

GLUCOSE OXIDATION BY *SERRATIA MARCESCENS*¹

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Abstract

Fresh cell suspensions of glucose-grown *Serratia marcescens* oxidize glucose, gluconic acid, and 2-ketogluconate with an uptake of 3.0, 2.5, and 2.0 μ moles of oxygen per μ M. substrate, respectively. Approximately two μ M. of CO₂ per μ M. of each substrate are evolved. 6-Phosphogluconate, glucose-6-phosphate, and hexose diphosphate do not appear to be intermediates in the normal pathway of glucose oxidation. Dried-cell preparations of *S. marcescens* oxidize glucose and gluconate in a two-stage pattern, with an initial uptake of 1.0 and 0.5 μ M. O₂/ μ M. substrate, respectively. 2-Ketogluconate was identified chromatographically in these solutions. In the second stage, both added and metabolically formed 2-ketogluconate are further oxidized to the same oxidation level attained with the whole cell, i.e., 3.0, 2.5, and 2.0 μ M. O₂/ μ M. glucose, gluconate, and 2-ketogluconate, respectively. An as yet unidentified acidic compound has been isolated from the solution of metabolites of a glucose oxidation by these organisms.

Introduction

In the first systematic investigation of glucose dissimilation by *Serratia marcescens* it was found that lactic acid was produced in large quantities. Substantial amounts of acetoin and 2,3-butylene glycol, and traces of ethanol, acetic acid, and formic acid were also formed (8). One strain of *Serratia marcescens* formed approximately 12% succinic acid. Further investigation of glucose metabolism (5), indicated that approximately 50% of the glucose went to form 2,3-butylene glycol. The remainder gave rise to lactic acid and ethanol, with traces of glycerol and succinic acid also being found. The production of hydrogen anaerobically led to a scheme for the division of the genus *Serratia* into three groups. In two of these groups 2,3-butylene glycol is the major end product, and in the third group glucose is dissimilated primarily to acetic acid (4, 6).

The metabolic pathways by which *S. marcescens* dissimilates glucose have not been investigated. However, with a cell-free extract of a 2-ketogluconate adapted strain of *S. plymouthicum* it has been shown that 2-ketogluconate was phosphorylated to 2-keto 6-phosphogluconate (2).

The studies reported here indicate that cells of glucose-grown *S. marcescens* dissimilate glucose via gluconic and 2-ketogluconic acids to an acidic end product which has not yet been identified.

Materials and Methods

A laboratory strain of *S. marcescens*, obtained from the Northern Utilization Research Branch, U.S. Department of Agriculture, was grown in a glucose-peptone-milk protein medium on a reciprocal shaker at 37° for 17 hr. Cell suspensions in water were adjusted to the required dry weight, using a

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precalibrated dry weight vs. turbidity curve prepared with a Beckman model B spectrophotometer. Freeze-dried preparations of the organism were obtained by freezing a thick cell suspension at -70° , and vacuum drying with a Hy-Vac pump.

Warburg determinations were carried out by conventional manometric techniques. Gas exchange values are reported as μM . of gas (O_2 or CO_2) per μM . of substrate. The data reported are the average values of three to six experiments each.

Chromatographic analysis of glucose, gluconate, and 2-ketogluconate was carried out by modification of the methods of Partridge (7) and White (11). The paper chromatograms were developed by ascending technique in methanol : ethanol : water (45 : 45 : 10) solvent. After drying, the paper was sprayed with triphenyltetrazolium chloride reagent (50 mgm. triphenyltetrazolium chloride dissolved in a mixture of: 5% sodium hydroxide, 10 ml.; *n*-butanol, 30 ml.; ethanol, 20 ml.). Color was developed by steaming the paper. Glucose and 2-ketogluconate appeared as cherry-red spots. Gluconate did not react with the reagent. After drying, the paper was sprayed with silver nitrate reagent (0.1 *N* silver nitrate in 5 *N* ammonium hydroxide). When the paper was free of ammonia, steam was again applied. Gluconate, with an R_f similar to that of 2-ketogluconate, appeared as a dark brown spot.

Acidic compounds were determined chromatographically by the method of Martin (3). Chromatographic analysis for phosphate esters was performed by the method of Bandurski and Axelrod (1). Quantitative colorimetric analyses were carried out by the methods of Somogyi (9) for glucose, Westerfeld (10) for acetone, and Winnick (12) for 2,3-butylene glycol.

Materials

Gluconolactone, from Eastern Chemical Corporation² was the source of gluconic acid used in these studies. 6-Phosphogluconate was obtained from the Delta Chemical Works, Inc. 2-Ketogluconate was supplied by the Northern Utilization Research Branch, Peoria, Illinois, through the courtesy of Dr. F. H. Stodola. Glucose-6-phosphate was obtained from the Nutritional Biochemical Corp. Hexose diphosphate was obtained from Schwarz Laboratories.

Results

The oxidative behavior of one preparation of a fresh cell suspension of *S. marcescens* on glucose and several possible intermediates is shown in Fig. 1. Glucose, gluconate, and 2-ketogluconate were oxidized with an uptake of approximately 3.0, 2.5, and 2.0 μM . O_2 respectively. Glucose-6-phosphate and 6-phosphogluconate were oxidized to a level of 1.0–1.5 μM . O_2 depending upon the cell preparation, while hexose diphosphate was metabolized to a level of about 0.7 μM . O_2 . Table I shows CO_2 evolution from these substrates and O_2 uptake values obtained simultaneously. Approximately 2 μM . of

²Mention of trade names or companies does not imply recommendation by the United States Department of Agriculture over similar products not mentioned.

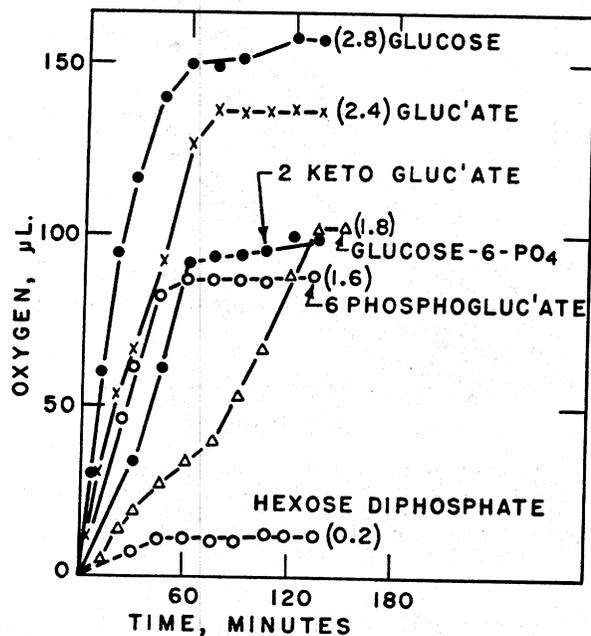


FIG. 1. Metabolic pattern of fresh *S. marcescens*. Each flask contains 0.5 ml. 0.05 M Tris (hydroxymethyl) amino methane buffer, pH 7.2; 100 γ inorganic phosphorus; 0.5 ml. cell suspension (containing 4 mgm. dry weight); 2.5 μ M. of appropriate carbohydrate; water to 2.0 ml. All values are corrected for endogenous metabolism. Numbers in parenthesis are μ M. O₂/ μ M. substrate.

TABLE I
GAS EXCHANGE DURING OXIDATION BY *S. marcescens*

	O ₂ *	CO ₂ *
Glucose	3.0	2.4
Gluconate	2.2	2.2
2-Ketogluconate	2.0	1.9
6-Phosphogluconate	1.3	2.1
Glucose-6-phosphate	1.0	1.5
Hexose diphosphate	0.7	0.8

* μ M. gas/ μ M. substrate.

Each flask contains 0.5 ml. 0.05 M Tris (hydroxymethyl) amino methane buffer, pH 7.2; 100 γ inorganic phosphorus; 0.5 ml. cell suspension (containing 4 mgm. dry weight); 2.5 μ M. of appropriate substrate; water to 2.0 ml. Values are corrected for endogenous metabolism. These values are the average of three to six experiments with several cell preparations.

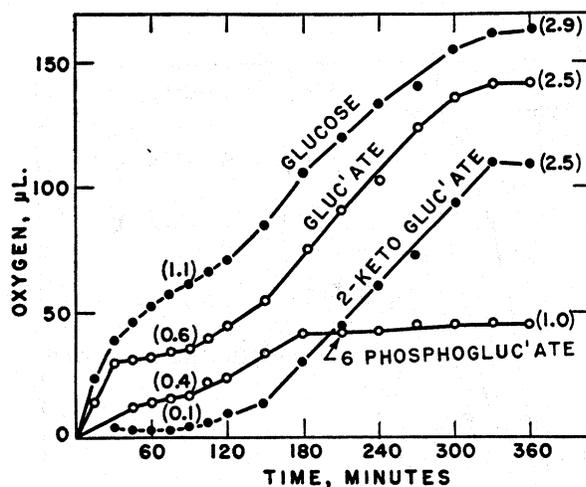


FIG. 2. Metabolic pattern of freeze-dried *S. marcescens*. Each flask contains 0.5 ml. 0.05 M Tris (hydroxymethyl) amino methane buffer, pH 7.2; 100 γ inorganic phosphorus; 0.5 ml. dried-cell suspension (containing 8 mgm. dry weight); 2.5 μ M. of appropriate carbohydrate; water to 2.0 ml. All values are corrected for endogenous metabolism. Numbers in parenthesis are μ M. O₂/ μ M. substrate.

CO₂ were formed from each μ M. of glucose, gluconate, 2-ketogluconate, and 6-phosphogluconate. Approximately 1 μ M. of CO₂ was obtained from glucose-6-phosphate and hexose diphosphate.

In an attempt to determine whether permeability was a factor in metabolic behavior of the phosphorylated intermediates, freeze-dried cells of *S. marcescens* were employed in a similar series of experiments. As shown in Fig. 2, the rates of oxidation of all substrates by the dried cells were generally lower than that of fresh cells. The oxidation of glucose and gluconate occurred in a two-stage pattern. First, glucose and gluconate were metabolized with an uptake of 1.0 and 0.5 μ M. O₂ respectively. 2-Ketogluconate was oxidized slowly, if at all. In the second stage, the rate of 2-ketogluconate oxidation increased, and the oxidation of glucose and gluconate were resumed. The rate of oxidation of glucose and gluconate paralleled that of the 2-ketogluconate oxidation, leading to the supposition that the same material was being utilized in all three instances. The final levels of oxidation for these substrates were the same as for fresh cell suspensions, namely 3.0 μ M. O₂ for glucose, 2.5 μ M. O₂ for gluconate, and 2.0 μ M. O₂ for 2-ketogluconate. Hexose diphosphate was not oxidized.

6-Phosphogluconate was metabolized slowly to a level of 0.5 μ M. O₂, then the oxidation continued to a final level of 1.0–1.5 μ M. O₂.

Chromatographic analysis of the metabolite solutions at stage one revealed the presence of 2-ketogluconate in the flasks originally containing glucose and gluconate and not in the solution containing 6-phosphogluconate. Analysis of the solutions at the completion of the experiments showed the complete absence of 2-ketogluconate in the solutions in which it had accumulated.

Chromatographic analyses for phosphate esters were carried out at various times during the course of oxidation, and at no time were any phosphorylated compounds found in the solutions containing glucose, gluconate, or 2-ketogluconate.

Colorimetric analysis of the solutions indicated that when glucose was used as substrate it disappeared by the completion of the experiment. Acetoin was not formed in the solutions originally containing glucose, gluconate, 2-ketogluconate, or 6-phosphogluconate. 2,3-Butylene glycol was not found in the oxidized solutions of glucose and 2-ketogluconate.

However, the gas exchange values indicate that glucose dissimilation was accomplished with 50% of the O_2 uptake and 33% of the CO_2 evolution expected if glucose was completely oxidized to CO_2 and water. The accumulation of an end product was suspected. Glucose, in the Warburg medium described under Fig. 1, was oxidized by a suspension of fresh cells until the reaction attained the rate of endogenous metabolism after taking up $3.0 \mu M. O_2$. Following the removal of the cells by centrifugation, and the halting of further enzymatic activity by either boiling the metabolite solution or adding 20% trichloroacetic acid, the solution was run through a cation exchange column (Dowex-50) and then through an anion exchange column (Duolite A-4). The neutral, basic, and acidic fractions were examined chromatographically. In the acidic fraction there was an accumulation of material great enough to be investigated. Using larger quantities of metabolite solution, an acidic, saturated, nonreducing compound was isolated. Chemical identification of this compound has not been completed, but chromatographically it does not appear to be identical to many acidic compounds normally found as end products of glucose metabolism.

Discussion

Fresh cell suspensions of *S. marcescens*, grown on a glucose-containing medium, oxidized glucose, gluconate, and 2-ketogluconate rapidly without an initial lag period. The final values of oxygen utilization— $3.0 \mu M.$ for glucose, $2.5 \mu M.$ for gluconate, and $2.0 \mu M.$ for 2-ketogluconate—were in the same relative order as the initial oxidative levels of these substrates, indicating that the same end product was formed from all three substrates. This relation was further confirmed by the evolution of approximately the same amount of CO_2 , $2.0 \mu M.$ for each of the substrates. If 6-phosphogluconate were an intermediate compound in the metabolic pathway it would be expected that the amount of O_2 taken up would be the same as that for gluconic acid. However, the fact that $1 \mu M.$ less O_2 was utilized suggests that 6-phosphogluconate is oxidized via another path. Impermeability of the cell to 6-phosphogluconate may be discounted, inasmuch as the initial rate of 6-phosphogluconate oxidation was as rapid as that of the other substrates. Glucose-6-phosphate was oxidized to the same level as 6-phosphogluconate. If glucose-6-phosphate was first oxidized to 6-phosphogluconate it should show an O_2 uptake of $0.5 \mu M.$ greater than that of 6-phosphogluconate. In addition, the initial rate

of glucose-6-phosphate was slow, suddenly increasing to parallel the rates of the other substrates. This lag could be caused by the conversion of glucose-6-phosphate into a more readily oxidizable compound, or by the need for the formation of enzymes capable of utilizing the substrate itself.

The low oxidation of hexose diphosphate may be explained by the impermeability of the cell to hexose diphosphate, or by the lack of enzymes capable of converting hexose diphosphate into a more utilizable form.

The utilization of freeze-dried preparations of *S. marcescens* further confirmed the fact that glucose was oxidized via gluconate and 2-ketogluconate. The cells thus treated oxidized glucose and gluconate to 2-ketogluconate, which accumulated temporarily. During the same time period the cells were able to oxidize 2-ketogluconate very slowly, if at all. Oxidation of 2-ketogluconate then began at the same time in all three substrate solutions and continued to the same oxidation level for each substrate attained by the fresh cells. It appears, therefore, that during the freeze-drying treatment the cells are injured to such an extent that their normal metabolic pattern is interrupted. The nature of this injury is unknown. Altered permeability may allow leakage of a cofactor necessary for 2-ketogluconate oxidation when the dried cells are restored in water. The lag period would be required for the cell to rebuild the missing component. This would be true also if the enzyme responsible for this oxidation were destroyed in freezing or dehydration.

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