THE BIOCHEMISTRY OF HYDROGENOMONAS

II. THE ADAPTIVE OXIDATION OF ORGANIC SUBSTRATES

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Because of their metabolic versatility, the facultative chemotrophic bacteria seem well suited for investigations dealing with the comparative biochemistry of autotrophic and heterotrophic growth. Among these organisms, those using hydrogen as the electron source for carbon dioxide reduction are especially attractive because of the ease of removing excess substrate (hydrogen), the absence of possible metabolic effects of substrate oxidation products, and the simple apparent stoichiometry of the over-all autotrophic process (Ruhland, 1924; Schatz, 1952).

As background for such a comparative study, some knowledge of the oxidative metabolism of the organism is needed. This paper reports observations on the respiratory potentialities of a facultative hydrogen autotroph, Hydrogenomonas facilis.

EXPERIMENTAL METHODS

Autotrophic growth conditions were essentially as described previously (Atkinson and McFadden, 1954). Mineral agar plates were inoculated from stock cultures maintained autotrophically on the same medium. Plates were incubated at room temperature (22 to 27 °C) under approximately 70 per cent hydrogen, 10 per cent carbon dioxide, and 20 per cent air for from 40 hours to four days, depending on the size of the inoculum. Cells were harvested in 0.067 M pH 7 phosphate. Since washing had little effect on the responses, it was usually omitted. Heterotrophic cells were grown under air on the same medium with the addition of one per cent of the desired substrate. The dry weight of cells used in experiments was estimated turbidimetrically.

Standard manometric methods were used in rate measurements which were made at 28 °C.

RESULTS

Cells of H. facilis grown under air on the complex organic medium of Schatz and Bovell (1952), which contains succinate, acetate, citrate, glutamate, yeast extract, and peptone, oxidize succinate, lactate, pyruvate, malate, and acetate but not glucose, citrate, glycerol, and various phosphorylated intermediates. When supplied as the sole carbon source, succinate, glutamate, lactate, or glucose supports growth at about the same rate as the complex medium. Growth is very slow on acetate and negligible on citrate.

Cells grown on succinate, glutamate, or lactate resemble those grown on the complex medium in that they oxidize any of these compounds, as well as pyruvate, malate, acetate, and oxalacetate, without appreciable lag. These cells will not oxidize glucose. Glucose grown cells take up oxygen linearly without lag when supplied glucose; with metabolic acids the rate of uptake is initially about equal to that with glucose but increases to a much higher value. Figure 1 illustrates these reciprocal effects in the case of glucose and glutamate. Results with the other metabolic acids are similar.

Autotrophically grown cells do not oxidize glucose, and no adaptation to glucose oxidation occurs within a four hour period in the Warburg vessel under air. The metabolic acids listed above are all oxidized by these cells. Cells which are harvested with little exposure to air, and which are either tested immediately after harvest or stored under hydrogen in the cold for a few days, exhibit a typically adaptive oxidative pattern. Representative curves are shown in figure 2. Oxygen uptake, initially slow or negligible, increases smoothly with time. Except for lactate and glutamate, the metabolic acids reproducibly give curves of the general shape shown in the figure although the time required to reach a

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Figure 1. A: Oxidation of glucose and glutamate by glucose grown Hydrogenomonas facilis cells. Warburg flasks contained 0.9 mg (dry wt) of cells in a final volume of 2 ml 0.067 M pH 7 phosphate; 5 μmoles of substrate added from side arm at 30 min. Endogenous uptake subtracted. B: Same for glutamate grown cells.

The constant rate of oxygen uptake is frequently somewhat shorter than in this experiment. The curve shown for lactate, which approximates two linear regions with the second having the greater slope, is obtained for both lactate and glutamate with some cell suspensions, while others adapt to these acids in the manner shown for succinate and malate. The reason for this discrepancy is not known. It is apparently not explainable by variations in age of culture, frequency of changing the gas phase during growth, washing or nonwashing of cells, or differences in exposure to air during a short interval between harvest and use of the cells.

Cells which have adapted to oxidation of any substrate are found to be adapted to all although on addition of the second substrate (after exhaustion of the first) there may be some further increase in rate of oxygen uptake over the initial rate. Cell suspensions aerated in the absence of added substrate also gain the ability to oxidize the metabolic intermediates tested. This "endogenous adaptation" is usually slower than adaptation in the presence of substrate.

From the data of figure 2, it may be estimated that from 40 to 60 per cent of the various substrates is assimilated. As would be expected, the addition of 2,4-dinitrophenol to preadapted cell suspensions reduces the degree of assimilation (increases the oxygen uptake per mole of substrate). Of greater interest is the effect of dinitrophenol on adaptation since "uncoupling" of phosphorylation by this agent should prevent or retard adaptations which involve actual enzyme synthesis (Monod, 1944). Typical dinitrophenol effects are shown in figures 3 and 4. Dinitrophenol at the concentration used is seen to block adaptation completely while having much less effect on the rate of oxygen uptake by preadapted cells.
When premixed with unadapted cells, tipped with substrate (these are not plotted), or added 20 minutes after substrate, dinitrophenol prevented significant oxygen uptake. When the inhibitor was tipped later, further adaptation was prevented while oxygen uptake continued approximately linearly at a tangent to the adaptive curve.

Since the response to dinitrophenol suggests that the adaptations may involve enzyme synthesis, the effect of an exogenous nitrogen source was investigated. Added ammonium ion strongly enhanced adaptation of washed cells to malate (figure 5). As expected, an additional nitrogen source had no effect on adaptation to glutamate.

Autotrophically grown cells of _H. facilis_ are unable to reduce fumarate at the expense of hydrogen (Schatz and Bovell, 1952). Cells which...
Adapt to succinate oxidation do not simultaneously acquire the ability to reduce fumarate (figure 6). The rate of hydrogen uptake in the presence of fumarate by either succinate adapted or nonadapted cells is negligible. Since aeration in the absence of hydrogen reduces the rate of the hydrogen-oxygen reaction somewhat, two flasks were placed under a 90 per cent hydrogen and 10 per cent air mixture after adaptation of the cell suspension to succinate oxidation (curve S-O₂, figure 6). The rapid gas uptake indicates that failure to reduce fumarate is not due to loss of any component of the system catalyzing the hydrogen-oxygen reaction.

**DISCUSSION**

The initial lag followed by an increasing rate of oxygen uptake observed when autotrophically grown *H. facilis* cells oxidize organic substrates contrasts with the observation of Schatz and Bovell (1952) that autotrophic cells of this species oxidize a variety of substrates without lag. These authors found glucose to be oxidized (although at a lower rate than the other substrates tested), in contrast to the present failure to demonstrate glucose oxidation by autotrophic cells. The reasons for these discrepancies are not clear, but differences in cultural conditions or in the treatment of cell suspensions between harvesting and using them seem likely possibilities. Exposure of cells to air during the latter period, for example, might explain the absence of lag in Schatz and Bovell's experiments. Curves presented by Wilson et al. (1953) indicate a slow adaptation to lactate oxidation by autotrophically grown *H. facilis* cells and linear oxygen uptake (or rapid adaptation) by peptone grown cells.

The inability of autotrophically grown cells to oxidize glucose suggests that free glucose does not arise during autotrophic metabolism. Although the nonutilization of a substrate by intact cells must be interpreted with caution, in the case of glucose permeability difficulties seem improbable.

Various interpretations might be proposed for the adaptive pattern of oxidations observed. Whatever the mechanism, it seems clear that autotrophically grown cells of *H. facilis* must alter their metabolic activities in some rather time-consuming manner when forced to obtain energy from the oxidation of organic substrates, whether of exogenous or endogenous origin. This result is of interest in view of the fact that the organism is a strict aerobe (Schatz and Bovell, 1952). Oxygen is utilized during growth; it seems at least reasonable from analogy that the component reactions of the oxidative sequence operate in the synthesis of cell constituents; and yet a slow adaptation is needed before added intermediates can be oxidized.

The possibility that the adaptations consist of permeability changes is not rigorously excluded. If, however, the adaptations involve actual enzyme synthesis, as is suggested by the effects of dinitrophenol and of ammonium ion, the seeming nonspecificity of the response is of interest. Adaptation apparently requires the activation either of a large number of reactions nearly simultaneously or of one reaction common to oxidation of all of these substrates. An obvious possibility is that adaptation consists of the acquisition or activation of some previously unused step in electron transport from organic substrates to oxygen. (Cultures which oxidize lactate and glutamate without lag, while adapt-
ing in the usual way to the other substrates, do not fit easily into this hypothesis.)

In the autotrophically metabolizing cell, electron pathways from hydrogen to oxygen and from hydrogen to carbon dioxide are obviously functioning; it is not necessary, however, that a pathway from organic substrates to oxygen be available. Although metabolic syntheses will certainly involve some oxidative steps, the net process from carbon dioxide to cell constituents is of course reductive. Any reduced cofactors arising in oxidative steps could thus be reoxidized in other necessary metabolic reactions and need not transfer electrons to oxygen. All of the reactions of the tricarboxylic acid cycle, for example, might function in the synthesis of cell components under autotrophic conditions although none of the electrons arising from oxidative steps was transferred to oxygen. (Operation of the cycle under anaerobic conditions has in fact been demonstrated in Escherichia coli (Swim and Krampitz, 1954).) Under aerobic conditions in the absence of hydrogen, the missing reaction or reactions needed for electron transport to oxygen might be activated, resulting in the observed simultaneous acquisition of ability to metabolize any of a wide variety of substrates.

This hypothesis requires the assumptions that the electron pathways from hydrogen to oxygen and from organic substrates to oxygen are not identical, and that under autotrophic conditions organic substrate oxidation (with attendant oxidative phosphorylation) does not function as an energy source. In other words, while expending energy in the production of reduced compounds from carbon dioxide, the cell may not need simultaneously to oxidize reduced substrate by another set of reactions as a source of metabolic energy. Support for the suggestion that an autotroph may use autotrophically derived energy directly is found in the observation of Calvin and Massini (1952) that \(^{14}C\) from labeled carbon dioxide does not appear in quantity in compounds of the tricarboxylic acid cycle during illumination of the green alga Scenedesmus. Apparently the energetic demands of the cell are satisfied by other means. Labeled carbon enters acids of the cycle rapidly when the light is removed.

The reduction of fumarate by Escherichia coli, using electrons obtained from the oxidation of organic substrates, was reported by Krebs (1937) and has been analyzed further in the case of acetate oxidation by Swim and Krampitz (1954). Succinate is presumably oxidized through fumarate in H. faciliis (although this was not demonstrated), and the reaction should be reversed by hydrogen if its cofactor is linked to hydrogen directly or indirectly. (The equilibrium ratio of succinate to fumarate under an atmosphere of hydrogen is about \(10^2\).) The absence of significant hydrogen uptake by succinate adapted cells when supplied fumarate thus suggests that at least one electron-transfer agent of succinate oxidation is neither involved in hydrogen oxidation nor linked enzymically with any cofactors of hydrogen oxidation. These results would be most readily explained by metabolic nonequivalence of electrons derived from hydrogen and those from organic substrates.

Further clarification may be expected from experiments with cell-free preparations. Suspensions resulting from sonic disruption of cells in phosphate buffer, which retain full ability to catalyze the hydrogen-oxygen reaction (Atkinson and McFadden, 1954), fail to oxidize any organic substrate tested, even when the cells have been previously adapted. Modifications of this procedure have not resulted in a cell-free preparation active toward organic substrates, but no extensive search for a satisfactory method of breaking cells has yet been made.

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SUMMARY

Freshly harvested autotrophically grown cells of Hydrogenomonas faciliis do not oxidize glucose, and oxidize various metabolic acids only after a lag and at an increasing rate. The suggestion of enzyme induction is supported by the effects on adaptation of 2,4-dinitrophenol (inhibitory) and of exogenous nitrogen (stimulatory).

Adaptation to any of the intermediates is accompanied by adaptation to the others. Cells incubated under air in the absence of added substrate also gain the ability to oxidize metabolic intermediates. When supplied hydrogen as substrate, these cells, whether adapted to organic substrates or not, exhibit rapid and linear oxygen uptake. Adaptation to succinate oxidation does
not confer ability to reduce fumarate at the expense of hydrogen.

Cells of the same organism grown heterotrophically with glucose as carbon source oxidize glucose linearly. Metabolic acids are oxidized without lag but at an increasing rate. Cells grown on glutamate, lactate, or succinate fail to metabolize glucose.

Possible implications of these results are discussed.

REFERENCES


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