REGULATION OF SUGAR ACCUMULATION BY ESCHERICHIA COLI

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1. Introduction

Mutants of *Escherichia coli* devoid of PEP-synthase activity (pps^-) do not grow on media containing C_3-acids, such as pyruvate, lactate or alanine, as sole sources of carbon, although they grow readily on hexoses, acetate, or on utilizable intermediates of the tricarboxylic acid cycle [1–3]. However, when pyruvate is added to cultures of such pps^--mutants growing on glucose, their subsequent growth is markedly inhibited; the growth rate returns to that normally observed with pps^+-organisms when the added pyruvate has been utilized [4]. This effect of pyruvate is observed also when pps^--mutants grow on fructose, mannose, mannitol, and sorbitol; in contrast, pyruvate does not inhibit growth on glucose-6-phosphate, fructose-6-phosphate, glycerol, maltose, galactose, ribose, or gluconate [5], which indicates that pyruvate exerts its effect not by inhibiting glycolysis but by interfering with the uptake of specific hexoses from the growth media. Since the growth on glucose plus acetate of pps^--mutants, which lack a component of the pyruvate dehydrogenase complex of enzymes (pdh^-) was not inhibited by pyruvate, but was when the pdh^+-allele was re-introduced, it further appears that the inhibition of hexose utilization is caused by a product of pyruvate oxidation and not by pyruvate itself [4].

It is the purpose of this communication to report that the addition of pyruvate inhibits the accumulation of specific sugars by *E. coli*, and to suggest that the inhibitory agent is acetyl-coenzyme A.

2. Experimental procedures

The organisms used are listed in table 1. Cultures were grown aerobically at 37° on synthetic media [6] containing the appropriate carbon source at 25 mM concentration. Cells were harvested in the middle log phase of growth (at about 0.4 mg dry wt/ml) by centrifugation at room temperature, and were washed with, and resuspended in, basal medium without carbon source at 37°. The suspension (2 ml, containing about 0.7 mg dry wt of cells/ml) was shaken at 37° in a reciprocating water bath for 15 min with pyruvate or acetate, or without addition, as indicated in the Results section of this paper. Radioactive substrates

Table 1
Organisms used in this study

Strain	Enzymic dysfunction	Reference
K1	none	[3]
K1-1	pps^-	[3]
K1-1/A10	pps^-, pdh^-	*
K2	none	[3]
K2-1-4	pps^-, $gltA^-$	[5]
R4-1-11	pps^-, pta^-	[5]

* K1-1/A10 is a transductant of K1-1 devoid of lipoyl reductase transacetylase activity; it was prepared and given to us by Professor U.Henning (Tübingen).

The abbreviations used indicate absence of pps^- = PEP-synthase, pdh^- = pyruvate dehydrogenase, $gltA^-$ = citrate synthase, pta^- = phosphotransacetylase.

were now added; samples (0.1 ml) were withdrawn at known times and pipetted directly onto "Millipore" membrane filters (0.45 μ pore size) to which suction was constantly applied from a vacuum line. The filters were washed with 5 ml of basal medium at 37° and were rapidly transferred to vials, containing 5 ml of Bray's fluid [7], for radioactive assay in a Packard Model 4000 scintillation spectrometer.

The accumulation of radioactive materials is expressed as the ratio of isotope/ml of cell water to the isotope/ml of external medium at that time; it is assumed that 1 mg dry wt of cells contains 4 μl of cell water.

3. Results and discussion

As shown in fig. 1, glucose-grown *E. coli* rapidly take up [U-^{14}C]glucose when this labelled substrate is added to a suspension of the cells in basal medium.

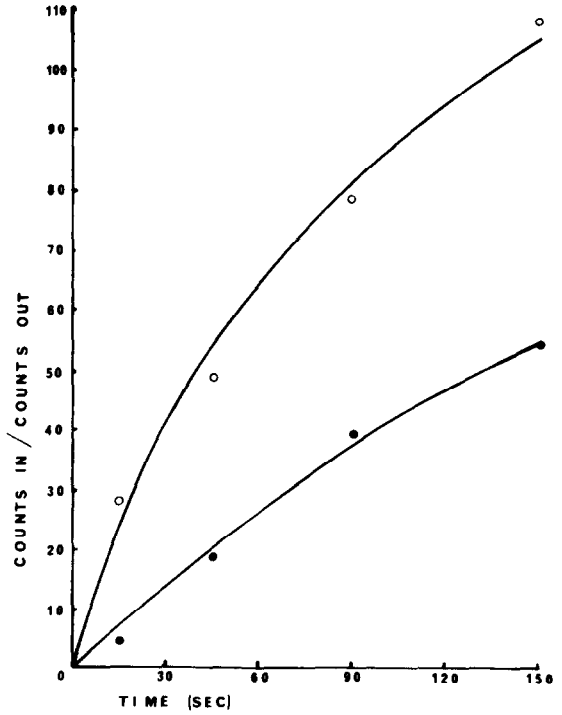

Fig. 1. Accumulation of [U-^{14}C]glucose by glucose-grown K1. Washed suspensions of the organism were shaken aerobically at 37° for 15 min alone (○) and with 10 mM pyruvate (●) before addition of 150 μM [U-^{14}C]glucose.

In the presence of pyruvate, this rapid apparent rate of glucose accumulation is considerably reduced: for example after 150 sec, only half as much isotope was accumulated by the cells as in the absence of pyruvate. As expected from the known effects of pyruvate on the growth of pps$^-$-mutants [5] (see Introduction), this inhibitory effect was not observed with gluconate-grown cells taking up sodium [U-^{14}C]gluconate; neither pyruvate nor glucose affected the rate of gluconate uptake (fig. 2). Although these experiments show that the processes of glucose and of gluconate uptake operate independently of each other, and that pyruvate inhibits the former but not the latter, it is difficult to quantitate the data obtained since the substrates taken up are both incorporated into cell materials and catabolized. This objection does not apply with equal force if non-catabolizable analogues of glucose are used instead of glucose.

As shown in fig. 3, the presence of pyruvate strongly inhibits the uptake of α-methyl [^{14}C]glucoside by acetate-grown cells; significantly, acetate

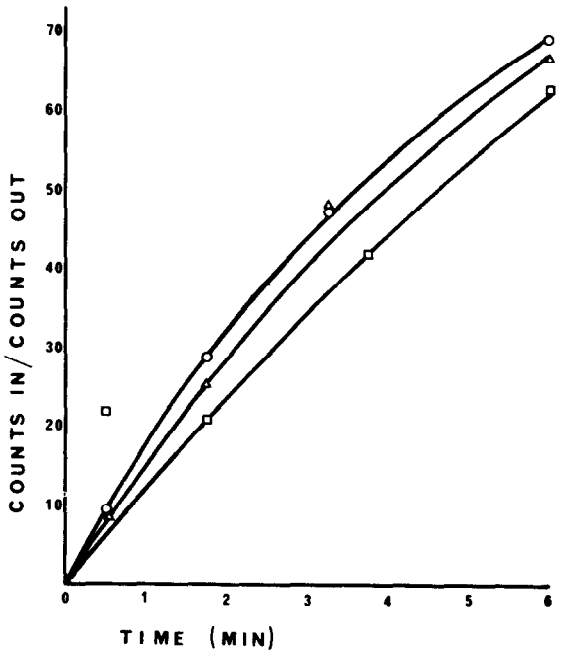

Fig. 2. Accumulation of sodium [U-^{14}C]gluconate by gluconate-grown K1-1. Washed suspensions of the organism were shaken aerobically at 37° for 15 min alone (○), with 10 mM pyruvate (△), and with 10 mM glucose (□) before the addition of 150 μM sodium [U-^{14}C]gluconate.

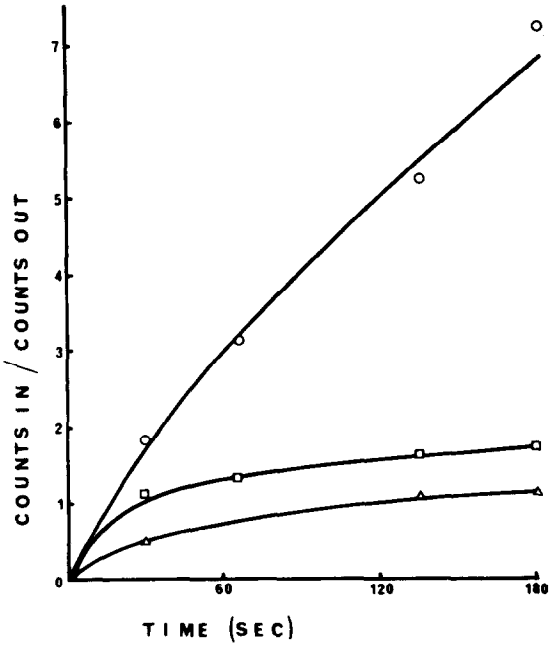

Fig. 3. Accumulation of α-methyl-[U-^{14}C]glucoside by acetate-grown K1-1. Washed suspensions of the organism were shaken aerobically at 37° for 15 min alone (○), with 10 mM pyruvate (△), and with 10 mM acetate (□), before the addition of 61 μM α-methyl[U-^{14}C]glucoside.

exerts a similar inhibitory effect. This not only confirms that α-methylglucoside uptake is similar to that of glucose [8] but suggests that the inhibitory action of pyruvate may be due to the acetyl-coenzyme A formed from it.

The accumulation of 2-deoxy-D-[U-^{14}C]glucose (2-DG), like that of α-methyl-[U-^{14}C]glucoside, is also markedly inhibited in the presence of pyruvate. As shown in table 2, pre-incubation of wild-type cells with 1 mM pyruvate lowered the rate of isotope accumulation by 36%, and 10 mM pyruvate lowered it by 70%. The suggestion that it is acetyl-coenzyme A rather than pyruvate itself that is the inhibitory agent is borne out by experiments with mutants affected in enzymes of acetyl-coenzyme A metabolism. Thus, the rate of accumulation of 2-DG by mutants devoid of the ability to form acetyl-coenzyme A from pyruvate (pdh$^-$) is no longer affected by the presence of pyruvate. On the other hand, mutants that lack citrate synthase activity and that are in consequence unable

Table 2
Effect of pyruvate on the accumulation of 2-deoxy-D-[U-^{14}C] glucose (2-DG) by *Escherichia coli* suspensions.

Enzymic dysfunction	Pyruvate concn. added (mM)	2-DG accumulated in the presence of pyruvate / 2-DG accumulated in the absence of pyruvate
none	10	0.30
none	1	0.64
pyruvate dehydrogenase$^-$	1	0.91
citrate synthase$^-$	1	0.23
phosphotransacetylase$^-$	1	0.50

Washed suspensions of the organisms were shaken aerobically at 37° for 15 min with or without pyruvate before addition of 44 μM 2-DG; the incubations were terminated 30 sec thereafter. For experimental conditions, see text.

to remove rapidly any acetyl-coenzyme A formed from pyruvate, are more sensitive to the presence of 1 mM pyruvate than are wild-type organisms to the presence of 10 times this concentration. The possibility that the inhibitor of 2-DG uptake is acetyl phosphate rather than acetyl-coenzyme A is excluded by the observation (table 2) that mutants devoid of phosphotransacetylase activity are still sensitive to the presence of pyruvate.

The inhibition of the uptake of specific hexoses by *E. coli*, mediated through a product of pyruvate oxidation, appears to be analogous to the effect of acetate on sugar utilization by *Aspergillus nidulans* [9]. This suggests that organisms that take up sugars against concentration gradients may, in part, regulate such processes through end-products of hexose catabolism.

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