Glucose Transport as Rate-Limiting Step in the Growth of *Escherichia coli* on Glucose

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Over a wide range of growth rates, two strains of *Escherichia coli* growing aerobically in continuous culture under glucose limitation utilized glucose at rates identical with those at which cells harvested from the chemostats transported [^{14}C]glucose.

Two types of observation suggest that the rates at which *Escherichia coli* grows on a variety of carbohydrates are limited by the rates at which these substrates are taken up by the cells. In one such observation, the rate at which a suspension of lactose-grown *E. coli* hydrolysed *o*-nitrophenyl galactoside was compared with that at which the same cells, rendered permeable with toluene, hydrolysed this lactose analogue; the former rate was found to be only about one-tenth of the latter (Rickenberg *et al.*, 1956). In a second type of experiment, mutants were used that could take up galactose (Horecker *et al.*, 1960) or gluconate (Pouysségur *et al.*, 1974), but could not metabolize these substances. The initial rates at which these carbohydrates were taken up by these mutants were identical with those at which they were taken up by the parent organisms, but, whereas the carbohydrates accumulated to considerable extents in the former, no such accumulation was detected in the latter. It follows that the overall rates of metabolism could not be less than those of the initial uptake steps.

All the carbohydrates used for these studies enter *E. coli* by active transport. Glucose, on the other hand, is taken up by group translocation, in which the phosphoenolpyruvate-dependent phosphotransferase system (Kundig *et al.*, 1964) plays an essential role. Kornberg & Reeves (1972*a*) showed that the rates at which the glucose analogue methyl α -D-glucoside was taken up by two strains of *E. coli* were closely similar to the rates at which this analogue was phosphorylated by the phosphotransferase system, measured with suspensions of these cells rendered permeable with toluene. Since methyl α -D-glucoside is phosphorylated, but not catabolized, this equivalence could be used to calculate, from measurements of phosphotransferase activity *in situ*, the true rates at which glucose and fructose enter *E. coli* under a variety of conditions; direct measurements of transport of [^{14}C]glucose and [^{14}C]fructose would be

in error, since an unknown portion of the labelled material taken up would be lost as $^{14}\text{CO}_2$. Such measurements showed that the K2.1t strain of *E. coli* was always rich in glucose phosphotransferase activity, and indicated that this activity was sufficient to account for the observed rate of growth of this organism on glucose (Kornberg & Reeves, 1972*b*); however, they also showed that cultures of another strain, B11, formed glucose phosphotransferase activity in significant amounts only after growth on glucose and that, even in cells thus grown, the activity was insufficient to account for the observed growth rate.

We here report experiments with these two strains, grown in continuous culture on glucose as limiting nutrient, which resolve this discrepancy and which show that, in either strain of *E. coli*, the overall rates of glucose utilization and those of glucose uptake are identical; above a threshold value of the dilution rate, these activities are inversely related to the doubling time.

Materials and Methods

Cultures of *E. coli* strains K2.1t (Brice & Kornberg, 1967) and B11 (Broda, 1967) were grown with vigorous aeration at 35°C in a 'Porton-type' chemostat of working volume 500 ml (Herbert *et al.*, 1965). The basal medium contained 20 mM- NaH_2PO_4 , 120 mM- NH_4Cl , 10 mM- K_2SO_4 , 1 mM-citric acid, Mg^{2+} and other trace elements as in Table 1 of Evans *et al.* (1970), and glucose at 5 g/litre. It was supplemented, for growth of strain B 11, with 0.85 mM-L-methionine, and for strain K2.1t with 0.5 mM-L-histidine, 1.5 mM-L-leucine and -arginine, and 2 mM-L-threonine. Cultures were maintained at $\text{pH } 7.0 \pm 0.05$ by the automatic pH-control system described by Evans *et al.* (1970). These media sufficed to maintain the densities of the cultures at about 2.5 mg dry mass/ml, over a wide range of flow rate. Growth was definitely glucose-limited, since samples removed from the growth vessels over a period of 3 weeks contained an excess of required amino acids, but no residual glucose

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was detected. This made it possible to measure the rates at which [^{14}C]glucose was taken up, and the rates at which this carbohydrate was removed from solutions thereof, without washing the cells. Preliminary experiments showed that washing, which is necessary with batch cultures containing excess of glucose, greatly decreased these rates. Samples (0.2–0.4 ml) of the growing cells were therefore taken from the growth vessels at intervals and diluted 5–10-fold at 35°C with a mixture of 75 mM- $\text{NaH}_2\text{PO}_4/\text{K}_2\text{HPO}_4$, pH 7.2, and chloramphenicol (100 $\mu\text{g}/\text{ml}$); 2 mM- $[\text{C}^{14}]$ glucose was added at zero time to give a final concentration of 0.1 mM. For measurement of glucose utilization, samples (0.5 ml) were filtered rapidly with syringes fitted with Millipore filters (1 cm diam., 0.20 μm pore size) into clean dry tubes; 0.1 ml of these filtrates was acidified with acetic acid, evaporated to dryness at 115°C and the residue was dissolved in Bray's (1960) scintillation fluid for assay of radioactivity. For measurement of glucose uptake, samples (0.2 ml) were filtered with suction through Millipore filters (2.5 cm diam., 0.45 μm pore size), washed at 35°C with 2 $\times$ 5 ml of 50 mM- $\text{NaH}_2\text{PO}_4/\text{K}_2\text{HPO}_4$, pH 7.2, and dissolved in 5 ml of Bray's (1960) scintillation fluid. Radioactivity was measured with a Philips Automatic Liquid-Scintillation Analyser, model 4510; radioactivities of all samples were counted for at least a total of 5000 counts and were corrected for background.

The difference in the ability of strains B11 and K2.1t to take up methyl α -D- $[\text{C}^{14}]$ glucoside from agar plates supplemented with this analogue at 10 μM was checked at intervals by the film technique described by Kornberg & Smith (1972). Under these conditions, all samples of strain K2.1t, taken from the chemostat and plated out, took up the labelled substrate to a much greater extent than did samples of strain B11, taken and plated out in this manner.

Discussion

The rate of uptake of [^{14}C]glucose by cell suspensions, taken from chemostat growth vessels and used soon thereafter, was constant over about 4 min (Fig. 1, open symbols). Although the lines, extrapolated to zero time, did not pass through the origin, there appeared to be no accurately identifiable discontinuity in rate between the last points, taken when the pools filled by [^{14}C]glucose must have been virtually saturated, and even the earliest sample. It must therefore be assumed that at no time was uptake itself being measured.

This difficulty cannot be overcome simply by washing the filtered cells at 0°C instead of at 35°C, in order to rapidly slow down metabolism. Not only might such a procedure lead to losses of material through cold shock (MacLeod & Calcott, 1976), but it would be ineffective. Samples filtered for measurements of

sugar uptake usually contain 0.05–0.1 mg dry mass of cells, which contain approx. 0.15–0.3 μl of cell water (Winkler & Wilson, 1966). Even if the uptake of 0.1 mM-glucose were to yield a glucose 6-phosphate concentration in the cell water tenfold higher than this, cells on a filter, utilizing glucose at 4 $\mu\text{mol}/\text{h}$ per mg dry mass (Fig. 2), would catabolize this quantity of glucose 6-phosphate in 1.5–3 s; if, as is likely, no such accumulation of glucose 6-phosphate occurs, these times would be of the order of 0.15–0.3 s. We found it impossible to filter cells in less than 3 s. Cooling of the filtered samples, by washing the cells at 0°C, would thus lower metabolism too late and with insufficient rapidity to enable any reliable estimates of the uptake process, divorced from catabolism, to be made.

The conclusion that the labelled material retained in the filtered cells at all times represented the difference between the glucose that had entered the cells and the glucose that had been lost, by oxidation to CO_2 and water, was borne out by measurements of the rates at which 0.1 mM- $[\text{C}^{14}]$ glucose was removed by the cells from the incubation medium. When plotted to permit direct comparison (Fig. 1, closed symbols), it is seen that, for every mol of glucose removed from the medium (by oxidation and incorporation into cell material), only 0.55–0.6 mol of glucose was measured as being taken up by the cells. The conventional determinations of carbohydrate transport, by measurement of radioisotope incorporation into cell suspensions, thus underestimate the 'true' rates by a factor of nearly 2. Since the glucose phosphotransferase activities of cells taken from continuous culture and rendered permeable by treatment with toluene (Kornberg & Reeves, 1972b) are nearly

Fig. 1. Uptake (open symbols) of glucose, and removal of glucose from 0.1 ml solutions (closed symbols), by *E. coli* *E. coli* strain B11 was grown at $D = 0.1 \text{ h}^{-1}$ (Δ , $\blacktriangle$) and $D = 0.4 \text{ h}^{-1}$ (∇ , $\blacktriangledown$), and strain K2.1t at $D = 0.1 \text{ h}^{-1}$ ($\circ$, $\bullet$) and $D = 0.4 \text{ h}^{-1}$ ($\square$, $\blacksquare$). For ease of comparison, all rates are plotted as nmol taken up or removed/unit mass of cells per 0.1 ml of incubation mixture, containing [^{14}C]glucose at 0.1 mM.

Fig. 2. Relationship of glucose uptake and overall utilization of glucose to the exponential growth rate at which *E. coli* strains B11 (Δ) and K2.1t ($\circ$) grow in continuous culture with glucose as limiting nutrient

The dilution rate is equal to the exponential growth rate, μ .

double the rates at which intact cells take up [14 C]-glucose (J. W. Keyte & H. L. Kornberg, unpublished work), there is evidence that the rates of glucose utilization (Fig. 1, closed symbols) are a measure also of glucose phosphotransferase activity *in vivo*.

Fig. 1 also shows that the rate of glucose utilization by cells harvested at an exponential growth rate (μ) of 0.4 h^{-1} is more than twice that of cells harvested at $\mu = 0.1 \text{ h}^{-1}$; the glucose phosphotransferase activity is thus intimately related to the rate at which cells utilize glucose for growth, under conditions where the glucose content of the media in the chemostat vessels is very low.

This relationship between growth rate and glucose phosphotransferase activity is emphasized by the data of Fig. 2. The symbols indicate the measured rates of [14 C]glucose utilization by suspensions of cells harvested at various exponential growth rates; the broken line is the calculated rate of glucose utilization needed to maintain cells at $2.5 \text{ mg dry mass/ml}$ at these various growth rates. This line was derived from the relationship

$$q_s = \frac{D(S_0 - S)}{x} \quad (1)$$

where q_s = amount of glucose utilized/h per mg dry mass of cells, D = dilution rate (the ratio of flow rate to culture volume), S_0 = glucose concentration of medium entering the culture vessel (5 mg/ml in

these experiments), S = glucose concentration of medium in the vessel (vanishingly small and taken to be zero) and x = density of cells in the vessel (measured at each D , but very close to $2.5 \text{ mg dry mass/ml}$ at all times). At any constant dilution rate, the organisms in the chemostat will grow up until the limiting nutrient is exhausted; the maximal growth rate, μ , under these conditions will thus also be constant and will be equal to the rate of flow of medium, D .

At values of D (and hence of μ) > 0.2 , there is a striking correlation between the rates at which glucose must be used to sustain the growth of the cells, calculated from eqn. (1), and the rates of glucose utilization by cells harvested at various values of μ and measured independently; these latter determinations are also measures of glucose transport and of glucose phosphotransferase activity at these points. The equivalence thus demonstrated, and the nearly threefold increase in the latter activity as μ rises from 0.2 to 0.5 , supports the view that it is the glucose phosphotransferase activity that sets the pace of overall glucose utilization.

The data of Figs. 1 and 2 reveal a further remarkable phenomenon. Whereas cultures of strain B11, grown in batch culture, contain much less glucose phosphotransferase activity than do similar cultures of strain K2.1t (Kornberg & Reeves, 1972*b*), this difference is not seen with cultures drawn from chemostat vessels; however, samples of these cells grown on nutrient agar overnight again showed it. It is thus likely that the low glucose phosphotransferase activity reported for batch cultures of strain B11 is indicative of an unusual sensitivity of the appropriate (Kornberg & Jones-Mortimer, 1975; Curtis & Epstein, 1975) glucose uptake system to the catabolite repression that accompanies growth in excess of glucose. This may also explain why even strain K2.1t, which forms the glucose phosphotransferase in high activity during batch culture on all carbon sources tested, forms less of this phosphotransferase during growth on glucose than during growth on fructose (Kornberg & Reeves, 1972*b*); fructose is likely to exert less catabolite repression.

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