# ENZYME INDUCTION AND REPRESSION BY GLUCOSE IN AEROBACTER AEROGENES

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It has been previously reported (Magasanik et al., 1959) that glucose- and glycerol-grown cells of Aerobacter aerogenes, strain 1033, oxidize glucose at the same rate when suspended in phosphate buffer, and that the addition of $Mg^{++}$ greatly stimulates the rate of glucose oxidation of the glucose-grown cells but not of the glycerol-grown cells. This additional $Mg^{++}$-dependent degradation of glucose produces in part gluconate and pyruvate which accumulate in the reaction mixture. The glucose oxidation by gluconate-grown cells is also stimulated by $Mg^{++}$.

Glucose and gluconate, in contrast to glycerol and most other carbon compounds, repress the formation of many inducible enzymes such as histidase, urocanase, and inositol dehydrogenase in A. aerogenes (Magasanik et al., 1958). A mutant of this organism, strain JF-4, differs from the parent strain in that glucose fails to repress these enzymes; gluconate, on the other hand, has its usual repressive effect (Neidhardt, 1960 a). Glucose-, gluconate-, and glycerol-grown cells of strain JF-4 suspended in phosphate buffer, oxidized glucose at approximately the same rate as similar suspensions of the parent strain 1033; however, JF-4 differed from 1033 in that the glucose oxidation of the glucose-grown cells failed to be stimulated by $Mg^{++}$. On the other hand, the oxidation of glucose by gluconate- and glycerol-grown cell suspensions of strain JF-4 was affected by $Mg^{++}$ in the same way as that of corresponding cell suspensions of the

parent strain: the gluconate-grown cells were stimulated, and the glycerol-grown cells were not.

In order to obtain additional information on the glucose metabolism of the two strains, cells were harvested from exponential growth in three liters of minimal medium containing 0.2% glucose, gluconate, or glycerol as the only source of carbon, washed with 0.05 M Tris buffer of pH 7.4, and disrupted in 15 ml. of buffer of the same composition by sonic oscillation in a 10 kc magnetostrictive oscillator (Raytheon Manufacturing Company)at 4° for 5 minutes. The cellular debris was removed by two centrifugations at 5500 g for 20 minutes each, and the supernatant fluids containing approximately 20 mg. of protein per ml. were examined for their ability to oxidize glucose and glucose-6-phosphate, and to convert gluconate to pyruvate under anaerobic conditions. The results of the oxidation experiments are summarized in Table I.

TABLE I

Oxidation of Glucose and of Glucose-6-Phosphate

| Substrate added: | | None | | Glucose | | Glucose-6-P | |
|---|---|---|---|---|---|---|---|
| TPN + Methylene Blue: | | - | + | - | + | - | + |
| Strain | Growth Medium | | | | | | |
| 1033 | Glucose | 45 | 61 | 206 | 347 | 234 | 390 |
| JF-4 | Glucose | 16 | 26 | 16 | 20 | 34 | 542 |
| 1033 | Glycerol | 28 | 31 | 44 | 104 | 120 | 674 |
| 1033 | Gluconate | 35 | 59 | 144 | 259 | 266 | 458 |
| JF-4 | Gluconate | 39 | 55 | 120 | 240 | 206 | 405 |

The consumption of oxygen was measured manometrically; the reaction mixture consisted of cell extract containing approximately 20 mg. of protein, where indicated 200 µg. of TPN and 100 µg. of methylene blue, 0.05 M Tris pH 7.4, and 25 µmoles of substrate added from the side arm, in a total volume of 2.0 ml. The rate of oxygen uptake was linear after the addition of the substrate, and is expressed as µl. of oxygen taken up in 30 minutes per 20 mg. of protein.

A striking difference between strains 1033 and JF-4 is immediately apparent: extracts of glucose-grown cells of 1033 oxidize glucose and glu-

cose-6-phosphate at rapid rates, which are further stimulated by the addition of TPN and methylene blue, while similar cell extracts of strain JF-4 fail to oxidize glucose, and oxidize glucose-6-phosphate only in the presence of TPN and methylene blue. This oxidation of glucose-6-phosphate by extracts of glucose-grown strain JF-4 was found to be absolutely dependent on TPN and to be stimulated by methylene blue. Extracts of glycerol-grown 1033 resemble those of glucose-grown JF-4 in their inability to oxidize glucose in the unsupplemented reaction mixture; however, they can oxidize glucose in the presence of TPN and methylene blue, and glucose-6-phosphate without this supplementation, though at a much slower rate than extracts of glucose-grown 1033. The rapid oxidation of glucose-6-phosphate in the presence of TPN and methylene blue by extracts of glucose- and glycerol-grown 1033 and JF-4 may be ascribed to the action of a glucose-6-phosphate dehydrogenase which could be demonstrated in these extracts by spectrophotometric assay (DeMoss, 1955). Similar extracts also showed equal hexokinase activities (Neidhardt, 1960b).

The extracts of gluconate-grown cells of either strain oxidized glucose-6-phosphate and glucose, though the latter more slowly than did the extract of glucose-grown 1033.

The results summarized in Table II show that with the exception of the extract prepared from glycerol-grown cells, all extracts could form pyruvate anaerobically from 6-phosphogluconate or from gluconate and ATP (Entner and Doudoroff, 1952). The extracts of gluconate-grown cells of both strains, and of glucose-grown 1033 could also form a small amount of pyruvate from gluconate in the absence of ATP.

The ability of the various cell extracts to oxidize glucose parallels the ability of the corresponding cell suspensions to oxidize glucose at an increased rate in the presence of $Mg^{++}$. The significance of this relation is emphasized by the observation that $4 \times 10^{-3}$ M EDTA completely inhibits the oxidation of glucose by the extract of glucose-grown

TABLE II

Formation of Pyruvate from Gluconate and 6-Phosphogluconate

| Substrate : | | Gluconate | Gluconate | 6-Phosphogluconate |
|---|---|---|---|---|
| ATP + $Mg^{++}$ : | | - | + | - |
| Strain | Growth Medium | | | |
| 1033 | Glucose | 1.2 | 7.4 | 4.5 |
| JF-4 | Glucose | 0 | 2.4 | 7.9 |
| 1033 | Glycerol | 0 | 0 | 0.1 |
| 1033 | Gluconate | 0.7 | 7.3 | 3.9 |
| JF-4 | Gluconate | 1.0 | 7.6 | 5.6 |

The reaction mixtures consisted of enzyme extract containing approximately 10 mg. of protein, 10 µmoles of substrate, and where indicated, 25 µmoles of neutralized ATP and 5 µmoles of $MgSO_4$, in a total volume of 0.75 ml. of 0.05 M Tris pH 7.4. After one hour of incubation at 37° under an atmosphere of nitrogen, the mixtures were deproteinized by the addition of 0.75 ml. of 10% trichloroacetic acid and centrifugation. The pyruvate present in the supernatant fluid was estimated by the method of Friedemann and Haugen (1943). The results are recorded in µmoles of pyruvate produced per 10 mg. protein in one hour.

strain 1033, and that this effect of EDTA can be overcome by $Mg^{++}$.

The other correlation that can be drawn is between the presence of the enzymes responsible for this $Mg^{++}$-dependent glucose oxidation, and the repression exerted by glucose on enzyme formation: in strain JF-4, glucose fails to induce this $Mg^{++}$-dependent glucose oxidation, and fails to repress the formation of the normally glucose-sensitive enzymes. Furthermore, cells of either strain during growth on glycerol lack the glucose-oxidizing mechanism and can form the glucose-sensitive enzymes, but during growth on gluconate possess this mechanism and cannot form these enzymes.

It is possible that the substance whose metabolism is actually responsible for the repression of the glucose-sensitive enzymes is gluconate. The effect exerted by glucose would then depend on the ability of the glucose-grown cells to oxidize glucose rapidly to gluconate by

the agency of the $Mg^{++}$-dependent enzyme system.

The observations reported previously and in this paper show that the metabolism of glucose is not entirely a constitutive property of the cell. Glucose induces the formation of enzymes which cause an increase in the rate of glucose oxidation and in the production of pyruvate from gluconate. This increased glucose metabolism appears to be responsible for the excessive production of repressors which prevent the formation of certain inducible enzymes (Magasanik et al., 1958).

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