Studies on the Uptake of Hexose Phosphates

I. 2-DEOXYGLUCOSE AND 2-DEOXYGLUCOSE 6-PHOSPHATE

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SUMMARY

2-Deoxy-D-glucose was taken up by Escherichia coli and was recovered from cell extracts largely as a material that behaved chromatographically as 2-deoxyglucose 6-phosphate. With 14C-labeled deoxyglucose, it was observed that radioactivity was taken up by the cell and partially released after 1 hour. No induction of transport for glucose 6-phosphate was observed on treatment of cells with 2-deoxyglucose, in spite of the apparent accumulation of intracellular 2-deoxyglucose 6-phosphate. By contrast, extracellular 2-deoxyglucose 6-phosphate, in low concentration, was an effective inducer of the hexose phosphate transport system.

2-Deoxyglucose inhibited growth of E. coli and also caused temporary growth stasis when glycerol or succinate was the carbon source. Cells growing in the presence of 2-deoxyglucose, in the lag period was prolonged for as long as several days. Often this was followed by rapid growth caused by spontaneous appearance of a mutant strain. The mutant was isolated and found to be resistant to growth inhibition by deoxyglucose.

There is a considerable literature on the uptake of 2-deoxy-D-glucose by mammalian cells (1) and by yeast (2-7). In yeast, the fermentation of glucose and the respiration of ethanol were inhibited by 2-deoxyglucose. Transported 2-deoxyglucose was recovered from yeast partly as the free sugar and partly as 2-deoxyglucose 6-phosphate (3-5). Under experimental conditions allowing cell growth, some incorporation of 2-deoxyglucose into uridine nucleotides could be observed (6); this incorporation seemed to be related to inhibition of cell growth. Under experimental conditions not allowing growth, the only significant conversion of deoxyglucose in yeast cells was a phosphorylation to 2-deoxyglucose 6-phosphate.

Very little work appears to have been done with deoxyglucose.

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in E. coli. We were able to locate only one reference specifically to its transport (8), in which it was claimed that E. coli are unable to transport deoxyglucose. In this investigation, it was found that 2-deoxyglucose inhibited phosphorylation and fermentation of glucose in lysosome lysates of E. coli B, but not in lysosome protoplasts or intact cells. However, another report (9) stated that 10 mM 2-deoxyglucose inhibited growth of E. coli by 50%, although the differential rate of inducible β-galactosidase synthesis was not reduced. This could be a surface effect, but it seems likely that 2-deoxyglucose entered the cell. Pogell and Maity (9) observed that 0.1 mM deoxyglucose in the growth medium more than doubled the lag period when glucose 6-phosphate was supplied as a source of energy for E. coli B. A similar effect was noted with 0.1 mM α-D-methylglucoside. These authors also observed that 2-deoxyglucose 6-phosphate appeared to be taken up by means of the glucose 6-phosphate transport system. In later work (10) it was noted that 32P-labeled 2-deoxyglucose 6-phosphate was not completely stable in E. coli but was slowly converted to other products.

In the present investigation, we observed that 2-deoxyglucose was taken up by E. coli and a material which behaved chromatographically like 2-deoxyglucose 6-phosphate accumulated in the cell; the amount decreased slightly after 1 hour. No induction of the hexose phosphate transport system occurred during the uptake of 2-deoxyglucose and its conversion intracellularly to the phosphate ester. However, when 2-deoxyglucose 6-phosphate was added to the medium it was an effective inducer.

Mutants have been isolated which are resistant to the growth inhibitory effects of 2-deoxyglucose.

EXPERIMENTAL PROCEDURE

Materials

Bacteria—Strain E15 (11) has a deletion for the alkaline phosphatase gene but is wild type with respect to transport.

Media—The synthetic medium (CRM) was similar to that described by Cohen and Rickenberg (12) and contained (per liter, pH 7.3, adjusted with KOH) 13.6 g of KH2PO4, 2.0 g of (NH4)2SO4, 0.2 g of MgSO4·7H2O, 0.5 mg of FeSO4·7H2O, and 1 ml of a trace metal solution containing 480 mg of FeCl3·6H2O, 280 mg of MnCl2·4H2O, 270 mg of CaCl2, 2000 mg of ZnCl2, 290 mg of H3BO3, and 130 mg of CoSO4 per liter. Tryptone broth consisted of 10 g of Difco Bacto-tryptone and 5 g of NaCl per liter.

Chemicals—14C-Labeled 2-deoxyglucose was from New England Nuclear. It was purified by chromatography on Whatman No. 1
measured by removing samples and measuring optical density at 540 nm. Strain E15 were grown to midlog phase in tryptone broth and a 1% inoculum was transferred to Medium CRM plus 0.015 M glycerol. Again the culture was grown to midlog phase and a 2% inoculum transferred into: Curve A, CRM plus 0.015 M glucose; Curve B, CRM plus 0.03 M glycerol; Curve C, CRM plus 0.03 M glycerol plus 9 X 10^-4 M 2-deoxyglucose; Curve D, CRM plus 0.03 M glycerol plus 9 X 10^-3 M 2-deoxyglucose. Turbidity was measured in a Klett colorimeter with No. 42 filter.

Bacteria were grown in a rotary shaker at 37°C. Growth was extended lag period. The culture was harvested by centrifugation, twice washed with Medium CRM (without a carbon source) at 23°C, and suspended in Medium CRM (up to 2.5 mg per ml, wet weight). Chloramphenicol was added to a final concentration of 40 μg per ml, and the mixture was incubated with gentle agitation for 4 min. The radioactive substrate (5 x 10^-4 M) was used to start the reaction and at indicated time intervals 0.2 ml portions were filtered through a Millipore membrane filter (0.45 μm pore size). The cells were washed on the filter with a cold solution consisting of 0.15 M NaCl, 5 x 10^-4 M MgCl₂, and 0.01 M Tris-HCl, pH 7.3. (Similar values were obtained when the cells were washed with Medium CRM.) The filters were glued to 3-cm aluminum planchets with rubber cement, dried under a heat lamp, and counted in a Nuclear-Chicago thin window counter. To determine specific activity of the substrate, samples were placed in 0.1 ml of water on an aluminum planchet, dried, and counted. In calculating specific activities no account was taken of self absorption by the cells on the Millipore filter. The fraction of substrate taken up was always less than 5%. In this assay the counts varied linearly with the number of bacteria over a 5-fold range.

The method of Lowry et al. (13) was used to determine protein.

**RESULTS**

**Effect of 2-Deoxyglucose on Growth of E. coli E15**—Cells growing in the presence of 0.015 M glycerol showed inhibition upon the addition of 9 X 10^-4 M 2-deoxyglucose; the doubling time was increased 2-fold. When cells in exponential phase growing on glycerol were transferred to fresh medium of the same composition but supplemented with 2-deoxyglucose, there was a prolonged lag period.

Even more striking effects were observed when glucose-grown cells were transferred to fresh medium containing glycerol or

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**Table I**

| 14C-Substrate                  | Uptake (μmol/mg protein/4 min) |
|--------------------------------|--------------------------------|
| 2.3 X 10^-5 M deoxyglucose     | 3.6*                           |
| 5.2 X 10^-5 M deoxyglucose     | 6.9                            |
| 5.0 X 10^-8 M glucose 6-phosphate | 0.2                          |
| 5.0 X 10^-7 M glucose          | 53.0                           |

* The rate of uptake of deoxyglucose was constant for nearly 10 min. A curve of accumulation of radioactivity plotted against time of incubation showed a 20% decrease between 10 and 60 min.

600 μg in the Gilford model 240 spectrophotometer. Alternatively, cell suspensions were shaken in 125 ml Erlenmeyer flasks equipped with a side arm, and growth was measured with a Klett, Klett Manufacturing Company, New York, New York, colorimeter equipped with a number 42 filter. The approximate relation between absorbance in the Gilford and number of bacteria per ml is: 0.12 = 0.9 X 10^8; 0.48 = 4.1 X 10^9; 0.70 = 5.5 X 10^9; 1.11 = 12 X 10^9.

All uptake assays were carried out at ambient temperatures. The culture was harvested by centrifugation, twice washed with Medium CRM (without a carbon source) at 23°C, and suspended in Medium CRM (up to 2.5 mg per ml, wet weight). Chloramphenicol was added to a final concentration of 40 μg per ml, and the mixture was incubated with gentle agitation for 4 min. The radioactive substrate (5 x 10^-4 M) was used to start the reaction and at indicated time intervals 0.2 ml portions were filtered through a Millipore membrane filter (0.45 μm pore size). The cells were washed on the filter with a cold solution consisting of 0.15 M NaCl, 5 x 10^-4 M MgCl₂, and 0.01 M Tris-HCl, pH 7.3. (Similar values were obtained when the cells were washed with Medium CRM.) The filters were glued to 3-cm aluminum planchets with rubber cement, dried under a heat lamp, and counted in a Nuclear-Chicago thin window counter. To determine specific activity of the substrate, samples were placed in 0.1 ml of water on an aluminum planchet, dried, and counted. In calculating specific activities no account was taken of self absorption by the cells on the Millipore filter. The fraction of substrate taken up was always less than 5%. In this assay the counts varied linearly with the number of bacteria over a 5-fold range.

The method of Lowry et al. (13) was used to determine protein.

**Methods**

Bacteria were grown in a rotary shaker at 37°C. Growth was measured by removing samples and measuring optical density at
After gentle agitation for 4 min at 23°C, purified 14C-2-deoxyglucose was added to a concentration of 3.0 × 10^{-5} M. Ten minutes later the uptake reaction mixture contained 40 μg per ml of chloramphenicol in Medium CRM. The uptake reaction mixture contained 40 μg per ml of chloramphenicol in Medium CRM. After gentle agitation for 4 min at 23°C, purified 14C-2-deoxyglucose was added to a concentration of 3.0 × 10^{-5} M. Ten minutes later the suspension was layered over 3 ml of 2% sucrose in 0.15 M NaCl, 8 × 10^{-4} M MgCl₂, pH 7.3, and centrifuged at 10,000 × g for 10 min in a cold room. The pellet was taken up in 2.5 ml of cold water and stirred for 5 min before filtering through a 0.45-μm Millipore filter. The shock fluid was lyophilized and taken up in 0.065 ml of H₂O. Chromatography was in Solvent 1, descending, on Whatman No. 1 paper. The chromatogram was cut into strips 0.5 cm wide which were glued to aluminum planchets and counted in a gas flow counter.

Fig. 3. Chromatographic evidence for the formation of 14C-2-deoxyglucose 6-phosphate from 14C-2-deoxyglucose by E. coli strain E15. Cells were grown in Medium CRM supplemented with 0.02 M glucose, harvested at an optical density of 0.360 (8 × 10⁶ cells per ml), washed twice at room temperature with 0.01 M Tris-HCl, pH 7.3, 4.03 M NaCl and resuspended (1 g, wet weight, per 40 ml) in 0.033 M Tris-HCl, pH 7.3. The uptake reaction mixture contained 40 μg per ml of chloramphenicol in Medium CRM. After gentle agitation for 4 min at 23°C, purified 14C-2-deoxyglucose was added to a concentration of 3.0 × 10^{-5} M. Ten minutes later the suspension was layered over 3 ml of 2% sucrose in 0.15 M NaCl, 8 × 10^{-4} M MgCl₂, pH 7.3, and centrifuged at 10,000 × g for 10 min in a cold room. The pellet was taken up in 2.5 ml of cold water and stirred for 5 min before filtering through a 0.45-μm Millipore filter. The shock fluid was lyophilized and taken up in 0.065 ml of H₂O. Chromatography was in Solvent 1, descending, on Whatman No. 1 paper. The chromatogram was cut into strips 0.5 cm wide which were glued to aluminum planchets and counted in a gas flow counter.

succinate as the carbon source (Fig. 1). In the presence of 9 × 10^{-4} M, or even 9 × 10^{-3} M deoxyglucose, no growth was observed for many hours. Finally the turbidity of the culture began to increase, but this was due to a spontaneous mutation. Single cell isolates were transferred successively to nutrient agar slants, tryptone broth, and Medium CRM supplemented with glucose. The new culture was then transferred to Medium CRM plus 0.015 M glycerol, and a portion of the culture was supplemented with 9 × 10^{-3} M 2-deoxyglucose. No effect of the analogue was observed (Fig. 2); the mutant was resistant to 2-deoxyglucose. The mutant was also resistant to the effect of 2-deoxyglucose on adaptation to succinate. The mutant was derived from the parent strain E15 and retained other properties of the parent such as being alkaline phosphatase negative. It grew on glucose and was able to transport glucose and 2-deoxyglucose.

**Uptake of Labeled 2-Deoxyglucose—14C-2-Deoxyglucose was taken up by E. coli and was converted to a material which behaved chromatographically like 2-deoxyglucose 6-phosphate (Table I, Fig. 3). The concentration of deoxyglucose 6-phosphate in cell water reached 1.6 × 10^{-3} M within 10 min and usually decreased by no more than 10% during the next hour. This accumulation of intracellular 2-deoxyglucose 6-phosphate did not cause induction of the glucose 6-phosphate transport system. By contrast, only 1 × 10^{-3} M 2-deoxyglucose 6-phosphate added to the growth medium did cause induction of transport activity. At concentrations above 5 × 10^{-3} M, 2-deoxyglucose 6-phosphate was as effective as glucose 6-phosphate for exogenous induction. However, at 1.5 × 10^{-3} M and 7.5 × 10^{-3} M concentrations, the deoxyglucose ester induced only one-third and one-fourth as much transport activity, respectively, as the glucose ester.**

**DISCUSSION**

When 2-deoxy-D-glucose was taken up by yeast cells it was recovered partly as free sugar and partly as 2-deoxy-D-glucose 6-phosphate (2). At low 2-deoxyglucose concentrations there was an uphill transport of the free sugar; concentration ratios of 20 to 30 indicated an active transport mechanism. Intracellular hydrolysis of accumulated 2-deoxyglucose 6-phosphate could be demonstrated and with a 3H-2-deoxyglucose-pulsing technique it was shown that the ester behaved as the precursor of intracellular free 2-deoxyglucose. Our studies showed that 2-deoxyglucose was taken up by E. coli and accumulated as a material that behaved chromatographically like 2-deoxyglucose 6-phosphate. We are not in a position to say whether 2-deoxyglucose 6-phosphate is the product of transport or whether it accumulates in the cell after penetration of the membrane by the free sugar. To answer this question with confidence, one should isolate a mutant lacking the periplasmic acid hexose phosphatase which conceivably could hydrolyze the ester if it were formed in the membrane.

2-Deoxyglucose had no effect on growth when glucose was the source of carbon for E. coli; apparently it was unable to compete with glucose for entry. The deoxy sugar also caused an inhibition of growth when the carbon source was pyruvate or glucose. On the other hand, cells growing in the presence of succinate or glycerol were sensitive to the effects of 2-deoxyglucose. We assume that the metabolism of succinate and glycerol involves an enzyme or enzymes sensitive to 2-deoxyglucose or a derivative.

Exogenous 2-deoxyglucose 6-phosphate was an effective inducer of the hexose phosphate transport system, although the endogenously formed ester was not active as an inducer. This transport system has been actively studied in recent years (14-17). Glucose 6-phosphate was a somewhat more efficient inducer but since it was metabolized the specific activity of the inducer became lower. Once the transport system was induced, it was maintained for a much longer period in growing cultures.

Pogell et al. (10) investigated the behavior of 32P-labeled 2-deoxyglucose 6-phosphate when taken up by E. coli cells. Only about 50% behaved chromatographically like unchanged 2-deoxyglucose 6-phosphate. The nature of the label did not give information on whether the 2-deoxyglucose moiety was metabolized or not. We did not observe any 3H-labeled regions on the chromatogram other than those corresponding to 2-deoxyglucose and 2-deoxyglucose 6-phosphate.

We have shown that 2-deoxyglucose interferes with adaptation of glucose-grown E. coli to glycerol or succinate, and mutants resistant to these effects were isolated. The nature of the mutation is unknown and will be investigated in the future. It is possible that these effects are related to the ability of 2-deoxyglucose to repress various inducible systems such as β-galactosidase, as reported by Tyler, Loomis, and Maasnenik (18). These authors observed a similar effect by α-methylglucoside.
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