Biochem. J. (1975) **150**, 123–128
Printed in Great Britain

Utilization of Gluconate by *Escherichia coli*

A ROLE OF ADENOSINE 3′:5′-CYCLIC MONOPHOSPHATE IN THE INDUCTION OF GLUCONATE CATABOLISM

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(*Received* 17 *March* 1975)

1. Cultures of *Escherichia coli* growing on gluconate use both gluconate and glucose when glucose is added. 2. Glycerol-grown cells adapt to gluconate utilization even in media containing glucose as well as gluconate. 3. The rates of gluconate utilization by cells growing on a mixture of glucose and gluconate, and the specific activities of the gluconate uptake system and of gluconate kinase, are greater if adenosine 3′:5′-cyclic monophosphate (cyclic AMP) is present in the medium than in its absence. 4. Growth on media containing gluconate and cyclic AMP is accompanied by the formation of methyl glyoxal and pyruvate, and progressive inhibition of growth. 5. A mutant devoid of adenylate cyclase activity (*cya*) grew well on glucose in the absence of exogenous cyclic AMP but grew only poorly on gluconate; neither the gluconate uptake system nor gluconate kinase was adequately induced. The addition of cyclic AMP promoted growth on gluconate and facilitated the induction of proteins required for gluconate catabolism. 6. Phage P1-mediated transduction of *cya*$^+$ into the *cya*-mutant also restored the wild-type phenotype in its ability to adapt to gluconate utilization.

When cultures of *Escherichia coli* are placed in growth media containing both glucose and any one of a wide range of carbohydrates, glucose is used preferentially and the proteins required for the uptake and catabolism of the other carbohydrate are inducibly formed only after the glucose has been virtually used up. This phenomenon, originally termed the 'glucose effect' (Monod, 1942), is made up of at least two components (Lengeler, 1966). By 'catabolite inhibition' (McGinnis & Paigen, 1969), glucose inhibits the uptake of other carbohydrates; by 'catabolite repression' (Magasanik, 1961), glucose (or some product of glucose metabolism) represses the synthesis of enzymes that would normally be induced by those other carbohydrates. It has been established that, for a number of inducible enzymes, the repressive effect of glucose is associated with a lowering of the intracellular concentrations of adenosine 3′:5′-cyclic monophosphate (cyclic AMP), and that the addition of cyclic AMP to the bacterial cultures permits induction of these enzymes to occur even in the presence of glucose (Ullman & Monod, 1968; Pastan & Perlman, 1970, 1971). However, it has also been reported that gluconate exerts a degree of catabolite repression on the synthesis of β-galactosidase even greater than does glucose (Okinaka & Dobrogosz, 1967), and that the repression produced by a combination of gluconate and glucose is so severe that external cyclic AMP is not able to reverse it (Goldenbaum & Dobrogosz, 1968). This not only indicates that gluconate can be utilized by *E. coli* simultaneously with glucose, but might be taken to imply that cyclic AMP does not play any major role in the catabolism of gluconate.

It is the main purpose of this paper to show that cyclic AMP is involved in the induction of gluconate utilization by *E. coli*.

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Experimental

Organisms used

All the organisms used were derivatives of the K12 strain of *E. coli*. Strain WZ 22 (F$^-$, *metB cya*855 *strA*) was the generous gift of Dr. Michael Yudkin (Department of Biochemistry, University of Oxford). *Cya*$^+$-transductants were obtained by infecting a culture of strain WZ 22 with phage P1, propagated on wild-type *E. coli*, and selecting colonies that grew rapidly on media containing lactose in the absence of cyclic AMP; one such colony, purified by repeated isolation of single colonies, was designated BB 130 (F$^-$, *metB strA*) and used in this work. The strain K2 (F$^-$, *his*

argH thr leu trp str) (Brice & Kornberg, 1967) was used as a'wild-type' strain.

Growth of cells

Cultures were grown aerobically at 37°C on defined media containing salts (Ashworth & Kornberg, 1966) and a carbon source at 10mM unless otherwise stated; where necessary, the media were supplemented with L-amino acids as required at 40–100μg/ml. Cyclic AMP, sterilized by filtration through 'Millipore' filters, was added where indicated at a final concentration of 5mM.

Cell growth was measured as the absorbance of cell suspensions at 680nm; the absorbance, measured with a Unicam SP.600 spectrophotometer in 3ml cells (1cm light-path), multiplied by 0.68 was taken to equal mg dry mass/ml (Ashworth & Kornberg, 1966; Kornberg & Reeves, 1972).

Assay of gluconate uptake

Cells, harvested from the growing cultures by centrifugation at 20000*g* for 5min at 20°C were washed with N-free basal-salts medium (Ashworth & Kornberg, 1966) and were resuspended in 2ml of this solution at 0.2mg dry mass/ml; this suspension was shaken at 25°C. The uptake reaction was started by the addition of sodium [U-^{14}C]gluconate (3.4Ci/mol) to a final concentration of 0.05mM. At appropriate times, from 10 to 70s after the addition of the labelled substrate, samples (0.5ml) were withdrawn, diluted into 2.5ml of 50mM-potassium phosphate buffer, pH7.0, at room temperature, filtered rapidly with suction through HA 'Millipore' filters (0.45μm pore size) and washed again with 2.5ml of the same buffer. The filters were dissolved in 5ml of Bray's (1960) fluid; the radioactivity of the resultant solutions was assayed with a Packard model 3385 liquid-scintillation spectrometer. The rates of gluconate uptake were expressed as nmol of labelled material retained/min per mg dry mass of cells.

Assay of gluconate kinase

Cells were harvested and disrupted by sonic oscillation, and the gluconate kinase activity of the cell extract was measured, as described by Pouysségur & Stoeber (1972). Enzymic activity is expressed as nmol of 6-phosphogluconate formed from ATP and gluconate/min per mg of protein under the conditions of the assay procedure.

Measurement of utilization of ^{14}C-labelled substrates

For the experiments of Figs. 1 and 2, sufficient sodium [U-^{14}C]gluconate or [U-^{14}C]glucose was added to cultures growing on media containing both 5mM-glucose and 5mM-sodium gluconate to give a final isotopic concentration of 0.2μCi/ml. The incorporation of the labelled substrates by the growing cells was measured as described by Kornberg & Soutar (1973).

Assay of oxo compounds

Oxo compounds were identified and assayed as pyruvate and methyl glyoxal by measurements of the spectra of their 2,4-dinitrophenylhydrazones, as described by Cooper & Anderson (1970).

Chemicals used

^{14}C-labelled glucose and sodium gluconate were purchased from The Radiochemical Centre, Amersham, Bucks., U.K. and cyclic AMP from Boehringer Corp. (London) Ltd., London W5, U.K. Other reagents were of the highest quality readily available commercially.

Results and Discussion

Effect of glucose on gluconate utilization

When glucose is added to cultures of *E. coli* strain K2, or of mutants derived from strain K2, that are growing on sugars such as fructose, lactose, or galactose, the continued growth of the organisms occurs predominantly at the expense of the added glucose (Kornberg, 1973). This does not appear to happen when gluconate is the carbon source: the addition of glucose to cultures of strain K2 that are growing on gluconate does not result in the overwhelming shift toward the utilization of glucose that was noted with other carbohydrates and, in the steady state, about twice as much carbon is derived from gluconate as is derived from glucose by the growing culture (Fig. 1*a*). It thus appears that glucose does not exert 'catabolite inhibition' over the continued entry of gluconate into the cells. However, the rate of gluconate utilization was initially more rapid than at later stages of growth: this could imply a progressive repression of some enzyme(s) of gluconate catabolism since, in the K2 strain of *E. coli*, the uptake of glucose does not require to be induced (Kornberg & Reeves, 1972).

When cultures of strain K2 were grown on glycerol overnight and were then transferred to flasks containing equimolar mixtures of glucose and sodium gluconate as carbon sources, the enzymes of gluconate catabolism were induced, and effected the uptake and metabolic utilization of gluconate, despite the presence of glucose in the culture media (Fig. 2*a*). It thus appeared as if glucose failed also to prevent the induction of the enzymes for gluconate catabolism and, hence, as if this metabolic route was not subject to the 'glucose effect'. However, analysis of the specific activity of the gluconate kinase in the cells, grown in the presence of glucose and gluconate, showed that this immunity from catabolite repression was more apparent than real. Whereas extracts of cells, grown for one and two doublings on gluconate as sole

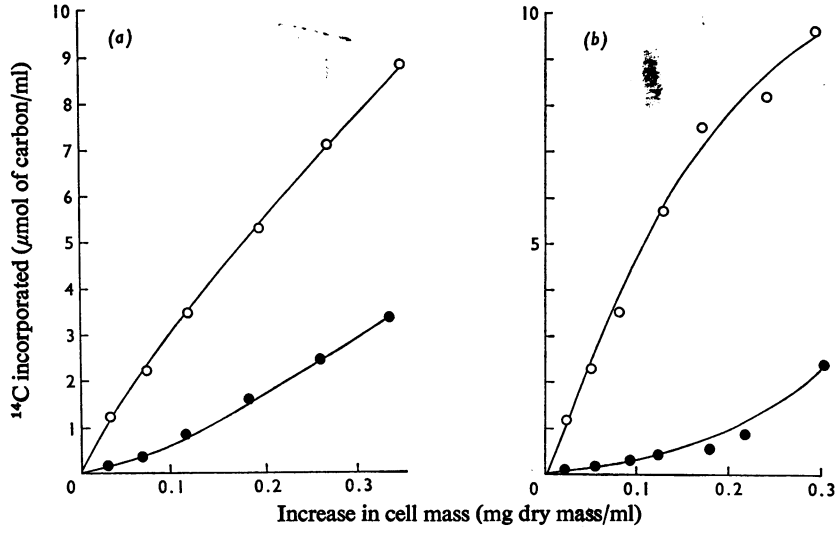

Fig. 1. *Utilization of glucose* (●) *and gluconate* (○) *by gluconate-grown E. coli growing on a mixture of these two substrates*

(*a*) Gluconate-grown cultures of strain K2 were placed in duplicate flasks containing 5mM-glucose and 5mM-sodium gluconate as carbon source for growth. To one of these flasks, [U-^{14}C]glucose was added; the other received sodium [U-^{14}C]-gluconate. (*b*) Conditions were identical with those of (*a*) except that the growth flasks also contained 5mM-cyclic AMP. The incorporation of labelled carbon was measured as described in the Experimental section, and is plotted against Δ cell mass.

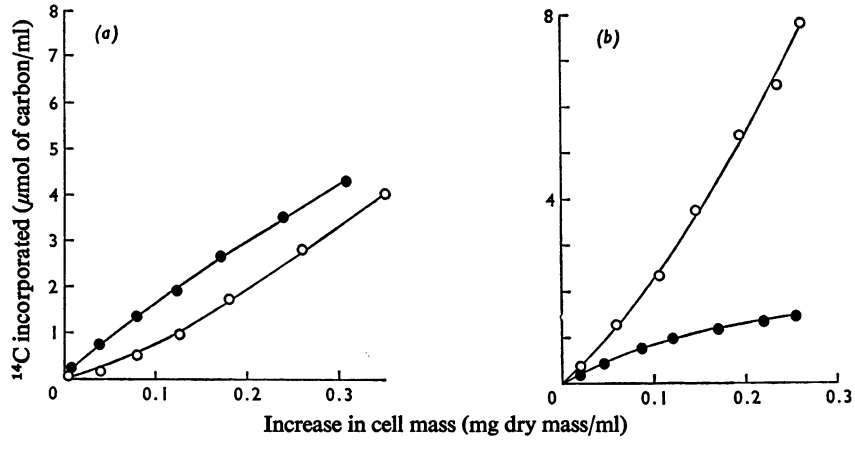

Fig. 2. *Utilization of glucose* (●) *and gluconate* (○) *by glycerol-grown E. coli growing on a mixture of these two substrates*

Glycerol-grown cultures of strain K2 were placed in duplicate flasks containing 5mM-glucose and 5mM-gluconate, with and without cyclic AMP, as described in the legend to Fig. 1.

carbon source, contained gluconate kinase activity at 148 and 214 nmol/min per mg of protein respectively, extracts of cells grown on glucose plus gluconate contained this enzyme at specific activities of only 11 after one, and 93 after two, doublings (Table 1). The presence of glucose also retarded the induction of the gluconate uptake system, and repressed its synthesis after two generations, to about the same extent as it affected the formation of gluconate kinase.

These results suggested that glucose, or some metabolite derived from it, interferes with the induction of the enzymes of gluconate catabolism. If this inter-

Table 1. *Formation of gluconate uptake and gluconate kinase activities by cultures of E. coli strain K2 during growth on different carbon sources*

Inocula were grown on 20mM-glycerol overnight and were then left to grow for one and two doublings on the carbon sources indicated. The gluconate uptake by washed cells harvested at these times, and the gluconate kinase activity in extracts of such cells, were measured as described in the Experimental section.

Carbon source (mM)	Gluconate uptake after:		Gluconate kinase activity after:	
	One doubling	Two doublings	One doubling	Two doublings
Glucose (10)	0.2	0.6	0.1	6.1
Gluconate (10)	11	11	148	214
Glucose (10)+gluconate (10)	0.9	4	11	93
Glucose (10)+gluconate+cyclic AMP (5)	6	11*	109	238*
Gluconate (10)+cyclic AMP (5)	11	9*	199	209*

* The growth of these cultures slowed progressively and virtually ceased after two doublings: this was accompanied by the accumulation of methyl glyoxal in cells and media.

ference were associated with the lowering of the intracellular concentration of cyclic AMP, which is known to be a consequence of glucose utilization (Makman & Sutherland, 1965; Buettner *et al.*, 1973) it would be expected that the provision of cyclic AMP in the media would overcome it. Such was indeed found to be the case. As shown in Fig. 1(*b*), the presence of cyclic AMP in the medium prevented the rapid decrease in the rate of gluconate utilization noted in its absence, and kept the proportion of cellular carbon derived from glucose down, for at least two doublings. Similarly, as shown in Fig. 2(*b*), approximately three times as much gluconate was incorporated by glycerol-grown cells placed into media containing gluconate, glucose, and cyclic AMP as was incorporated by cells growing in this medium but lacking cyclic AMP; conversely, the utilization of glucose was initially less and progressively declined. Clearly, glucose is not a preferred carbon source under these conditions, and, in the presence of cyclic AMP, no longer interfered with the induction of the components required for gluconate utilization. This conclusion is borne out by the analyses shown in Table 1: cells grown on the mixture of glucose and gluconate in the presence of cyclic AMP exhibited both the gluconate uptake and the gluconate kinase activities shown by cells grown on gluconate alone, or on gluconate in the presence of cyclic AMP.

One consequence of the addition of 5mM-cyclic AMP to cultures growing on gluconate is that, after the first doubling, growth progressively slows down and virtually ceases after two generations. This inhibition of growth is accompanied by the appearance of large quantities of oxo compounds in the cultures: after two doublings on gluconate plus cyclic AMP, 0.23mM-methyl glyoxal and 1.7mM-pyruvate was present in the samples of cells plus medium analysed, and after two doublings on the mixture of glucose, gluconate and cyclic AMP, 0.32mM and 2.6mM respectively of these materials were measured by their reaction with 2,4-dinitrophenylhydrazine. Similar accumulations of methyl glyoxal and growth stasis have been observed after addition of cyclic AMP to cells growing on hexose phosphates, on xylose and on arabinose (Ackerman *et al.*, 1974); methyl glyoxal has been reported also to be formed after the addition of gluconate to a mutant constitutive for the enzymes of gluconate catabolism (Rekarte *et al.*, 1973).

Role of cyclic AMP in the induction of gluconate utilization

The interference by glucose with the induction of the components required for gluconate catabolism, and its reversal by cyclic AMP, suggested that cyclic AMP played a role in this induction. This was tested directly with a mutant WZ 22 of *E. coli* devoid of adenylate cyclase activity; for comparison, similar experiments were carried out with a *cya*$^+$-transductant BB 130 derived from WZ 22 and hence (apart from those genes transduced into WZ 22 together with *cya*$^+$ by the P1-phage) isogenic with it.

When cultures of the *cya*-mutant, grown on 10mM-lactose plus 5mM-cyclic AMP, were transferred to medium containing gluconate as sole carbon source, the adaptation to growth on this new substrate was slow and, even after exponential growth was achieved, the doubling time was well over 5h (Fig. 3*a*). In contrast, the mutant grew on glucose with a doubling time of under 2h. That this reluctance to use gluconate was associated with the adenylate cyclase lesion of the mutant, and hence with its inability to achieve satisfactory cellular concentrations of cyclic AMP, was shown in two ways. In the first place, transduction of the wild-type *cya*$^+$-gene into strain WZ 22 yielded strain BB 130: this strain adapted rapidly to growth on gluconate and its doubling time on that substrate was 1.4h (Fig. 3*b*). Secondly, the addition of 5mM-cyclic

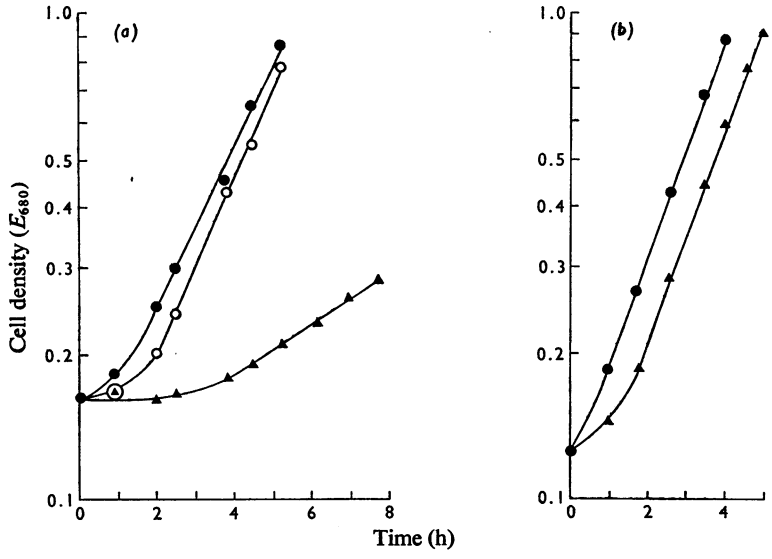

Fig. 3. *Growth of a cya-mutant of E. coli (a) and its cya⁺-transductant (b)*

(*a*) A culture of the *cya*-mutant WZ 22, grown on 10mM-lactose +5mM-cyclic AMP overnight, was placed in growth media containing 10mM-glucose (●), 10mM-gluconate (▲) or 10mM-gluconate plus 5mM-cyclic AMP (○). Growth was measured at 37°C. (*b*) A similar culture of the *cya*⁺-transductant BB 130 was placed in growth media containing 10mM-gluconate (▲) or 10mM-gluconate +5mM-cyclic AMP (●). Growth was measured as above.

Table 2. *Specific activities of gluconate uptake and gluconate kinase after growth on different carbon sources*

Cells were grown on 10mM-lactose plus 5mM-cyclic AMP overnight, harvested and left to grow for two doublings on the carbon sources indicated. The uptake of gluconate by washed cells, and the gluconate kinase activity of cell extracts, were measured as described in the Experimental section.

Strain	Carbon source	Gluconate uptake	Gluconate kinase
WZ 22 (*cya*)	Glucose	2.7	16
	Gluconate	3.1	21
	Gluconate +cyclic AMP	20	600
BB 130 (*cya*⁺)	Glucose	1.7	11
	Gluconate	11	420

AMP to cultures of strain WZ 22 also shortened greatly the time required to adapt to growth on gluconate, and decreased the doubling time on that substrate to 1.7h (Fig. 3*a*).

Analysis of the uptake of gluconate by these cells, and of the gluconate kinase activity of extracts derived from them, confirmed this conclusion (Table 2). After growth on gluconate, the *cya*⁺-transductant BB 130 was induced for gluconate uptake and gluconate kinase to the same extent as had been found with wild-type *E. coli* (Table 1). In contrast, the gluconate-grown *cya*-mutant exhibited little activity of gluconate uptake, and extracts contained gluconate kinase in only slightly greater activity than that present in glucose-grown (uninduced) cells. But growth on gluconate in the presence of cyclic AMP elevated both these activities above those observed with wild-type cells grown on gluconate.

The results presented in the present paper explain the ability of gluconate to augment the catabolite repression exerted by glucose. Unlike that of many other carbohydrates, the uptake of gluconate by *E. coli* is not subject to catabolite inhibition (McGinnis & Paigen, 1969). Gluconate can thus enter cells previously induced for the enzymes of gluconate catabolism (Fig. 1), and can induce previously uninduced cells (Fig. 2), even though glucose is present. However, the apparent immunity from catabolite inhibition does not confer immunity from catabolite repression: the rates of gluconate utilization by cells growing in the presence of glucose, and the specific activities of components of the gluconate catabolic route, are greatly increased by the provision of cyclic AMP in the medium. The inability of the *cya*-mutant WZ 22 to induce adequately these components even when exposed for several hours to gluconate as sole carbon source, and the abolition of this difficulty either by transducing into WZ 22 the *cya*⁺-gene or by the addition of cyclic AMP, support the view that cyclic AMP plays a necessary role in the induction of the components of gluconate catabolism.

We thank Dr. Maurice Jones-Mortimer for many helpful discussions, Dr. Michael Yudkin for the gift of the *E. coli* mutant WZ 22, and the Science Research Council for support under Grant B/SR/72462. B. B. thanks the Schweizerischer Nationalfonds for a Fellowship, held during part of this work.

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