COORDINATE REGULATION OF DNA-DEPENDENT CELL-FREE SYNTHESIS OF URIDYLTRANSFERASE AND GALACTOKINASE

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1. Introduction

Zubay and his coworkers have developed a system for the DNA-dependent cell-free synthesis of an active bacterial enzyme, β-galactosidase of *Escherichia coli* and have shown that this synthesis is regulated by control mechanisms known to be operative in the intact cell [1–3]. We have modified this system for the efficient cell-free synthesis of the enzymes of the galactose operon of *Escherichia coli* [4]. We have demonstrated that this synthesis also requires the addition of cyclic adenosine 3′:5′ monophosphate (AMP) and that it is estimated by D-fucose, which is known as an inducer of the galactose operon [5, 6]. After completion of our study, Parks et al. [7] reported the cell-free synthesis of galactokinase. While these authors investigated the stimulation of galactokinase synthesis by cyclic AMP, they did not demonstrate an effect of D-fucose.

Here we report further evidence for the activity of the *gal*-repressor in our *in vitro* system by extending our experiments to another enzyme of the galactose operon, thus demonstrating coordinate induction of galactokinase (EC 2.7.1.6) and uridyltransferase (EC 2.7.7.12). Furthermore, inhibition of this induction was achieved by TMG (methyl-β-D-thiogalactopyranoside), known as an antiinducer from *in vivo* studies [5].

2. Materials and methods

The protein for the cell-free systems was prepared from the following strains: (1) $TN_{102}/F'102$ [8] carrying the strong-polar insertion mutation N_{102} in the transferase gene both on the chromosome and on the F_8 *gal* episome. This strain has no transferase activity, but a small residual kinase activity. (2) F_{i165}, carrying a deletion of the galactose operon.

DNA was prepared from strain $\lambda pgal_8$ [9] and from $\lambda dgal\ 0^c_{10}$ [10] carrying an operator constitutive mutation. All other methods employed were described previously [4].

3. Results and discussion

With improved preparations of bacterial extracts the synthesis of the two enzymes galactokinase and uridyltransferase in response to DNA concentrations as low as 1 μg/ml (table 1) could be measured. Although the amounts of enzyme synthesized decrease with decreasing DNA concentrations, the induction ratio is larger at the smaller DNA concentrations. Both enzymes behave coordinately over the whole range of DNA concentrations employed. The inverse relationship between induction ratio and DNA concentration may be due to the titration of few active repressor molecules by increasing amounts of operator-carrying DNA [3, 4], though quantitatively the results seem to be more complicated than would be expected for a simple interaction between one molecule each of repressor and operator-carrying DNA. The slight stimulation by D-fucose at high DNA concentrations is unexplained, but resembles the observation that *in vivo* a similar stimulation is seen even in strains in which the gene for the repressor is deleted [11].

The stimulation by D-fucose of uridyltransferase synthesis is true induction, because it is not observed

Table 1
Coordinate induction by D-fucose of *gal*O^+-DNA directed cell-free synthesis of uridyltransferase and galactokinase.

DNA concentration (μg/ml)	Enzyme activity synthesized *de novo*/30 min incubation (radioactivity of product, cpm/test)					
	Uridyltransferase			Galactokinase		
	+Fucose (5×10^{-3} M)	−Fucose (5×10^{-3} M)	Induction-ratio column 2 / column 3	+Fucose (5×10^{-3} M)	−Fucose (5×10^{-3} M)	Induction ratio column 5 / column 6
0.65	416	30	13.9	380	–	–
1.25	920	100	9.2	660	80	8.3
2.5	1,820	290	6.3	1,280	220	5.8
5	3,640	840	4.3	2,020	500	4.0
10	5,900	1,950	3.0	3,970	1,210	3.3
20	10,480	4,290	2.4	8,010	2,610	3.1
30	10,920	4,640	2.4	9,530	2,960	3.2
50	17,280	9,080	1.9	13,660	5,860	2.3
80	17,880	10,260	1.7	11,820	5,560	2.1
100	16,120	12,080	1.3	13,020	6,040	2.2

Protein synthesis was for 30 min at 37° with a cell-free extract from TN_{102}/F'_{102} and DNA from λp*gal*$_8$. 10 μl aliquots were incubated for the enzyme tests (30 min at 37° in 0.10 ml final volume). The following blank-values were substracted: 100 cpm found after 0 min of incubation of assays of both kinase and transferase and 510 cpm for the residual kinase activity contributed by the bacterial extract. No such correction is necessary in the case of transferase. The radioactivity thus measured is given in the table. Aliquots smaller than 10 μl had to be used for the enzyme assay at DNA concentrations higher than 5 μg/ml but results are expressed here for 10 μl aliquots. The values obtained with 50 μg/ml of DNA in the presence of D-fucose represent the synthesis of 28.6 units [4] of uridyltransferase and of 20.4 units [4] of galactokinase per ml of the mixture incubated for protein synthesis.

Table 2
Constitutive synthesis of uridyltransferase directed by *gal*O^c-DNA.

DNA concentration (μg/ml)	Uridyltransferase activity synthesized: 30 min incubation (radioactivity of UDPgal, cpm/test)		
	+Fucose (5×10^{-3} M)	−Fucose (5×10^{-3} M)	Induction ratio
5	8,600	6,900	1.2
10	12,000	10,900	1.1
20	21,800	20,900	1.0
30	24,000	24,000	1.0
40	25,200	24,000	1.1
50	26,600	26,600	1.0

The DNA was prepared from λd*gal* O^c_{10} [10]. The extract used and all details are as described in table 1.

Table 3
Effects of methyl-β-D-thiogalactopyranoside (TMG) on galactokinase induction.

Addition	Galactokinase activity synthesized/30 min incubation (radioactivity of Gal-1P, cpm/test)	
	+Fucose (10^{-3} M)	−Fucose (10^{-3} M)
−TMG	2,580	650
+TMG (2.5×10^{-2} M)	1,090	1,010

The protein extract was prepared from strain F_{i165}. DNA from λp*gal* O^+ was used at a concentration of 5 μg/ml. No correction for residual kinase activity was necessary since the extract is prepared from a strain carrying a deletion of the *gal* operon. All other details are the same as in table 1.

in a cell-free system with DNA carrying an operator-constitutive mutation (table 2). The higher yield of enzyme synthesized with DNA carrying an O^c mutation is reproducible, and cannot be explained at present. The stimulatory effect of D-fucose is largely reversed by the addition of TMG (table 3). In our *in vitro* system this antiinducer appears to act as a weak inducer in the absence of D-fucose. This property of TMG may be obscured *in vivo* by its predominant effect to suppress the internal induction of the galactose operon [12]. All these observations support our conclusion that in the cell-free system the galactose repressor can be detected without any purification. The concentration of active repressor, however, is low and requires the use of low DNA concentrations. This finding is in agreement with the interpretation of Parks et al. [7] that an effect of D-fucose cannot be detected in a protein system that requires higher concentrations of DNA.

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