Glucose Transport of *Escherichia coli* Growing in Glucose-Limited Continuous Culture

By IAIN S. HUNTER and HANS L. KORNBERG

Department of Biochemistry, University of Cambridge, Tennis Court Road, Cambridge CB2 1QW, U.K.

(Received 8 September 1978)

Dilute cultures of wild-type *Escherichia coli* K12 and of derivatives impaired in one or other Enzyme-II component of the glucose phosphotransferase system were grown in continuous culture under glucose limitation. Cells harvested from the chemostat took up [U-¹⁴C]glucose from 0.1 mM solutions at rates directly related to the rates at which those cells had grown; the activity of the phosphotransferase system in those cells, rendered permeable with optimal accounts of toluene, parallels the ability of the cells to take up glucose. The capacity of these systems was rate-limiting for growth under the negligibly low glucose concentration in the chemostat, but was adequate to account for the stimulation of respiration observed when the cells were presented suddenly with excess glucose.

Several reports in apparent conflict with each other have been published on the variation with growth rate in the activity of the phosphoenolpyruvate-linked phosphotransferase systems (Kundig *et al.*, 1964) of enteric bacteria growing on limiting glucose in continuous culture. Herbert & Kornberg (1976) observed that, over a 5-fold range of growth rate, two strains of *Escherichia coli* harvested from the culture vessel transported [¹⁴C]glucose at rates which, above a threshold value, increased linearly with growth rate and which corresponded to the rates at which glucose was utilized by the growing cultures. Since external glucose enters the metabolic pathways of *E. coli* predominantly via the phosphotransferase system (Kornberg & Jones-Mortimer, 1975; Curtis & Epstein, 1975), it was concluded that 'it is the glucose phosphotransferase activity that sets the pace of overall glucose utilization'. Carter & Dean (1977) came to a similar conclusion for a number of strains of *Klebsiella aerogenes* after estimating the activities of hexokinase in disrupted cells and of glucose phosphotransferase in cells rendered permeable with toluene (Kornberg & Reeves, 1972*a,b*). The phosphotransferase activities of all strains were similar and linearly dependent on growth rate, whereas hexokinase activities varied over a wide range.

On the other hand, Neijssel & Tempest (1976) found that the sudden addition of excess glucose to glucose-limited cultures of *K. aerogenes* resulted in immediate stimulation of respiration. This implied that the system responsible for glucose transport was not saturated before further provision of glucose and hence that it was 'not the pace-maker reaction in regulating the rate of cell synthesis

particularly at slow dilution rates'. This work was extended to include two wild-type strains of *E. coli* (Neijssel *et al.*, 1977), where, again, the addition of excess glucose to the glucose-limited cultures resulted in increased respiratory activity. The authors concluded that 'the capacity to transport the growth-limiting nutrient (glucose) into the cell is not the overall growth-limiting step'.

We now report measurement of glucose uptake, glucose utilization, glucose phosphotransferase activity and respiration rates by glucose-limited cultures of *E. coli* that resolve this apparent conflict.

Experimental

E. coli strains HK 24 (formerly designated K2.1t; Brice & Kornberg, 1967) PtsG⁺PtsM⁺, and its derivatives HK 510 (formerly designated K2.1.21; Ferenci & Kornberg, 1971) PtsG⁺PtsM⁻, and HK 63 (formerly designated K2.1.22a; Kornberg & Jones-Mortimer, 1975) PtsG⁻PtsM⁺, are laboratory stock. They were grown on glucose (1.44 g/l) in continuous culture (volume 300 ml) at 37°C under forced aeration (300 ml/min). The minimal medium of Ashworth & Kornberg (1966) was supplemented with L-arginine, L-histidine, L-leucine and L-threonine (all at 160 µg/ml) to satisfy the amino acid requirements of these bacterial strains. At all growth rates used, growth was shown to be limited by glucose, since no glucose could be detected in the culture effluent and since cell yields were increased by increasing the glucose content of the inflowing medium. Samples of the cultures were screened routinely for contaminants by checking the auxotrophic markers, and for spontan-

eous mutants by checking growth rate and phosphotransferase activity during subsequent batch culture.

Growth was estimated turbidimetrically as A_{680} in a Unicam SP.600 spectrophotometer. With the instruments and cultures used, an A_{680} of 1 was found to be equivalent to a bacterial dry mass of 500 $\mu\text{g/ml}$.

Glucose was estimated spectrophotometrically by using a glucose oxidase test (Werner *et al.*, 1970).

Uptake and utilization of $[\text{U-}^{14}\text{C}]$ glucose by cell suspensions were determined at 37°C as described previously (Amaral & Kornberg, 1975). Since cells taken from the chemostat had grown under limiting glucose it was not necessary to remove residual traces of sugar by washing the bacteria before estimation of transport activity, as is necessary with cells harvested from batch cultures. Indeed, washing was found to impair the capacity of the bacteria to incorporate glucose. The phosphotransferase activity of cells rendered permeable with toluene was measured at 37°C essentially by the method of Kornberg & Reeves (1972*a,b*). However, as was pointed out to us by Professor D. W. Tempest, the toluene concentration necessary to release optimal phosphotransferase activity from bacteria grown in continuous culture is dependent on the culture growth rate. We readily confirmed this. Consequently, fresh solutions of toluene in ethanol were prepared each day, with toluene contents ranging from 8% (v/v) to 34% in 2% steps. Samples (2.5 ml) of culture were removed from the growth vessel and chilled on ice for 20 min. The chilled suspensions were then agitated vigorously on a vortex mixer (Fisons, Loughborough, U.K.) while 25 μl (0.01 vol.) of toluene/ethanol were added by micropipette. Agitation was continued for 60 s. The suspensions thus obtained could be kept on ice for up to 5 h without change in phosphotransferase activity. Furthermore, no additional phosphotransferase activity was released when agitation was continued for longer.

Respiration of the bacteria was measured by the method of Hamilton & Holms (1970).

Results and Discussion

Rates of glucose utilization for growth and maintenance of E. coli

In previous work (Herbert & Kornberg, 1976), the rates at which glucose must be used to sustain the growth of *E. coli* over a range of growth rates (μ) were calculated from the relationship:

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

where q_s is the rate of glucose utilization (mmol/h per g of cell dry mass), D is the dilution rate (the ratio

of flow rate to culture volume and equal to μ ; h^{-1}), S_0 is the glucose concentration of medium entering the culture vessel (mm), S is the glucose concentration in the vessel (mm), in this case taken to be zero, and x is dry mass of cells (mg/ml).

To account also for the quantities of glucose utilized for purposes other than growth (the so called maintenance energy), we used a different procedure

Fig. 1. Glucose-limited culture of *E. coli* strain HK 24 (*PtsG⁺PtsM⁺*)

(a) Concentration of toluene in ethanol (0.01 vol. per assay) necessary to release optimal phosphotransferase activity from cells grown at various growth rates in glucose-limited continuous culture. (b) Δ , Uptake of $[\text{U-}^{14}\text{C}]$ glucose by cells harvested at various growth rates; $\square$, glucose phosphotransferase activity of such cells rendered permeable with the optimal amounts of toluene; $\bullet$, stimulation of oxygen uptake by cultures after addition of 5 mM-glucose. ----, Calculated rate of glucose utilization by the chemostat cultures.

for determining the rates of glucose utilization during growth. The Pirt (1965) equation for growth in continuous culture may be modified to give:

$$q_s = m_s + \frac{\mu \cdot 10^3}{Y_s^{\max}}$$

where m_s is the maintenance requirement of culture (mmol of glucose/h per g and $Y_s^{\max}$ is the maximum molar growth yield (g/mol).

Therefore q_s may be calculated for any given μ provided that m_s and $Y_s^{\max}$ are known.

The broken lines of Figs. 1(b) and 2(b) show such a relationship, using growth yield and maintenance constants obtained for the prototrophic K10 strain of *E. coli* K12 by growth and gas-exchange measurements during glucose-limited growth (Hunter, 1977). These broken lines therefore represent the rate *in vivo* of glucose utilization by the growing cultures.

Rate of [U - ^{14}C]glucose incorporation and uptake by cells harvested at different growth rates

As reported previously (Herbert & Kornberg, 1976), the initial rates of incorporation of ^{14}C into cells, taken directly from the culture vessels and exposed to 0.1 mM-[U - ^{14}C]glucose for up to 2 min, were linearly related to the rates at which those cells had been growing before harvest. This applied equally to a $PtsG^+PtsM^+$ strain (Fig. 1b) and to its $PtsM^-$ derivative (Fig. 2b), in which one of the two ports of entry for glucose (Kornberg & Jones-Mortimer, 1975; Curtis & Epstein, 1975) did not function.

However, the rates of incorporation of ^{14}C thus measured are not equal to the rates at which the labelled glucose enters the cells, since a large proportion of the glucose taken up will be oxidized and hence lost as $^{14}CO_2$. From comparisons of the rates of [U - ^{14}C]glucose incorporation by cells harvested from the chemostat used by Herbert & Kornberg (1976) and the rates at which suspensions of such cells removed glucose from 0.1 mM-glucose solutions, these authors estimated the 'true' rates of glucose uptake by approximately doubling the rates at which glucose was incorporated into cell material. Although we have confirmed this relationship with cells harvested from batch cultures, it did not hold for cells grown at the much more dilute cell concentrations maintained during our present experiments. The rates at which ^{14}C from [U - ^{14}C]glucose was incorporated into cells harvested from cultures maintained at about 0.5 mg of dry mass/ml were only 30% of those at which such cell suspensions removed glucose from 0.1 mM-glucose solutions. This difference suggests that, under the conditions used by Herbert & Kornberg (1976), where cultures were maintained at 2.5 mg of dry mass/ml, a degree of aeration necessary to ensure that glucose was totally oxidized was not achieved; key enzymes of the tricarboxylic acid

Fig. 2. Glucose-limited culture of *E. coli* strain HK 510 ($PtsG^+PtsM^-$)

(a) Concentration of toluene in ethanol (0.01 vol. per assay) necessary to release optimal phosphotransferase activity from cells grown at various growth rates in glucose-limited continuous culture. (b) Δ , Uptake of [U - ^{14}C]glucose by cells harvested at various growth rates; $\square$, glucose phosphotransferase activity of such cells rendered permeable with the optimal amounts of toluene. ----, Calculated rate of glucose utilization by the chemostat cultures.

cycle may thus not have been totally de-repressed and some of the glucose taken up might have been fermented only to products such as acetate, which would not have been detected in the analyses. If this interpretation is correct, the 'true' rates of glucose uptake in the earlier experiments would have been higher than those reported.

Evidence to this effect is provided in Fig. 3 (open symbols), which records the rates at which $PtsG^+PtsM^+$ cells, harvested from our more dilute chemo-

Fig. 3. Comparison between the calculated rates of glucose utilization by chemostat cultures (----), the rate at which suspensions of the *PtsG*⁺*PtsM*⁺ strain HK 24 remove glucose from 0.1 mM-glucose solution (○), and the maximal activity of the *PtsG* system of toluene-treated suspensions of strain HK 510 (●)

stat cultures growing over a 3-fold range of μ , removed glucose from 0.1 mM-glucose solutions. Like the rates of [^{14}C]glucose incorporation, these rates of glucose uptake increased linearly with increase in growth rate. But in all cases these latter rates were somewhat higher than those at which glucose was calculated to be utilized in the chemostat (Fig. 3, broken line). This shows that the activity of the system(s) responsible for the uptake of glucose by these cells was proportional to the rate at which those cells grew, but their system(s) were not saturated with glucose at the low concentrations of this substrate present in the medium of glucose-limited cultures.

Glucose phosphotransferase activity in cells rendered permeable with toluene

That the system(s) for glucose uptake by *E. coli* indeed involved the phosphotransferase system was confirmed by measurements of the rates at which cells harvested from the chemostats and rendered permeable by treatment with dilute toluene solutions (Kornberg & Reeves, 1972a,b) effected the phosphoenolpyruvate-dependent conversion of glucose or

methyl α -glucoside into pyruvate and the respective hexose phosphates. Studies with a number of *E. coli* strains grown in batch culture confirmed that phosphotransferase activity was revealed optimally by treating the cells with 0.01 vol. of toluene in ethanol (1:9, v/v). A similar result was obtained by Clark & Holms (1976). However, Professor D. W. Tempest kindly informed us that a greater concentration of toluene was required for the release of maximal phosphotransferase activity of *K. aerogenes* grown in continuous culture. A similar effect is observed with *E. coli*. As shown in Fig. 1(a), the concentration of toluene necessary to give maximal release of phosphotransferase activity of the *PtsG*⁺*PtsM*⁺ strain HK 24 was inversely related to growth rate. Fig. 2(a) shows that virtually identical results were obtained with its *PtsM*[−] derivative HK 510. The lowered sensitivity to toluene by cells growing at slow growth rates probably reflects changes in the composition of their cell membranes. Interestingly, this change in sensitivity is not shown by *PtsG*[−]*PtsM*⁺ strains; in these mutants, maximal phosphotransferase activity was released by 14% (v/v) toluene/ethanol (0.01 vol.) at all values of μ measured. This indicates that of the two Enzyme-II components of the glucose phosphotransferase system, only the release of activity of the *PtsG* system by toluene is affected by changes in growth rate.

The optimal toluene concentration for release of maximal activity was found to be independent of the dry mass of cells treated, at least over the range 0.25–2 mg/ml measured by us. The different toluene concentrations needed to render cells, grown at different rates, permeable are thus not merely a reflection of differences in membrane surface area resulting from differences in cell size.

The glucose phosphotransferase activity of strain HK 24 increased with growth rate to $\mu = 0.3 \text{ h}^{-1}$; thereafter it decreased slowly (Fig. 1b). Since in this strain both the *PtsG* and *PtsM* systems are functioning, the resultant activity is due to their summed rates. As expected from this view, the phosphotransferase activity of strain HK 510 (*PtsM*[−]) was always less than that of strain HK 24, but increased linearly throughout the range of growth rates tested (Fig. 2b). When methyl α -glucoside, as specific substrate for the *PtsG* system, replaced glucose in the assay of phosphotransferase activity of the *PtsG*⁺*PtsM*⁺ strain HK 24, the rates measured were identical with those obtained with the *PtsG*⁺*PtsM*[−] strain HK 510, either with glucose or with methyl α -glucoside as substrate. The 'extra' phosphotransferase activity of strain HK 24 relative to strain HK 510 was therefore due to the activity of its *PtsM* system. That this was the case was confirmed by growing the *PtsG*[−]*PtsM*⁺ strain HK 63 in glucose-limited continuous culture and estimating its maximal phosphotransferase

activity in cells rendered permeable with toluene. Maximal activity was low but constant at 48 nmol/min per mg at growth rates up to 0.3 h^{-1} ; thereafter activity decreased with increasing μ . A curve virtually identical with that for strain HK 24 (PtsG⁺PtsM⁺; Fig. 1*b*) resulted if the activities determined for strains HK 510 (PtsG⁺PtsM⁻; Fig. 2*b*) and HK 63 (PtsG⁻PtsM⁺) were summed.

The finding that in both PtsM⁺ and PtsM⁻ strains the glucose phosphotransferase activity, measured in the presence of saturating amounts of phosphoenolpyruvate and the appropriate hexose [Figs. 1*b*) and 2*b*), □], was always in excess of that required to account for the utilization of glucose by the glucose-limited cultures [Figs. 1*b*) and 2*b*), broken lines] shows that the glucose phosphotransferase system(s) were not saturated with substrate under the conditions of glucose-limited growth. Moreover, there is a striking correspondence between the rates at which cells harvested from the chemostat cultures at various growth rates removed glucose from 0.1 mM-glucose solution (Fig. 3, open symbols) and the activity of the PtsG system of those cells (Fig. 3, closed symbols). And when excess glucose was added to cultures of strain HK 24 at three growth rates, in a repetition of the experiments of Neijssel *et al.* (1977), the stimulation of respiration observed corresponded to a remarkable degree to the activity of the phosphotransferase system, now fully saturated with glucose (Fig. 1*b*, ●). We are thus led to maintain our view that the activity of the phosphotransferase system in toluene-treated cells parallels the ability of the whole cells to take up glucose; that the activity of the glucose uptake system(s) vary with growth rate in glucose-limited cells; and that the capacity of these system(s) is not only rate-limiting for glucose utilization in the carbon-limited chemostat, but is also adequate to account for the stimulation of respiration observed (Neijssel *et al.*, 1977) when the glucose concentration in the medium is suddenly raised.

Our findings further support the view (Kornberg & Jones-Mortimer, 1975) that the PtsM system plays a quantitatively minor role in the utilization of glucose, and that this may become negligibly small in glucose-limited growth. Measurement of the rates of [^{14}C]glucose uptake and of glucose

phosphotransferase activity with a PtsG⁻PtsM⁺ strain of *E. coli* showed that the PtsM system had poor affinity for glucose, with K_m of about 1.3 mM. This system could thus contribute little to glucose utilization even at 0.1 mM-glucose (Fig. 3), but would be brought into play when excess glucose is added to chemostat cultures (Fig. 1*b*, ●).

We thank Professor D. W. Tempest (University of Amsterdam) for informing us of his work before publication, and Dr. W. H. Holms (University of Glasgow) for the use of his facilities for measuring respiration during continuous culture. This work was supported by grant G/RA 4801.3 from the Science Research Council.

References

- Amaral, D. & Kornberg, H. L. (1975) *J. Gen. Microbiol.* **90**, 157–168
- Ashworth, J. M. & Kornberg, H. L. (1966) *Proc. R. Soc. London Ser. B* **165**, 179–188
- Brice, C. B. & Kornberg, H. L. (1967) *Proc. R. Soc. London Ser. B* **168**, 281–292
- Carter, I. S. & Dean, A. C. R. (1977) *Biochem. J.* **166**, 643–646
- Clark, B. & Holms, W. H. (1976) *J. Gen. Microbiol.* **95**, 191–201
- Curtis, S. J. & Epstein, W. (1975) *J. Bacteriol.* **122**, 1189–1199
- Ferenci, T. & Kornberg, H. L. (1971) *FEBS Lett.* **13**, 127–130
- Hamilton, I. D. & Holms, W. H. (1970) *Lab. Practice* **19**, 795–798
- Herbert, D. & Kornberg, H. L. (1976) *Biochem. J.* **156**, 477–480
- Hunter, I. S. (1977) Ph.D. Thesis, University of Glasgow
- Kornberg, H. L. & Jones-Mortimer, M. C. (1975) *FEBS Lett.* **51**, 1–4
- Kornberg, H. L. & Reeves, R. E. (1972*a*) *Biochem. J.* **126**, 1241–1243
- Kornberg, H. L. & Reeves, R. E. (1972*b*) *Biochem. J.* **128**, 1339–1344
- Kundig, W., Ghosh, S. & Roseman, S. (1964) *Proc. Natl. Acad. Sci. U.S.A.* **52**, 1067–1074
- Neijssel, O. M. & Tempest, D. W. (1976) *Arch. Microbiol.* **110**, 305–311
- Neijssel, O. M., Hueting, S. & Tempest, D. W. (1977) *FEMS Microbiol. Lett.* **2**, 1–3
- Pirt, S. J. (1965) *Proc. R. Soc. London Ser. B* **163**, 224–231
- Werner, W., Rey, H. G. & Wielinger, H. (1970) *Z. Anal. Chem.* **252**, 274–278