STUDIES ON THE OXIDATION OF GLUCOSE BY PSEUDOMONAS FLUORESCENS[1]

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Lockwood, Tabenkin, and Ward (1941) showed that many strains of fluorescent pseudomonads, grown in complex media containing a high concentration of glucose, will convert this sugar to gluconic acid and in some cases further to 2-ketogluconic acid, often with yields approximating 90 per cent of theory. These observations suggest that the metabolism of glucose by fluorescent pseudomonads may involve a direct oxidative attack, rather than a degradation via the glycolytic pathway; but the evidence cited above cannot be considered as conclusive proof that oxidation via gluconic acid is the only pathway for the breakdown of glucose. The extremely high concentrations of glucose employed, (over 10 per cent) might well have caused a derangement of normal carbohydrate metabolism, and called into operation a shunt mechanism not functional, as a rule, to any appreciable extent. The following experiments were designed to investigate the nature of glucose metabolism by *Pseudomonas fluorescens* under more normal physiological conditions.

METHODS

Pseudomonas fluorescens, strain A, 3.12, was employed exclusively. Cultures were grown on a medium of the following composition: NH_4NO_3 0.1 per cent, K_2HPO_4 0.1 per cent, $MgSO_4$ 0.05 per cent, carbon source (glucose or asparagine) 0.15 to 0.25 per cent, agar 2.0 per cent, pH adjusted to 7.0. Incubation was at 30 C. Cells for experimental use were harvested from cultures 24 hours old, washed once, and resuspended in buffer (M/30 phosphate, pH 7.0, unless otherwise indicated).

Respiratory gas exchanges were determined with the Warburg apparatus in the usual manner. Acid production was estimated by measuring the carbon dioxide released from bicarbonate buffer, the observed values being corrected for the accompanying respiratory gas exchange which was determined by simultaneous measurements on cells in phosphate buffer. All manometric experiments were conducted at 30 C.

In some experiments it was necessary to block adaptive enzyme formation without disturbing the pre-existing enzymatic activities of the cells. This was done by exposure of cell suspensions to ultraviolet irradiation, a technique whose selective inhibitory effect on adaptations by *P. fluorescens* was discovered by Lederberg and Stanier (unpublished). The light source employed was a commercial ultraviolet lamp of undetermined intensity. The dosage necessary to

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prevent adaptation was ascertained empirically, by exposing aliquots of a cell suspension to irradiation for different periods of time and subsequently testing their capacity to adapt. Since the effect of ultraviolet light on the adaptive process is in part photoreactivable, it is advisable to use a dose somewhat larger than the minimum dose needed to inhibit adaptation, unless subsequent experiments are conducted in the dark. In our experience, the amount of irradiation necessary to cause appreciable damage to pre-existing oxidative enzyme systems is far greater than that required to inhibit adaptation; consequently, the working range of exposures for selectively blocking adaptive enzyme formation is a fairly wide one. Once the required dosage had been determined empirically as previously described, identical working conditions (distance of lamp, period of

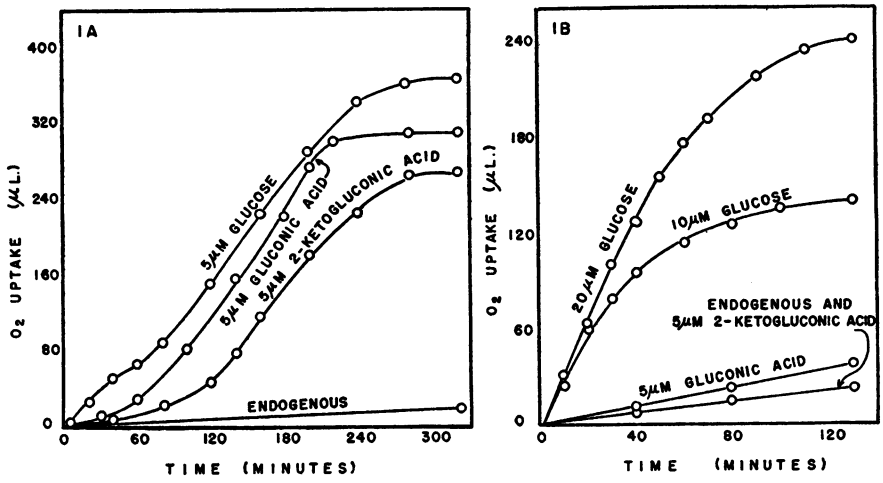

Figure 1. Oxidation of glucose, gluconic acid, and 2-ketogluconic acid by asparagine-grown cells; (A) nonirradiated, (B) after irradiation with ultraviolet light.

illumination, density and volume of cell suspension, degree of agitation during exposure) were employed in all subsequent experiments.

RESULTS

Oxidative behavior of asparagine-grown cells. Figure 1A shows the oxygen uptake by asparagine-grown cells of *P. fluorescens*, when furnished with limiting amounts of glucose, gluconic acid, and 2-ketogluconic acid. Attack on the two acids is characterized by a marked adaptive lag, although eventually both are oxidized at fairly high rates. Glucose, on the other hand, gives rise to an immediate rapid uptake of oxygen which declines after a small total volume has been consumed, and then increases again to a rate that is eventually almost identical with the maximal rate of oxidation of gluconic acid. The total oxygen consumption at the time of the first break with glucose is very close to the theoretical value (one atom per mole of substrate) for an oxidation to gluconic acid, and there is a negligible production of carbon dioxide during this period. The data in figure 1A

are thus most reasonably interpreted by assuming that asparagine-grown cells are not adapted to oxidize either gluconic or 2-ketogluconic acid, but contain the enzyme system necessary to oxidize glucose to gluconic acid. This interpretation was confirmed by a study of the oxidative behavior of asparagine-grown cells that had been exposed to ultraviolet irradiation (figure 1B). Such cells are incapable of adaptation and show a negligible capacity to oxidize either gluconic or 2-ketogluconic acid even after 2 hours; their capacity to carry out the initial rapid oxidation of glucose is unaffected, although the later, secondary oxygen uptake shown by nonirradiated cells in the presence of glucose is completely suppressed. As can be seen from figure 1B, the total oxygen consumption by irradiated cells at the expense of glucose is very close to one atom per mole of substrate. The oxidation of glucose under these circumstances proceeds with an R.Q. of zero, and is accompanied by the formation of one equivalent of acid per mole

TABLE 1

Manometric data on the oxidation of glucose by cells grown on asparagine and then exposed to ultraviolet irradiation

Glucose oxidized: 10 micromoles

	OBSERVED VALUE, MICROLITERS	MOLES PER MOLE OF GLUCOSE OXIDIZED
O_2 uptake, endogenous	23	
O_2 uptake with substrate	141	
Net O_2 uptake with substrate	118	0.53
CO_2 output, endogenous	23	
CO_2 output with substrate	23	
Net CO_2 output with substrate	0	0.00
Acid formation with substrate	236	1.05

of glucose decomposed (table 1), in full accordance with the requirements of the reaction:

$$\text{glucose} + 0.5\ O_2 \rightarrow \text{gluconic acid.}$$

In order to characterize the product, a large batch of cells was grown in liquid asparagine medium, washed, resuspended in 275 ml of phosphate buffer, and irradiated. To the irradiated suspension 275 mg of glucose were added, and the mixture was incubated at 30 C on a mechanical shaker under aerobic conditions until all the glucose had disappeared, the course of the oxidation being determined manometrically upon an aliquot of the suspension. Then the cells were removed by centrifugation, and the supernatant liquid was concentrated *in vacuo* to a small volume. The phenylhydrazide of gluconic acid was prepared from the concentrate; following recrystallization, it melted at 197 to 200 C (uncorr), and the melting point was not depressed by mixture with the phenylhydrazide prepared from commercial gluconic acid.

Oxidative behavior of glucose-grown cells. The oxidation of glucose, gluconic and 2-ketogluconic acids by glucose-grown cells proceeds in a radically different

manner, shown in figure 2A. There is a much more extensive rapid oxygen uptake at the expense of glucose than that observed with asparagine-grown cells, and gluconic acid also gives rise to a considerable immediate rapid oxygen consumption; 2-ketogluconic acid, on the other hand, is attacked at a comparatively low rate, which sometimes increases slightly during the course of the oxidation. As shown in figure 2B, exposure to ultraviolet irradiation has relatively little effect on the oxidative patterns of glucose-grown cells, except to stabilize the rate of oxidation of 2-ketogluconic acid. The initial rapid rate of oxygen uptake with glucose or gluconic acid eventually declines to a rate that is far higher than the endogenous, paralleling closely the rate of oxidation of 2-ketogluconic acid; the parallelism is more evident with irradiated than with nonirradiated cells as a

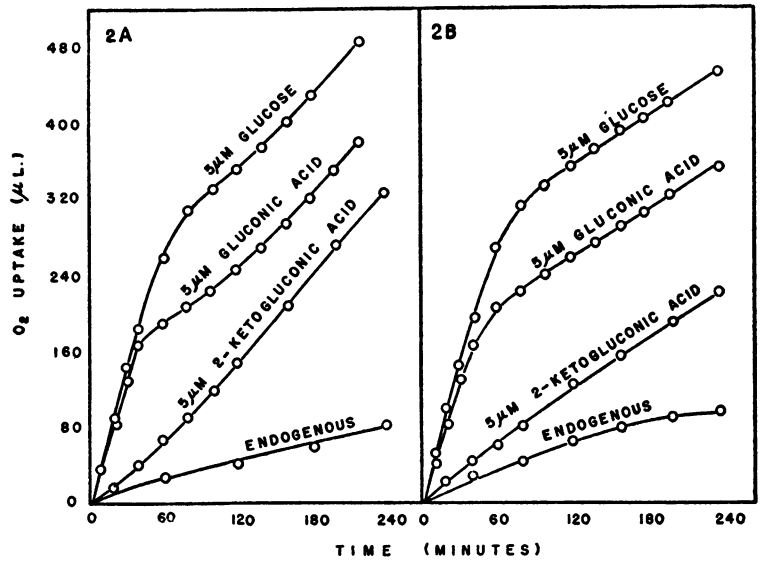

Figure 2. Oxidation of glucose, gluconic acid, and 2-ketogluconic acid by glucose-grown cells; (A) nonirradiated, (B) after irradiation with ultraviolet light.

result of the stabilization of the rate of oxidation of 2-ketogluconic acid. This suggests that 2-ketogluconic acid accumulates during the initial rapid oxidation of both glucose and gluconic acid. The formation of 2-ketogluconic acid from gluconic acid was confirmed by an experiment analogous to the one conducted for the isolation of gluconic acid, as described in the preceding section. Glucose-grown cells were used, and the oxidation was terminated when the characteristic decline in the initial rapid rate of oxygen consumption occurred in an aliquot followed manometrically. After removal of the cells, the supernatant liquid showed a strong furfural reaction, a high reducing value, and an increased laevorotation. It was concentrated *in vacuo*, and the quinoxyline derivative of 2-ketogluconic acid was prepared from the concentrate according to the method of Ohle (1934). Following recrystallization, this derivative melted at 198 to 199 C, and the melting point was not depressed by mixture with the corresponding derivative prepared from pure 2-ketogluconic acid (mp 198 to 199 C).

DISCUSSION

The "semiadapted" behavior of asparagine-grown cells of *P. fluorescens* with respect to glucose has made possible an unambiguous demonstration that such cells oxidize glucose entirely via gluconic acid, a glycolytic mechanism playing no measurable role. The fact that glucose-grown cells of *P. fluorescens* are adapted to gluconic acid suggests that even in such cells glucose dissimilation proceeds largely through gluconic acid, although the possibility of some glycolysis under these circumstances is not excluded.

Our data on the metabolism of 2-ketogluconic acid by glucose-adapted cells show that it cannot be an essential member of the main pathway for glucose oxidation. The initial rate of oxidation of glucose and gluconic acid by glucose-adapted cells is approximately ten times as rapid as the rate of oxidation of 2-ketogluconic acid, and continues to a level far higher than that required for a total conversion of either glucose or gluconic acid to 2-ketogluconic acid. As shown in figure 2B, the decline in the rates of oxidation of glucose and gluconic acid occurs after the total oxygen uptakes have reached values of 300 and 190 microliters respectively, corresponding to the consumption of 5.3 atoms of oxygen per mole of glucose, and 3.4 atoms per mole of gluconic acid; whereas quantitative conversion to 2-ketogluconic acid would require only 2 atoms of oxygen per mole of glucose, and 1 atom per mole of gluconic acid. Furthermore, there is a substantial evolution of carbon dioxide (R.Q. > 0.5) during this period. On the other hand, it is clear that 2-ketogluconic acid is formed and accumulates as a result of the oxidation of glucose and gluconic acid by glucose-grown cells, even when these substrates are supplied at the extremely low concentrations employed in our manometric experiments (approximately 0.002 M). This observation can be interpreted in two possible ways: either by assuming that a derivative of 2-ketogluconic acid (e.g, a phospho-2-ketogluconic acid) is a member of the main pathway, and that this active substance is split in part to 2-ketogluconic acid, which must be reactivated in a slow reaction before it can re-enter the pathway; or by assuming that there are two divergent pathways for glucose dissimilation, one proceeding via 2-ketogluconic acid, below which a partial block exists.

Our data do not permit a decision between these two interpretations. The recent report of Stokes and Campbell (1951) that dried cells of *Pseudomonas aeruginosa* catalyze a nonphosphorylative conversion of glucose to 2-ketogluconic acid might be considered to lend support to the latter hypothesis.

SUMMARY

After growth on asparagine, *Pseudomonas fluorescens* is unadapted to oxidize gluconic and 2-ketogluconic acids but can immediately oxidize glucose.

By the ultraviolet irradiation of asparagine-grown cells, subsequent adaptation to gluconic and 2-ketogluconic acids can be prevented; under these circumstances, the oxidation of glucose results in a quantitative accumulation of gluconic acid.

After growth on glucose, *P. fluorescens* oxidizes glucose and gluconic acid at

immediate high rates, whereas 2-ketogluconic acid is oxidized very slowly at first, the rate subsequently increasing to a slight extent as a result of adaptation. 2-ketogluconic acid accumulates during the oxidation of glucose or gluconic acid by glucose-grown cells, but the manometric evidence shows that the oxidation of glucose and gluconic acid cannot proceed chiefly via 2-ketogluconic acid.

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