GENETIC CONTROL OF THE REGULATION OF ISOCITRITASE AND MALATE SYNTHASE IN ESCHERICHIA COLI K 12.

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The metabolic pathways through which microorganisms satisfy their needs in carbon and energy, vary with the type of carbon source available. Under aerobic conditions, the carbohydrates (and their C_3 carbon glycolytic derivaties) are ultimately oxidized through the tricarboxylic acid cycle, which also serves a biosynthetic function. This latter function of the tricarboxylic acid cycle, however, involves the additional participation of a Wood and Werkman ($C_3 + CO_2$) type of condensation to replenish cyclic intermediates (Wiame, 1957). The crucial role of the $C_3 + CO_2$ condensation has received further support from the recent discovery of a mutant of Salmonella typhimurium in which the absence of this reaction leads to the inability to grow on glucose as the only carbon source (Theodore and Englesberg, 1962).

A similar situation was found to occur in a strain of E. coli K 12 (strain VG) which was used in the present work. In E. coli, the utilisation of acetate or glycollate for growth is possible through the occurence of inducible pathways added to the tricarboxylic acid cycle; neither of them involves the $C_3 + CO_2$ condensation.

Thus, growth on acetate is made possible by the glyoxylic shunt; growth on glycollate by the glyceric pathway (Kornberg, 1961). The glyoxylic cycle is composed of isocitritase (Olson, 1954) and malate

synthase (Wong and Ajl, 1957); this last enzyme participates also in the glyceric pathway. Most important is that each system is present only when the corresponding substrate is supplied (Kornberg, 1961).

Confirming Kornberg's data, table I shows the high level of the two aforementioned enzymes after growth on acetate and the inhibition of their formation if either glucose or succinate are present in addition to acetate. Growth on glycollate induces the formation of malate synthase, the change in isocitritase being comparatively much lower. (see table I).

Strain VG mentioned above (obtained from R. Lavallé) has the following properties. It does not grow on glucose as sole carbon source, but grows on succinate, glutamate, malate or fumarate or, after adaptation, on acetate or glycollate.

Since glycolysis as well as the oxidation of pyruvate are active in this strain, the mutation is supposed to be at the $C_3 + CO_2$ condensation. The most remarkable property of this strain is that growth on acetate (but not on glycollate) is completely inhibited by glucose (0.5 %). Inhibition of growth occurs also with gluconate, glycerol, pyruvate, lactate, glycine and serine. The enzymatic activities after growth on the different carbon sources are given in table II.

The inhibition of growth by glucose is due to repression of the enzymes of the glyoxylic cycle (Table I) in spite of the fact that the glucose cannot support growth. That glucose does not affect growth on glycollate agrees with the observation that activity of malate synthase is not repressed by glucose when the bacteria are grown on glycollate. Thus, the malate synthase activities which occur on acetate or glycollate are regulated in two different ways, suggesting the existence of two different malate synthases.

A mutant, VGD_5 resistant to glucose inhibition has been obtained by selection on solid media with acetate and glucose. This strain is able to grow on glucose; it is not a revertant to wild type since the

TABLE I

ENZYMATIC ACTIVITIES OF E. COLI STRAIN W 678* AFTER GROWTH ON DIFFERENT CARBON SOURCES

Source of carbon	Enzymatic activities (µM. mg protein^{-1} hour^{-1})	
	Isocitritase	Malate Synthase
Glucose	0.4	0.5
Succinate	1.1 ; 1.5	2.1
Acetate	14.5.; 17.8	17.6
Glycollate	4.8	48.0
Acetate + Glucose	1.9	3.7
Acetate + Succinate	1.7	4.6

1.- Growth conditions. Cells are grown at 36°C aerobically in the following medium : mineral minimum medium pH 7 containing the carbon source at 0,5 % final concentration and the required growth factors : per liter, DL-thréonine, 100 mg; L-leucine, 80 mg; L-histidine, 100 mg; Thiamine 200 µg.

2.- Preparation of extracts. The bacteria from 100 ml of culture are resuspended in 3 ml phosphate buffer 0,1 M at pH 7 and sonically disrupted for 10 min. in a Mullard sonic vibrator apparatus. Cell debris are discarded by centrifugation at 10.000 g for 10 min.

3.- Assay procedures (Dixon and Kornberg, 1959) (Reeves and Ajl, 1960)
Isocitritase : reaction mixture contains, in 2,5 ml, phosphate buffer at pH 7, 200 µmoles; Mg Cl_2, 15 µmoles; neutralized phenylhydrazine 10 µmoles; neutralized cysteine 6 µmoles; DL-isocitrate 10 µmoles and 0,10-0,05 ml of suitably diluted extract. The rate of formation of the glyoxylate phenylhydrazone is followed at 324 mµ in a Beckman recording spectrophotometer.
Malate synthase ; the rate of disappearance of acetyl CoA is followed at 232 mµ in the same apparatus, under the following conditions : in a final volume of 3 ml. Tris buffer pH 8,250 µmoles; $MgCl_2$, 10 µmoles; acetyl Coenzyme A, 1 µmole; Na glyoxylate, 2 µmoles; and 0,10-0,05 ml of the diluted extract.

* This strain is not the direct parent of strain VG used further; therefore, their enzymatic activities are not absolutely comparable with those given in Table II for VG or VGD_5.

utilisation of acetate by cells pre-grown on succinate does not require an adaptation and the enzymatic activities of both enzymes are high, irrespective of the carbon source and higher than in the corresponding cases in the original VG strain. (see table II).

TABLE II

ENZYMATIC ACTIVITIES OF E. COLI K 12 STRAIN VG AND VGD_5

Strain	Source of Carbon	Growth	Enzymatic activities ($\mu M.$ mg protein^{-1} hour^{-1})	
			Isocitritase	Malate Synthase
VG	Succinate	+	2.6	0.5
	Glucose	none	-	-
	Acetate	+	34.0	9.2
	Glycollate	+	6.2	23.2
	Acetate + Glucose	none	-	-
	Glycollate + Glucose	+	4.9	28.0
VGD_5	Succinate	+	38.6	14.4
	Glucose	+	60.1	26.4
	Acetate	+	65.1	18.0
	Glycollate	+	34.6	39.0
	Acetate + Glucose	+	71.0	28.0
	Succinate + Glucose	+	31.4	16.8

For conditions, see table I.

The most likely explanation of the behaviour of VGD_5 is that it has lost the regulation (repression) of the glyoxylic cycle by glucose or succinate. Presumably, this strain is a genetically derepressed mutant which has lost the ability either to produce an active cytoplasmic repressor or to form the corresponding operator (Cohen and Jacob, 1959; Jacob and Monod, 1961; Maas, 1961). This mutation leads simultaneously

to the loss of sensitivity (or most of it) to glucose as well as to succinate; the synthesis of both enzymes are derepressed in parallel. Growth on glucose is possible through the formation of pyruvate and acetate as intermediates. The activities of malate synthase under different conditions and specially after growth on glycollate deserves special mention; the activity is not only higher than in any of the other cases but is almost the sum of that obtained on acetate or succinate (approx. 16 units), and that which occurs in the parent VG after growth on glycollate.

This, again, suggests that there are two independent malate synthases, one of them corresponding to the glyoxylic cycle (derepressed at the 16 units level), the other still regulated and induced by the presence of glycollate (23 units). The occurence of these two enzymes independently regulated would be another illustration of the phenomenon first observed by Umbarger and Brown, 1957; Stadtman and al, 1961; and De Hauwer and al, 1962.

The two types of malate synthase activities, as well as the genetics of this mutation are under studies.

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