The Metabolism of 2-Oxoglucuonate by *Pseudomonas aeruginosa*

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**SUMMARY**

The 2-oxoglucuonate kinase and 2-oxoglucuonate 6-phosphate reductase of *Pseudomonas aeruginosa* were purified approx. 100-fold. The activities of these enzymes, and a 2-oxoglucuonate transport system, were induced when the organism was grown on glucose, gluconate or 2-oxoglucuonate but were absent when the organism was grown on glycerol, succinate or citrate. Gluconate dehydrogenase is membrane-bound and acts extracellularly.

**INTRODUCTION**

The ability of a variety of micro-organisms to grow on 2-oxoglucuonate as sole carbon and energy source was demonstrated by De Ley & Vandamme (1955). De Ley (1954) had previously shown that *Aerobacter cloacae* converts 2-oxoglucuonate by an ATP-dependent kinase [EC. 2.7.1.63] to the corresponding 6-phosphate ester which is then reduced by 2-oxo-6-phosphoglucuonate reductase [EC. 1.1.1.69] to 6-phosphoglucuonate. The kinase and reductase have also been demonstrated in *A. aerogenes* (Frampton & Wood, 1961a), *Pseudomonas fluorescens* (Frampton & Wood, 1961b) and *Leuconostoc mesenteroides* (Cifferri & Blakely, 1959).

Although *Pseudomonas aeruginosa* can grow on 2-oxoglucuonate, the precise pathway of its metabolism in this organism has not been defined, other than a chromatographic demonstration of an ATP-dependent production of 2-oxoglucuonate 6-phosphate (De Ley & Vandamme, 1955). We have therefore investigated the roles of the kinase and reductase enzymes and measured their specific activities in extracts of the organism grown on various carbon sources including glucose and gluconate.

*Pseudomonas aeruginosa* possesses parallel pathways of glucose and gluconate catabolism that converge at 6-phosphoglucuonate (Fig. 1), the non-phosphorylative (direct oxidative) and phosphorylative pathways respectively. We have previously shown (Midgley & Dawes, 1973) that glucose dehydrogenase, the first enzyme of the non-phosphorylative pathway, acts extracellularly. We now report that the second enzyme of this sequence, gluconate dehydrogenase, also acts extracellularly and thus, when growing on glucose or gluconate the organism produces 2-oxoglucuonate in the medium by the extracellular activity of these enzymes.

**METHODS**

**Organism.** The organism used was *Pseudomonas aeruginosa*, strain PAO, kindly provided by Professor B. W. Holloway. Previous work in this laboratory has used *P. aeruginosa* 2F32 and the major features of regulation of glucose catabolism reported with this strain (Hamilton & Dawes, 1959, 1960, 1961; Hamlin, Ng & Dawes, 1967; Ng & Dawes, 1967, 21-2.
1973; Midgley, 1972; Midgley & Dawes, 1973) have now been confirmed with strain PAO (P. H. Whiting, M. Midgley & E. A. Dawes, unpublished observations).

**Growth and maintenance of cultures.** Routine maintenance of cultures, growth of the organisms for determination of enzyme specific activities, harvesting and preparation of bacterial extracts were as previously described (Ng & Dawes, 1973; Midgley & Dawes, 1973). Large (20 l) batch cultures were grown in a fermenter operated with an air flow of 5 l/min at 37 °C and with pH controlled to 7-1.

**Preparation of bacterial extracts.** Bacterial extracts, for fractionation into membrane and soluble components, were prepared with a French pressure cell (Milner, Lawrence & French, 1950) operated at 27.6 MN·m\(^{-2}\) or by a technique employing carbenicillin and cycloserine to produce osmotically fragile cells, which were then lysed by exposure to hypotonic medium. Bacteria in mid-exponential phase in gluconate minimal medium (50 ml), were diluted, at 37 °C, with 200 ml of medium containing sucrose (50 g), carbenicillin (200 mg), cycloserine (20 mg), sodium gluconate (2.5 g) and MgSO\(_4\)·7H\(_2\)O (0.74 g), made up in the nutrient yeast broth medium of Isaac & Holloway (1968). After incubation for 6 to 8 h at least 50 to 70 % of the population showed deformed morphology when examined microscopically. The bacteria were harvested by centrifuging at 23000g for 5 min and resuspended in Mg\(^{2+}\)-glycylglycine buffer (18 ml of 120 mm-buffer, pH 7.1, plus 2 ml of 0.1 m-MgSO\(_4\)). DNase (approximately 50 μg) was added to the viscous solution which was incubated at 37 °C until free-flowing. Intact bacteria were removed by centrifuging twice at 9000g for
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5 min at 0 °C. The bacterial extract was then fractionated by centrifuging at 25000g for 1 h. The pellet was resuspended in the original volume of Mg²⁺-glycylglycine buffer and recentrifuged as before. The washed pellet was resuspended in Mg²⁺-glycylglycine buffer at a density of 0.1 to 0.2 mg protein/ml. Extracts prepared with the French Press were fractionated by ultracentrifuging as previously described (Midgley & Dawes, 1973).

The assay of enzymes of 2-oxoglucenate metabolism. De Ley & Vandamme (1955) demonstrated qualitatively an ATP-dependent production of 2-oxoglucenate 6-phosphate in extracts prepared from Pseudomonas aeruginosa. We were initially concerned with developing a quantitative assay for this kinase which could be used to determine specific activities in crude bacterial extracts containing various interfering enzymes, such as high ATPase activity, that made the results of indirect assays (e.g. those based on acid production) inaccurate. Preliminary experiments in which 2-oxoglucenate 6-phosphate was generated in situ from 2-oxoglucenate and ATP revealed that 2-oxoglucenate 6-phosphate reductase could use NADPH. As crude extracts possessed only low NADPH oxidase activity, accurate assays could be obtained by coupling the kinase and reductase activities, especially if partially purified preparations of the two enzymes, showing no cross-contamination, were available. The assay described by Brown & Romano (1969), though inapplicable for accurate studies with crude extracts because of high ATPase and high NADH oxidase activity, was used initially to follow the course of kinase purification. Once partially purified kinase preparations were available, they were used to locate reductase-containing fractions and so permitted assessment of reductase purification. The following assay systems, in which either excess reductase or excess kinase was added were devised: tris-HCl buffer (120 mM, pH 7.5), 1 ml; MgCl₂ (0.6 M), 0.1 ml; NADPH (2 mM), 0.1 ml; ATP (36 mM, pH 7.5), 0.3 ml; partially purified 2-oxoglucenate 6-phosphate reductase, 21 units; Na₂-2-oxoglucenate (50 mM), 0.1 ml; bacterial extract, 0.1 ml; and water to 3 ml. 2-Oxoglucenate 6-phosphate reductase was assayed in a similar reaction mixture except that partially purified 2-oxoglucenate kinase (2.2 units) replaced the reductase. All other enzymes were assayed as described by Ng & Dawes (1973) except that gluconate dehydrogenase was assayed in the presence of phenazine methosulphate (2 mM final concentration). All assays were conducted at 37 °C and corrected for endogenous activity. Specific activities are expressed as μmol/h/mg protein. One unit of enzyme is defined as the amount of enzyme which will catalyse the conversion of 1 μmol of substrate/h at 37 °C.

Enzyme purification. Operations were conducted at 4 °C. Bacterial extracts, prepared by sonication (Midgley & Dawes, 1973) of suspensions of Pseudomonas aeruginosa strain PAO harvested from 20 l of gluconate medium, were treated with 20 % (v/v) protamine sulphate (20 mg/ml) and the precipitate discarded. To the supernatant ammonium sulphate was added to 20% saturation, the precipitate was recovered and dissolved in EDTA (10⁻³ M, pH 7.6). This solution was adjusted to pH 6.0 with 1 M-NaOH and applied to a DEAE-cellulose column (25 × 2.5 cm). The column was washed with 65 ml of 0.06 M-phosphate buffer (10⁻³ M with respect to EDTA, pH 6.0). This was followed by gradient elution with 100 ml 0.06 M-phosphate buffer in the mixing chamber and 300 ml 0.12 M-phosphate buffer in the reservoir, both 0.005 M with respect to EDTA and pH 6.7.

Fractions (10 ml) were collected and assayed for 2-oxoglucenate kinase and 2-oxoglucenate 6-phosphate reductase. The fractions containing kinase were pooled and the kinase precipitated with ammonium sulphate (30% saturation) and redissolved in 5 ml EDTA (10⁻³ M, pH 7.6). Similarly the reductase was precipitated with 20% ammonium sulphate.

Characterization of the 2-oxoglucenate kinase fraction. Purified kinase (2.2 units) was i-
cubated in the assay system developed, except that NADPH and purified reductase were
omitted. After 90 min incubation the reaction was terminated by the addition of ethanol
(2 ml), precipitated protein removed by centrifuging and the concentration of 2-oxo com-
ounds of the supernatant was determined.

Characterization of the 2-oxoglucanate 6-phosphate reductase fraction. Purified reductase
(2 units) was incubated in the assay system developed, with the addition of purified kinase
(2·2 units) and NADPH (4 µmol). After 2 h incubation at 37 °C, when \( E_{340} \) measurements
indicated that the NADPH had been consumed, the reaction mixture was deproteinized
by the addition of ethanol (2 ml), centrifuged, and the supernatant assayed for content
of 2-oxo compounds.

Transport studies. These were performed with radioactive substrates and washed bacterial
suspensions in the presence of chloramphenicol, as described by Midgley & Dawes (1973).
Organisms were separated from the suspending medium by rapid filtration and subsequently
washed with \( \text{Mg}^{2+} \cdot (0·1 \text{ %, w/v}) \)-phosphate (0·067 M) buffer, pH 7·1, containing 2 % (w/v)
NaCl, a concentration of NaCl found necessary to overcome the effect of temperature shock
on organisms of Pseudomonas aeruginosa strain PAO.

Radiochemical techniques. The assay of radioactivity in samples from transport studies,
and on electrophoretograms or chromatograms, was as described by Midgley & Dawes
(1973).

Separation methods. Electrophoresis was carried out with either (i) 50 mM-sodium borate
(pH 9·0) or (ii) 25 mM-glycine/sodium citrate buffer (pH 1·5) on a Shandon high-voltage
electrophoresis apparatus operated at 4 kV for 30 to 60 min. The following chromatographic
solvent systems were employed: solvent system 1, pyridine:butanol:H₂O (1:1:1 by vol.),
descending; and solvent system 2, pyridine:NH₃OH (sp.gr. 0·88):H₂O (6:2:1, by vol.),
descending. All separations were effected on Whatman no. 1 paper. Marker compounds
were made visible by the methods of Midgley & Dawes (1973).

Preparation of 2-oxo[¹⁴C]gluconate. This substrate was prepared enzymatically in Warburg
manometers from [¹⁴C]gluconate (3·4 mCi/mm, Radiochemical Centre, Amersham,
Buckinghamshire) using a crude bacterial extract prepared from gluconate-grown organ-
isms. The manometer flasks contained in the main compartment: bacterial extract (20 mg
protein/ml), 1·5 ml; water, 0·8 ml; and in the side arm: sodium gluconate, 10·9 µmol in
0·5 ml, or 0·5 ml of water (for measuring the endogenous rate of oxygen consumption). The
centre well contained a roll of Whatman no. 541 filter paper and 0·2 ml of 20 % (w/v) KOH.
Oxygen uptake was followed for 6 h, after which period the endogenous and reaction flasks
showed the same rates. The reaction mixture was deproteinized by the addition of 1·5 M-
HClO₄ (1 ml) and centrifuged. The supernatant was neutralized with a solution containing
(final concentrations) KOH (5 M), EDTA (0·4 M), imidazole buffer (1 M) and the precipitate
removed by further centrifugation at 0 °C. The solution was then concentrated to 1 ml by
rotary evaporation under reduced pressure at 40 °C and applied as a streak on to a chroma-
togram that was then developed in solvent system 1 for 46 h. Appropriate markers of gluco-
nate and 2-oxoglucanate were also applied. After drying the chromatogram the 2-oxo[¹⁴C]-
gluconate was eluted from the appropriate areas. Preliminary electrophoresis of this material
in system (i) revealed contamination with gluconate, to the extent of 5 %. This gluconate was
removed by electrophoresis with system (i) of all the material derived from the chromato-
gram. 2-Oxoglucanate was eluted from the electrophoretogram with water (20 ml). Incuba-
tion of suitable samples of this material, to which unlabelled gluconate (0·36 µmol) was
added as a carrier, with gluconokinase followed by electrophoresis of a sample of the reaction
mixture using system (ii), failed to detect any radioactive gluconate 6-phosphate.
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Table 1. Purification of 2-oxoglucunate kinase and 2-oxoglucunate 6-phosphate reductase

<table>
<thead>
<tr>
<th></th>
<th>Supernatant from protamine sulphate precipitation</th>
<th>0-20 % (NH₄)₂SO₄ precipitate</th>
<th>(NH₄)₂SO₄ precipitate from pooled DEAE-cellulose column eluate</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Bacterial extract</td>
<td></td>
<td>Kinase</td>
</tr>
<tr>
<td>Volume (ml)</td>
<td>160</td>
<td>103</td>
<td>5</td>
</tr>
<tr>
<td>Total protein (mg)</td>
<td>2320</td>
<td>1991</td>
<td>103</td>
</tr>
<tr>
<td>Total units</td>
<td></td>
<td>1991</td>
<td>1991</td>
</tr>
<tr>
<td>Kinase</td>
<td>2157</td>
<td>1.52</td>
<td>103</td>
</tr>
<tr>
<td>Reductase</td>
<td>1995</td>
<td>12.91</td>
<td>96</td>
</tr>
<tr>
<td>Specific activity (μmol/h/mg protein)</td>
<td>0.93</td>
<td>1.42</td>
<td>0.86</td>
</tr>
<tr>
<td>Recovery (%)</td>
<td></td>
<td>36</td>
<td>96</td>
</tr>
<tr>
<td>Purification</td>
<td></td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Kinase</td>
<td></td>
<td>93</td>
<td>36</td>
</tr>
<tr>
<td>Reductase</td>
<td></td>
<td>93</td>
<td>32</td>
</tr>
</tbody>
</table>

Control experiments demonstrated that under the conditions employed there was full recovery of added gluconate.

Chemicals. Calcium 2-oxoglucunate was obtained from Sigma Chemical Co., St Louis, Missouri, U.S.A. and converted to its sodium salt by treatment with Dowex 50 (H⁺ form) followed by subsequent neutralization with 10 M-NaOH. Cycloserine was also supplied by Sigma. Carbenicillin was obtained from Beecham Research Laboratories, Brentford, Middlesex.

Analyses. Protein was determined by either the biuret reagent (Gornall, Bardawill & David, 1949) or the method of Lowry, Rosebrough, Farr & Randall (1951). 2-Oxoglucunate was determined as described by Lanning & Cohen (1951). Gluconate was determined with gluconokinase and 6-phosphogluconate dehydrogenase, purchased from Boehringer und Soehne (Mannheim, Germany) and used as described in their handbook.

RESULTS

The enzymes of 2-oxoglucunate metabolism. Preliminary experiments demonstrated the presence of 2-oxoglucunate kinase and 2-oxoglucunate 6-phosphate reductase in extracts prepared from Pseudomonas aeruginosa grown either on 2-oxoglucunate or gluconate. The purification of these enzymes is described in the Methods section and Table 1. DEAE-cellulose chromatography effectively separated the two activities (Fig. 2) and yielded approximately 100-fold purified enzymes. Since the transformations that these fractions catalyse, when incubated with either 2-oxoglucunate plus ATP or 2-oxoglucunate 6-phosphate plus NADPH, are critical for any interpretation of the data obtained by their use, the reactions were characterized.

Characterization of the 2-oxoglucunate kinase fraction. Fig. 3(a) shows an electrophorotogram of the products after incubation of partially-purified 2-oxoglucunate kinase with 2-oxo[¹⁴C]gluconate and ATP. The compound at the origin was unchanged 2-oxoglucunate while the second compound detected, which accounted for approximately 50% of the applied radioactivity, had the mobility of a hexose phosphate. Quantitative recovery of
Fig. 2. Separation of 2-oxoglucuronate kinase and 2-oxoglucuronate 6-phosphate reductase by DEAE-cellulose chromatography. The procedure described in Methods and Table I was followed. ○, Protein; □, 2-oxoglucuronate kinase activity; △, 2-oxoglucuronate 6-phosphate reductase activity.

Fig. 3. (a) Electrophoresis of 2-oxoglucuronate kinase reaction mixture. Purified kinase (2.2 units) was incubated with ATP and 2-oxoglucuronate as described in Methods, except that 2-oxo[14C]gluconate (5 mM final concn., 0.1 μCi/μmol) was used. The reaction was terminated with ethanol after incubation for 90 min at 37°C. Electrophoresis was carried out with buffer system (ii). ——, Reaction terminated at zero time; ———, reaction terminated after 90 min. 6PG, 6-phosphogluconate; 2-OG, 2-oxoglucuronate.

(b) Chromatography of 2-oxoglucuronate kinase reaction mixture. The procedure described in the legend to Fig. 3(a) was followed except that the incubation period was 30 min. Solvent system 2 was used.

applied radioactivity was recorded. Chromatography of a similar reaction mixture demonstrated that a compound with a mobility similar to 6-phosphogluconate was produced (Fig. 3b). This compound was employed as a marker since authentic 2-oxo-6-phosphogluconate was not available. The compound produced, however, was not 6-phosphogluconate since there was no disappearance of the '2-oxo' function after 90 min incubation under phosphorylating conditions. The addition of 6-phosphogluconate dehydrogenase and NADP to an incubation mixture in which the kinase had been allowed to act did not produce any increase in $E_{340}$, though addition of authentic 6-phosphogluconate did. The production
of the phosphorylated derivative was ATP-dependent, and there was no contamination of the preparation with reductase activity.

**Characterization of 2-oxoglutarate 6-phosphate reductase fraction.** Purified 2-oxoglutarate kinase was used to generate 2-oxoglutarate 6-phosphate in situ. The product of the reductase activity was characterized as 6-phosphogluconate by the observation that the ‘2-oxo’ function disappeared stoichiometrically with concomitant NADPH consumption and the demonstration that the product was a substrate for 6-phosphogluconate dehydrogenase. With the techniques described in Methods (when 5 µmol of 2-oxoglutarate, ATP and purified kinase were incubated with 4 µmol of NADPH), when all the NADPH was oxidized, 0·6 µmol of ‘2-oxo’ function remained. That the reduced product was a substrate for 6-phosphogluconate dehydrogenase was shown by the addition of 6-phosphogluconate dehydrogenase to the reductase assay system, after reaction (to the extent of 80 %) had been allowed to occur; an immediate increase in $E_{340}$ was recorded. The absence of 2-oxoglutarate kinase from this fraction was demonstrated by incubation with 2-oxoglutarate, ATP and NADPH in the absence of added kinase, when NADPH oxidation was not observed.

**Specific activities of the enzymes of 2-oxoglutarate metabolism.** Specific activities of these enzymes in extracts derived from bacteria grown on a variety of media are in Table 2. The activities detected were directly proportional to the amounts of bacterial extract added to the assay system. Since we desired to relate these activities to the activities of the enzymes of the major glucose catabolic pathways, the other enzymes shown were also assayed.

**Transport of 2-oxoglutarate and gluconate.** The ability of organisms grown on different media to transport 2-oxoglutarate or gluconate was assessed by measuring the initial rate of uptake of $^{14}$C substrate as Midgley & Dawes (1973) described for glucose transport (Table 3).

**The role of gluconate dehydrogenase.** Midgley & Dawes (1973) have shown that glucose dehydrogenase acts extracellularly to produce gluconate, and the possibility that gluconate dehydrogenase might act in a similar manner was important for understanding the role of the enzymes of 2-oxoglutarate metabolism in this organism. A previous investigation (Campbell, Hogg & Strasdine, 1962) suggested that the enzyme was not membrane-bound but our present studies indicate that it is, although the exact proportion that is demonstrably membrane-bound depends on the method of breakage (Table 4). The highest proportion of membrane-bound gluconate dehydrogenase was observed with membranes prepared by the carbenicillin-cycloserase procedure, and this ranged between 40 and 80 % with different preparations.

That gluconate dehydrogenase acts extracellularly was established by the application of the criterion previously applied to glucose dehydrogenase activity, i.e. the rate of uptake of $^{14}$C from $[^{14}$C]gluconate is far less than the rate of gluconate disappearance from the medium.

The initial rate of transport was measured in duplicate over the first min of incubation, and the rate of gluconate consumption was determined with an identical incubation mixture from which samples (0·5 ml) were removed over a 20 min period; the initial gluconate concentration was 5 mM and the bacterial density in each case was 460 µg/ml. The rates of $^{14}$C uptake and of gluconate utilization were respectively, 30 and 440 µmol/g dry wt/min. The apparent $K_m$ (1 mm) of gluconate dehydrogenase, determined from a conventional double reciprocal plot, is identical with that previously measured for glucose dehydrogenase acting on glucose (Midgley & Dawes, 1973). This value was obtained with a crude bacterial extract (0·14 mg protein/assay) produced by sonication of gluconate-grown organisms, and prepared as described by Midgley & Dawes (1973).
Table 2. Assay of enzymes of 2-oxoglucunonate metabolism of Pseudomonas aeruginosa PAO grown on various carbon sources

The pairs of figures are the values obtained with independently derived bacterial extracts.

<table>
<thead>
<tr>
<th></th>
<th>Glucose dehydrogenase</th>
<th>Glucose 6-phosphate dehydrogenase</th>
<th>2-Oxoglucunonate dehydrogenase</th>
<th>2-Oxoglucunonate 6-phosphate kinase</th>
<th>2-Oxoglucunonate 6-phosphate reductase</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-Oxoglucunonate</td>
<td>2.9</td>
<td>85.5</td>
<td>21.6</td>
<td>2.0</td>
<td>1.8</td>
</tr>
<tr>
<td>Glucose</td>
<td>2.7</td>
<td>78.5</td>
<td>17.8</td>
<td>2.3</td>
<td>1.9</td>
</tr>
<tr>
<td>Gluconate</td>
<td>2.4</td>
<td>71.8</td>
<td>30.6</td>
<td>1.8</td>
<td>1.6</td>
</tr>
<tr>
<td>Succinate</td>
<td>2.3</td>
<td>76.4</td>
<td>30.6</td>
<td>1.8</td>
<td>1.5</td>
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<tr>
<td>Citrate</td>
<td>2.2</td>
<td>86.2</td>
<td>30.4</td>
<td>1.8</td>
<td>1.4</td>
</tr>
<tr>
<td>Glycerol</td>
<td>2.5</td>
<td>84.9</td>
<td>30.4</td>
<td>1.8</td>
<td>1.6</td>
</tr>
<tr>
<td>Phenazine methosulphate not added.</td>
<td>0.1</td>
<td>1.3</td>
<td>1.2</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>Citrate</td>
<td>0.1</td>
<td>2.3</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>Glycerol</td>
<td>1.9</td>
<td>78.1</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
</tbody>
</table>

* Phenazine methosulphate not added. n.d., Enzyme was not detected.

Table 3. Transport of 2-oxoglucunonate and gluconate by Pseudomonas aeruginosa PAO grown on various carbon sources

Transport rate was measured over the first min after the addition of substrate (2-oxoglucunonate 1 mM final concn, specific activity 0.1 μCi/μmol; gluconate, 5 mM final concn, 0.3 μCi/μmol) from four samples taken during this period. The values are the averages of duplicate determinations in which there was less than 10% variation. Different bacterial suspensions were used for each determination. The rate was linear over the period of measurement in every case.

<table>
<thead>
<tr>
<th>Growth substrate</th>
<th>Uptake of [14C] substrate (μmol/min/g dry wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-Oxoglucunonate</td>
<td>37.5</td>
</tr>
<tr>
<td>Glucose</td>
<td>18.1</td>
</tr>
<tr>
<td>Gluconate</td>
<td>36.3</td>
</tr>
<tr>
<td>Citrate</td>
<td>n.d.</td>
</tr>
<tr>
<td>Glycerol</td>
<td>n.d.</td>
</tr>
<tr>
<td>n.d., Not detected; n.p., not performed.</td>
<td></td>
</tr>
</tbody>
</table>

DISCUSSION

Our results show that 2-oxoglucunonate is metabolized by the route shown in Fig. 1. Confirmation that this represents the sole pathway of 2-oxoglucunonate dissimilation awaits the isolation of mutants unable to grow on this compound and an analysis of their enzyme defect. Specific activities similar to those recorded here for the kinase have been demonstrated for the kinase of Pseudomonas fluorescens (Narrod & Wood, 1956).
Table 4. Comparison of the effect of different methods of bacterial breakage on the amount of membrane-bound gluconate dehydrogenase obtained

<table>
<thead>
<tr>
<th>Method of producing cell extract</th>
<th>Specific* activity</th>
<th>Total units</th>
<th>Supernatant</th>
<th>Pellet</th>
<th>Washed pellet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sonication</td>
<td>380</td>
<td>16 500</td>
<td>12 600†</td>
<td>—</td>
<td>4000</td>
</tr>
<tr>
<td>French press</td>
<td>330</td>
<td>26 800</td>
<td>24 000†</td>
<td>—</td>
<td>4400</td>
</tr>
<tr>
<td>Penicillin-cycloserine</td>
<td>54</td>
<td>340</td>
<td>128‡</td>
<td>194</td>
<td>176</td>
</tr>
</tbody>
</table>

* μmol substrate utilized/h/mg protein. † From 90 min at 105 000g. ‡ From 60 min at 25 000g.

The kinase and reductase enzymes, and also 2-oxogluconate transport, are present when the organism grows on glucose or gluconate. The role of these enzymes under such conditions is clarified by the demonstration that the two enzymes of the non-phosphorylative pathway, glucose and gluconate dehydrogenases, act extracellularly, thus generating 2-oxogluconate. When the organism grows on glucose the amount of glucose carbon committed to the phosphorylative pathway is presumably determined by the activity of the transport systems for glucose and gluconate. We have previously characterized the glucose transport system (Midgley & Dawes, 1973) but our evidence for the existence of a unique gluconate transport system at present is based upon the observation that glycerol-grown Pseudomonas aeruginosa can transport [14C]gluconate in the presence of chloroamphenicol (Table 3). Table 2 reveals that glycerol-grown organisms possess neither the enzymes of 2-oxogluconate metabolism nor a 2-oxogluconate transport system (Table 3), and therefore the intracellular accumulation of gluconate by such bacteria indicates a means of uptake other than by extracellular conversion to 2-oxogluconate and its subsequent transport. The apparent \( K_a \) of both enzymes of the non-phosphorylative pathway is \( 1 \) mM, and although the transport systems act at lower external concentrations, e.g. the \( K_a \) for glucose uptake is \( 8 \) μM (Midgley & Dawes, 1973), the excess of these activities (10- to 20-fold) over the transport activities is sufficient to commit a major portion of the carbon of glucose and gluconate to the non-phosphorylative pathway. Indeed, this property has allowed the isolation of mutants defective in glucose dehydrogenase, since these organisms do not now produce an acidic reaction on a suitable indicator medium (Midgley & Dawes, 1973).

MacKechnie & Dawes (1969) showed that Pseudomonas aeruginosa gives approximately the same molar growth yield for glucose and gluconate. If it is assumed that the extracellular conversion of glucose to gluconate yields no energy to the organism, then growth on glucose is almost equivalent to growth on gluconate since such a large proportion of glucose is converted to gluconate by glucose dehydrogenase. However, if similar assumptions are made concerning the extracellular conversion of gluconate to 2-oxogluconate it would be expected that similar molar growth yields would have been observed for these compounds. A significant difference was recorded (MacKechnie & Dawes, 1969), thus the question of whether any energy is available for these extracellular conversions remains unanswered. However, our results do offer some explanation for the apparent needless conversion of glucose and gluconate to 2-oxogluconate, which is then converted to 6-phosphogluconate, a compound which can be more directly produced from glucose and gluconate. The two pathways, phosphorylative and non-phosphorylative, are found in separate compartments, inside and outside the organism respectively.

As might be expected from previous studies concerning the regulation of the major
glucose-catabolizing enzymes (Ng & Dawes, 1973), the enzymes of 2-oxoglucconate metabolism are absent when the organism is grown on organic acids such as citrate and succinate (Table 2). These enzymes, and the 2-oxoglucconate transport system, are probably controlled independently from the previously studied glucose-catabolizing enzymes since the glycerol-grown organism is unable to metabolize 2-oxoglucconate and yet shows high specific activities of the glucose-catabolizing enzymes, including the enzymes of the non-phosphorylative pathway.

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REFERENCES


Metabolism of 2-oxogluconate by P. aeruginosa


