THE CR MUTATION AND CATABOLITE REPRESSION IN ESCHERICHIA COLI

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In a series of papers Loomis and Magasanik and Tyler, Loomis, and Magasanik (1965, 1967, 1967a) described mutants of Escherichia coli in which, according to the authors, the formation of the enzymes coded by the lac operon had become resistant to catabolite repression. The authors postulated a gene CR (for catabolite repression), which, when in the mutated form, CR^-, rendered the expression of the lac operon insensitive to catabolite repression. CR mapped near the tryptophan locus and distant from the lac operon. According to Loomis and Magasanik (1967) a mutation at the CR locus had no detectable metabolic effect other than rendering the lac operon resistant to catabolite repression. They also found that the wild type CR allele was trans dominant.

In an earlier publication (Hsie and Rickenberg, 1967) we described a mutant of E. coli which had lost sensitivity to catabolite repression when glucose served as source of carbon but was fully sensitive to catabolite repression when glucose-6-phosphate (G-6-P) or a mixture of glucose and gluconic acid were employed. A number of similar mutants have been isolated in this laboratory recently, and it was found that in all of them several enzymes normally subject to catabolite repression had become largely resistant to the effect of glucose but were still fully sensitive to G-6-P or the mixture of glucose and gluconic acid. For comparative purposes we decided to examine CR^- mutants kindly furnished by Dr. Loomis. Our

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Table 1

Catabolite Repression of β-Galactosidase Synthesis

Strain	Source of Carbon	Generation Time	β-Galactosidase, Specific Activity		
			Non-induced	Induced with TMG	Induced with IPTG
AB 257-7 (CR^+)	Glycerol	69	20	30,800	30,000
"	Glucose	49	8	2,800	7,800
"	G-6-P	44	3	30	3,300
K-12 3000 (CR^+)	Glycerol	100	27	30,500	23,000
"	Glucose	60	11	3,900	10,700
"	G-6-P	60	3	2,450	3,000
L9 (CR^-)	Glycerol	75	8	27,000	35,000
"	Glucose	75	7	27,800	29,900
"	G-6-P	48	4	400	3,700
LA12G (CR^-)	Glycerol	100	35	23,500	24,200
"	Glucose	60	17	22,200*	17,600
"	G-6-P	60	10	660	3,400

*In some experiments glucose inhibited β-galactosidase synthesis as much as 40% in strain LA12G.
Growth was at 37° C. In the case of strain AB 257-7 medium "56" was supplemented with 4×10^{-4} M L-methionine and in the case of strains L9, LA12G, and K-12 3000 with 5 μg/ml of thiamine. Glycerol was employed at a concentration of 2×10^{-2} M and glucose and glucose-6-phosphate (G-6-P) at 10^{-2} M. Cultures were induced with either 5×10^{-4} M TMG (methyl-1-thio-β-D-galactoside) or 10^{-3} M IPTG (isopropyl-1-thio-β-D-galactoside) and grown for a minimum of ten generations in the presence of the inducer. Unit of β-galactosidase activity: mμmoles of 2-nitrophenyl-β-D-galactoside hydrolized/min/mg of protein at 37° C.

findings indicate that in the CR^- mutants studied the synthesis of tryptophanase, and amylomaltase, as well as that of β-galactosidase was relatively resistant to inhibition by glucose but that the formation of the three enzymes was still inhibitable by G-6-P (or the mixture of glucose and gluconic acid; results not reported here).

Two of the mutants described by Loomis and Magasanik (1967), L9 and LA12G, as well as strains AB 257-7 and K-12 3000, wild type with respect to catabolite repression, were employed in this study. In preliminary experiments we compared the medium used by Loomis and Magasanik with medium "56" employed rou-

Table II

Catabolite Repression of Tryptophanase Synthesis

Strain	Source of Carbon	Tryptophanase, Specific Activity Non-induced	Induced
AB 257-7 (CR^+)	Glycerol	1.0	60
"	Glucose	0.8	3.2
"	G-6-P	0.3	3.1
K-12 3000 (CR^+)	Glycerol	2.5	134
"	Glucose	0.5	2.2
"	G-6-P	0.3	1.4
L9 (CR^-)	Glycerol	0.7	108
"	Glucose	0.7	106
"	G-6-P	0.5	1.2
LA12G (CR^-)	Glycerol	0.4	84
"	Glucose	0.5	21
"	G-6-P	0.4	14

Cultures were induced by growth for ten generations in the presence of 4×10^{-3} M L-tryptophan. Unit of tryptophanase activity: mμmoles of indole liberated/min/mg of protein at 37° C. (Intact bacteria were employed in the assay and 100 μg of chloramphenicol/ml were added to the assay mixture in order to prevent induction of tryptophanase during the assay).

Table III

Catabolite Repression of Amylomaltase Synthesis

Strain	Source of Carbon	Amylomaltase, Specific Activity Non-induced	Induced
AB 257-7 (CR^+)	Glycerol	45	407
"	Glucose	33	100
"	G-6-P	22	33
K-12 3000 (CR^+)	Glycerol	24	205
"	Glucose	22	30
"	G-6-P	17	14
L9 (CR^-)	Glycerol	29	250
"	Glucose	27	190
"	G-6-P	13	12
LA12G (CR^-)	Glycerol	28	275
"	Glucose	24.5	106
"	G-6-P	11	31

Cultures were induced by growth for at least ten generations in the presence of 10^{-2} M maltose added to the source of carbon indicated in the table. Bacteria were treated with toluene prior to the assay for amylomaltase activity. In other respects the procedure was identical with that of Loomis and Magasanik (1965). Unit of amylomaltase activity: mμmoles of glucose liberated/min/mg of protein at 37° C.

tinely in this laboratory. Since no significant differences related to the use of the two media were detected, we employed medium "56" in the experiments reported here. This medium, as well as the determination of growth and β-galactosidase activity were described in an earlier communication (Müller-Hill, Rickenberg, and Wallenfels, 1964). Tryptophanase activity was assayed according to the procedure of McFall and Mandelstam (1963) and amylomaltase according to that of Loomis and Magasanik (1965).

The data summarized in Tables I, II, III, and IV demonstrate clearly that the mutants isolated by Loomis and Magasanik were highly resistant, in the case

Table IV

Catabolite Repression

Summary

Strain	Source of Carbon	Generation time	Specific activity (mμmoles/min/mg protein) β-galactosidase	Tryptophanase	Amylomaltase
AB 257-7 (CR^+)	Glycerol	69	30,800 (100)	60 (100)	407 (100)
"	Glucose	49	2,800 (9.1)	3.2 (5.3)	100 (24.5)
"	G-6-P	44	30 (0.1)	3.1 (5.2)	33 (8.1)
K-12 3000 (CR^+)	Glycerol	100	30,500 (100)	134 (100)	205 (100)
"	Glucose	60	3,900 (12.8)	2.2 (1.7)	30 (14.6)
"	G-6-P	60	2,450 (8)	1.4 (1)	14 (6.9)
L9 (CR^-)	Glycerol	75	27,000 (100)	108 (100)	250 (100)
"	Glucose	75	27,800 (103)	106 (98)	190 (76)
"	G-6-P	48	400 (1.5)	1.2 (1.1)	12 (4.8)
LA-12G (CR^-)	Glycerol	100	17,600 (100)	125 (100)	275 (100)
"	Glucose	60	10,600 (60)	54 (43)	106 (38.5)
"	G-6-P	60	945 (5.4)	13.2 (10.5)	31 (11)

Conditions as described in legends to preceding tables. β-Galactosidase was induced with 5×10^{-4} M TMG. The results represent average values derived from at least three experiments. Numbers in parentheses represent per cent of residual enzyme activities.

of strain L9, and relatively resistant, in the case of LA12G, to catabolite repression when glucose served as source of carbon. The fact that the formation of β-galactosidase, of tryptophanase, and of amylomaltase was largely unaffected by glucose indicates that the mutation of the CR gene is not specific for the lac operon. Furthermore the finding that in the mutants the synthesis of β-galactosidase, tryptophanase, and amylomaltase was still inhibited by G-6-P suggests strongly that the mutation has in some manner affected either the formation or the maintenance of a steady state concentration of a derivative of glucose, the presumptive "effector" of catabolite repression.

Loomis and Magasanik (1965) also stated that in mutants L9 and LA12G, unlike in wild type, CR^+, strains, the residual formation of β-galactosidase observed in the absence of an exogenous source of nitrogen, was resistant to inhibition by glucose. Our own experiments, not reported in detail here, showed that the residual β-galactosidase formation in the absence of exogenous nitrogen was, in fact, sensitive to inhibition by glucose provided that the mutants were starved of nitrogen in the presence of glucose for a period of one hour prior to the addition of the inducer.

Loomis and Magasanik (1967) postulated a gene CR mapping distant from the lac operon and affecting specifically the sensitivity of that operon to catabolite repression. The findings described here do not support this contention and suggest that the CR gene affects the metabolism of glucose.

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