of fucose. At any concentration of fucose, the repression was reversed by increasing the concentration of the inducer, IPTG. Fucose repression of β-galactosidase formation and its reversal by IPTG were quantitatively similar in i^+ and i^- cells. The ability of an i^- mutant to be repressed and then re-induced is not unique to

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[4] These compounds do not inhibit growth nor do they inhibit β-galactosidase at the concentrations present during assay of the enzyme. Although the galactosides act at much lower concentrations than fucose, fucose is more readily available and has been the most extensively studied compound. The sample used was chromatographically pure in thin layer chromatography in three solvent systems under conditions that would have revealed the presence of 0.2% contaminant.

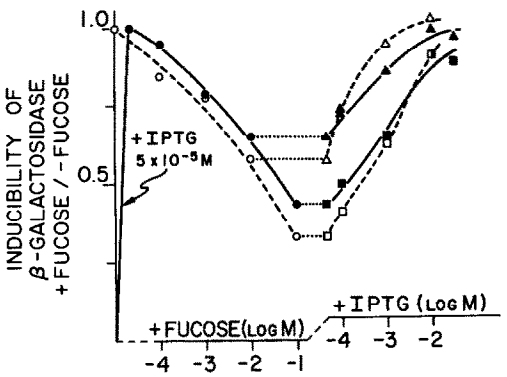

Figure 1. Repression of β-galactosidase synthesis by fucose and reversal of repression by IPTG. Cultures of i^+ cells induced with 5×10^{-5} M IPTG (● - ● - ●) and i^- cells in the absence of inducer (O - O - O) were repressed with increasing concentrations of fucose. At 10^{-2} M (Δ - Δ, ▲ - ▲) and 10^{-1} M (□-□, ■ -■) fucose, higher concentrations of IPTG were added to cultures of both strains. Results are expressed as the rate of synthesis in the presence of fucose compared with a control culture grown at the same IPTG concentration in the absence of fucose.

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this strain, but occurs in other i^- strains as well (W1317, ML35 and a series of mutants isolated from AB257 by Dr. H. V. Rickenberg) and is also characteristic of the double mutant $i^s i^-$.

In addition to super-inducing the o^c mutant, paradoxical compounds can weakly induce the wild-type strain E. coli 3000 (Table 2). The greater effectiveness of these compounds in o^c as compared to wild-type cells does not result from the prior presence in o^c cells of galactoside permease or other gene products of the lac operon. Pre-induced wild-type cells were induced by these compounds to the same differential rate of synthesis as were wild-type cells which had never been exposed to a conventional inducer, showing that the effectiveness of these compounds in o^c is related to the o^c mutation itself.

Repression of both wild-type and i^- cells begins immediately at the

TABLE 2: INDUCTION OF β-GALACTOSIDASE IN WILD-TYPE CELLS BY PARADOXICAL COMPOUNDS, ANTI-INDUCERS AND IPTG

Compound	Conc. (M)	Rate of enzyme formation
no addition	-	.002
fucose	10^{-1}	.015
phenyl-β-D-galactoside	10^{-2}	.750
p-aminophenyl-β-D-galactoside	10^{-3}	.740
p-aminophenyl-β-D-thiogalactoside	10^{-2}	.260
IPTG	5×10^{-5}	3.00
o-nitrophenyl-fucoside	10^{-3}	.002
phenyl-β-D-thiogalactoside	3×10^{-3}	.002

time of addition of fucose to a growing culture and is linear with growth for a period of at least 3 generations. The paradoxical compounds do not repress by preventing the entry of IPTG into the lac$^+$ cell since they are equally effective in lac i$^-$ cells where there is no exogenous inducer (Fig. 1). Because fucose neither contributes carbon to the intermediary pool (Buttin, 1963; Rotman, personal communication), nor inhibits utilization of the pool as measured by growth rate, and is able to both induce and repress β-galactosidase formation, it does not seem possible to ascribe its effects to catabolite repression. The same arguments apply to p-aminophenyl-β-D-thiogalactoside.

Our results lead to the following experimental conclusions.

1) Despite their close structural similarities, the biological activities of paradoxical compounds are readily distinguished from those of inducers and anti-inducers.

2) The i^- and $i^s i^-$ mutants retain the capability of being regulated by both paradoxical compounds and inducers.

This latter conclusion raises the possibility that the i locus, by analogy with the kinase locus in the gal operon (Jordan et al., 1962), may be the structural gene for an enzyme whose normal action is either the destruction of an internal inducer or the production of a low molecular weight repressor. The available evidence does not permit us to choose between this interpretation and the conventional one of the i gene as controlling the synthesis of a macromolecular repressor.

We wish to defer any detailed attempt to construct a model to explain the behavior exhibited by paradoxical compounds until a further account of their properties can be presented.

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