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The Oxidative Pathway of Carbohydrate Metabolism in *Escherichia coli*

2. QUANTITATIVE STUDIES OF GLUCOSE 6-PHOSPHATE DEHYDROGENASE AND 6-PHOSPHOGLUCONATE DEHYDROGENASE

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In this paper data are presented on the activities of glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase extracted from *Escherichia coli* in an effort to estimate the proportion of carbohydrate which might be metabolized by the enzymes during bacterial growth under aerobic conditions. Enzymes were extracted which accounted for approximately 40 % of the total carbohydrate metabolized by the cell.

METHODS

As will be shown, the amounts of these enzymes in *Esch. coli* varied according to the conditions of growth. The following procedure was followed in all experiments presented in this paper. *Esch. coli*, strain B, from the stock culture in synthetic medium (Cohen, 1949), was used to inoculate 100 ml. of synthetic medium containing 1 mg. glucose/ml. After 17 hr. aerobic growth at 36° the glucose was exhausted and the culture contained 9×10^8 cells/ml.; 50 ml. were then inoculated into each of two roller flasks containing 250 ml. of synthetic media with the desired concentration of carbohydrate as sole carbon source. The flasks were rotated at 300–400 rev./min. in the incubator at 36°. While spinning at this rate the fluid formed a band completely around the flask, thus assuring maximal aeration of the culture. The

growth of the culture was followed by measurement of the turbidity at 420 m μ . in the Klett photometer and sometimes by plate count. Fig. 1 shows the correlation of turbidity with plate count during growth of the bacteria on glucose or potassium gluconate.

The cells were harvested either during exponential growth or when the carbon source, or the nitrogen source, or both, were exhausted. The culture was immediately chilled to 4°. Centrifugation, washing, and extraction were carried out at 2–4°. The bacteria were disrupted by grinding with alumina (McIlwain, 1948) and extracted two or three times with water or tris(hydroxymethyl)aminomethane buffer (Tris) at pH 8.0. Determination of the enzyme activities was made by measurement of the reduction of triphosphopyridine nucleotide (TPN) in the presence of glucose 6-phosphate (G 6-P) or 6-phosphogluconate (PG) by the methods described in the preceding paper (Scott & Cohen, 1953).

RESULTS

At the beginning of growth on glucose in the roller flask there was either no lag phase or it was very short. With gluconate there was usually a lag of about 20–40 min., presumably for formation of the adaptive enzyme, gluconokinase (Cohen & Scott, 1950; Cohen, 1951a).

With 1 mg. glucose/ml. in the medium the bacteria exhausted the substrate when the turbidity was 190 and the count 9×10^8 cells/ml. With greater concentrations of glucose, growth, that is increase in turbidity and in cell count, was exponential and the extent of growth was proportional to the initial glucose concentration up to the limit of the nitrogen source in the medium (Monod, 1942). The medium contained 21.2 mg. N/ml. as ammonium sulphate and with excess glucose allowed exponential growth up to populations of 3×10^9 cells/ml. At this point, the cells continued to divide at least once, although the rate of increase in turbidity fell off to zero.

Fig. 1. Correlation of turbidity with plate count during growth of *Esch. coli* in glucose or gluconate. O—O, 10 mg./ml. glucose; x—x, 11 mg./ml. potassium gluconate.

The mass and cell doubling time during the exponential phase of growth was 45–55 min. with an average of 50 min. on either glucose or gluconate. However, it can be seen in Fig. 1 that cultures grown in gluconate may have 20% lower turbidity than those prepared in glucose, although both may contain the same numbers of cells.

The substrate consumption per cell is constant during growth, i.e. both are exponential (Monod, 1942). Therefore, during the last 50 min. of exponential growth, the bacteria have doubled in turbidity and plate count, and have used half the available substrate. The number of molecules of carbohydrate used/cell/min. was determined by the moles of glucose or gluconate used/ml./min. $\times 6.06 \times 10^{23}$ and divided by the number of cells/ml. Table 1 gives the values calculated from turbidity

and plate counts which were determined experimentally. The mean values were 8.5×10^7 molecules of glucose/cell/min. or 6.3×10^7 molecules of potassium gluconate/cell/min.

In the extracts prepared from the cells the total activities of both glucose 6-phosphate and phosphogluconate dehydrogenase were determined in terms of moles of substrate oxidized/min. This value, multiplied by 6.06×10^{23} and divided by the total number of cells from which the extract was prepared, gave the number of molecules of substrate oxidized/cell/min.

Data given in Table 1 show the following:

(1) Extracts from cells harvested during the exponential phase on glucose contained enzymes sufficient to account for oxidation at the rate of 2.6 – 6.9×10^7 molecules of glucose 6-phosphate/cell/min., and 2.0 – 3.7×10^7 molecules of phosphogluconate/cell/min. As was noted previously (Scott & Cohen, 1953), the lower values were usually obtained with a water extract. Later experiments in which the disrupted cells were extracted with Tris gave values of 6.0 – 6.9×10^7 molecules of glucose 6-phosphate/cell/min.

(2) Cells which had exhausted their nitrogen source but not their glucose, and were still dividing, were smaller but had a high content of enzymes, especially of glucose 6-phosphate dehydrogenase.

(3) In extracts of cells which had ceased to grow because of exhaustion of glucose or gluconate the activities of both enzymes were low.

(4) When gluconate was the carbon source, extracts made from cells harvested during the exponential phase of growth contained enzymes sufficient to account for oxidation at the rate of 2.2 – 5.2×10^7 molecules of glucose 6-phosphate/cell/min. and 1.5 – 2.6×10^7 molecules of phosphogluconate/cell/min.

DISCUSSION

The apparent content of glucose 6-phosphate dehydrogenase in cells harvested during exponential growth on glucose could account for 31–86% of the glucose metabolized as glucose 6-phosphate. But the extractable activity of phosphogluconate dehydrogenase accounted for a maximum of 44% of the glucose metabolized. The difference between the apparent amounts of the two dehydrogenases in the cell may have one or more of several explanations.

(1) Since the activity of glucose 6-phosphate dehydrogenase is so extremely sensitive to factors of dilution, of protein concentration, of ion concentration and especially of substrate concentration, perhaps our optimal conditions of assay represent more nearly the maximum capability of the enzyme rather than the functioning activity of the cell *in vivo*. The pH should not be considered a factor here,

Table 1. *Correlation of metabolic and enzymic activities*

Substrate (mg./ml.)	Growth phase when harvested	Turbidity	Extractant	Cells Molecules substrate used/cell/ min. $\times 10^7$	Extracted enzymes			
					Molecules G 6-P† oxidized/ cell/ min. $\times 10^7$	Amount of sub- strate accounted for (%)	Molecules PG‡ oxidized/ cell/ min. $\times 10^7$	Amount of sub- strate accounted for (%)
Glucose								
2	Exponential	310	Water	8.3	4.5	54	2.8	34
5		510	Water	8.5	3.6	41	3.1	37
10		635	Water	8.4	2.6	31	2.0	24
10		640	Water	8.0	4.0	50	2.1	26
10		600	Water	9.2	4.3	47	3.8	41
10		475	Tris	8.0	6.9	86	3.2	40
5		515	Tris	8.2	6.1	74	3.7	44
10	Exhaustion of N	750	Water	7.8*	6.2	86	3.0	38
5	Exhaustion of N	800	Tris	8.2*	6.0	73	3.1	38
2	Exhaustion of substrate	435	Water	8.3*	2.2	—	1.5	—
5	Exhaustion of N and substrate	776	Water	8.5*	1.3	—	1.3	—
Gluconate								
2.7	Exponential	320	Water	5.3	2.2	41	1.5	28
6.0	Exponential	395	Water	6.6	2.7	41	1.8	27
11.4	Exhaustion of N	650	Water	6.2*	2.4	39	1.5	24
6.0	Exponential	585	Tris	7.2	5.2	72	2.6	36
2.5	Exhaustion of substrate	345	Water	5.8*	2.1	—	1.1	—
2.0	Exhaustion of substrate	260	Water	6.8*	1.1	—	1.2	—

* Calculated during exponential growth.

† G 6-P=glucose 6-phosphate.

‡ PG=6-phosphogluconate.

since at the pH of assay (8.5) the enzyme has within 10 % of the activity at the pH of growth (7.8).

(2) It may be that not all the phosphogluconate which is formed from glucose 6-phosphate is degraded by phosphogluconate dehydrogenase. Perhaps some of the phosphogluconate is split by a mechanism similar to the cleavage by *Pseudomonas saccharophila* into pyruvate and triose phosphate (Entner & Doudoroff, 1952). This reaction has not yet been shown to occur in *Esch. coli*, but according to preliminary experiments, non-oxidative breakdown of phosphogluconate by extracts cannot be ruled out. However, the rate of this breakdown seems to be too slow in the extracts to account for the difference in activity of the two enzymes.

(3) The method of determining glucose 6-phosphate dehydrogenase in extracts may allow a slight reduction of TPN by the phosphogluconate generated. This effect would give falsely high values for glucose 6-phosphate dehydrogenase. This possibility is difficult to rule out, but from experiments with mixtures of the purified enzymes it appears that this effect must be small under the established conditions of assay.

(4) The phosphogluconate dehydrogenase may have been less completely extracted from the cells than the first enzyme or may have suffered greater

inactivation during extraction. However, it was noted that the amount of phosphogluconate dehydrogenase obtained in successive extractions of the same cells was proportional to the amount of total protein extracted. Phosphogluconate dehydrogenase is less sensitive to inactivation than glucose 6-phosphate dehydrogenase when conditions are not optimal.

The values for the activity of phosphogluconate dehydrogenase are estimated to account for a mean of 36 % and a maximum of 44 % of the total carbohydrate utilization, which agrees remarkably well with the maximal figure found by the isotope technique (Cohen, 1951*b*) of about 38 % of glucose metabolized via the oxidative pathway. From this it might be inferred that the measurement of the activity of the phosphogluconate dehydrogenase in extracts more nearly represents the enzyme activity in the cell than does the measurement of glucose 6-phosphate dehydrogenase which may merely be present in excess.

Extracts of bacteria grown on potassium gluconate contained less of both enzymes than those grown in glucose. However, these bacteria used less substrate for growth, and the glucose 6-phosphate dehydrogenase extracted accounted for 30–72 %, and the phosphogluconate dehydrogenase for 24–36 % of the substrate metabolized.

Isotope studies showed 85–95 % recovery of the ^{14}C as carbon dioxide, with cells growing on $[1-^{14}\text{C}]\text{gluconate}$ and 100 % recovery with cells metabolizing $[1-^{14}\text{C}]\text{gluconate}$ without a nitrogen source for growth (Cohen, 1951b). It was considered that the overall reaction:

was practically irreversible in intact bacteria under physiological conditions. Glucose dehydrogenase, for example, has been shown to be slowly reversible at pH 3.5 but essentially irreversible at pH 7 (Strecker & Korkes, 1952). Similarly, glucose 6-phosphate is probably not formed from 6-phosphogluconate and reduced TPN by a reversible reaction. It would, nevertheless, be expected that during utilization of gluconate some glucose 6-phosphate would be formed in the cell, arising from condensation products of two-carbon fragments (Dische, 1952; Horecker, 1951; De la Haba & Racker, 1952) or perhaps from triosephosphates derived from the splitting of pentose phosphates or from the cleavage of phosphogluconate (Entner & Doudoroff, 1952). Such a formation of glucose 6-phosphate in the cell metabolizing gluconate may explain the presence of the glucose 6-phosphate dehydrogenase, since the substrate may stimulate the production of the enzyme.

An explanation for a maximal content of phosphogluconate dehydrogenase sufficient to account for only 36 % of the gluconate used despite the 85–100 % recovery of the C-1 of gluconate as carbon

dioxide might lie in the adaptive formation of the cleaving system of Entner & Doudoroff (1952). The pyruvate formed by this cleavage contains the C_1 of gluconate in the carboxyl group. Decarboxylation of the pyruvate would produce labelled carbon dioxide.

SUMMARY

1. *Escherichia coli*, strain B, growing on glucose metabolized 8.5×10^7 molecules of glucose/cell/min.

2. Enzymes extracted from cells grown on glucose oxidized glucose 6-phosphate at a rate equivalent to $2.6\text{--}6.9 \times 10^7$ molecules/cell/min., and phosphogluconate at a rate equivalent to $2.0\text{--}3.8 \times 10^7$ molecules/cell/min.

3. *Esch. coli* growing on potassium gluconate metabolized 6.3×10^7 molecules of gluconate/cell/min.

4. Enzymes extracted from cells grown on potassium gluconate oxidized glucose 6-phosphate at a rate equivalent to $2.2\text{--}5.2 \times 10^7$ molecules/cell/min. and phosphogluconate at a rate equivalent to $1.5\text{--}2.6 \times 10^7$ molecules/cell/min.

5. These enzymes are still present though decreased in amount in cells which have exhausted their carbon source.

6. Thus enzymes of the oxidative pathway have been extracted from growing cells which account for approximately 40 % of the total metabolism of carbohydrate.

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