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The Induced Synthesis of Hydrogenase by *Hydrogenomonas facilis*

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SUMMARY: *Hydrogenomonas facilis* lack hydrogenase activity when grown heterotrophically, but these organisms develop hydrogenase activity when incubated, in phosphate buffer, under an atmosphere of 95 % (v/v) hydrogen + 5 % (v/v) air. The development of hydrogenase activity is inhibited by 2:4-dinitrophenol and by chloramphenicol. The development is also inhibited by the addition of sodium lactate or sodium acetate unless a nitrogen source (e.g. ammonium sulphate) is present.

It has frequently been observed that hydrogen bacteria, grown autotrophically in an atmosphere of hydrogen + oxygen + carbon dioxide, show strong hydrogenase activity; yet the same bacteria, when grown heterotrophically, show little or no hydrogenase activity (Kluyver & Manten, 1942; Kistner, 1954; Kluyver & Verhoeven, 1954; Packer & Vishniac, 1955). Schatz & Bovell (1952) found that *Hydrogenomonas facilis* had some hydrogenase activity when grown in air on a variety of organic media, although the activity was much less than that of autotrophically grown bacteria. However, Wilson, Stout, Powelson & Koffler (1953) and Atkinson & McFadden (1954) were unable to demonstrate hydrogenase activity in *H. facilis* grown heterotrophically in air although Wilson *et al.* found that it was developed in organisms grown heterotrophically under diminished oxygen concentration (5 % O₂ + 5 % CO₂ + 90 % N₂). We also found that *H. facilis* had no hydrogenase activity when grown heterotrophically in air, but activity developed when these inactive organisms were incubated in a mixture of hydrogen and oxygen. Such behaviour suggests the induced synthesis of hydrogenase in the presence of hydrogen. The effect of some substances on the development of the hydrogenase activity is reported in this paper.

METHODS

The culture of *Hydrogenomonas facilis* was obtained from Dr A. Schatz. It was grown on the organic agar medium of Schatz & Bovell (1952) in air at 25° and harvested 45 hr. after inoculation. The organisms were washed and then suspended in sterile M/30 Sørensen phosphate buffer (pH 7.2) by passing a stream of either hydrogen or oxygen-free nitrogen.

A suspension of organisms (20 ml.) which never showed any hydrogenase activity initially, was incubated in sterile 250 ml. flasks which were filled with the appropriate gas mixture after evacuation. The flasks were shaken at 25° during the incubation period. In some experiments a continuous gas stream was passed through the cultures throughout the incubation period.

Hydrogenase activity was measured manometrically, on 2 ml. samples of suspension, by determining the hydrogen uptake from an atmosphere of pure hydrogen at 25°, with $m/125$ methylene blue as hydrogen acceptor. Hydrogen was purified before use by passage through 10 % (w/v) KOH, 10 % (w/v) CuCl_2 , 0.1 M- AgNO_3 and finally through water. All determinations were made in duplicate.

The dry weight of suspensions, both before and after incubation, was determined turbidimetrically by the aid of a calibration curve.

RESULTS

The induced formation of hydrogenase

Heterotrophically grown *Hydrogenomonas facilis* showed no hydrogenase activity when harvested but this activity developed when the organisms were incubated in an atmosphere of 95 % (v/v) H_2 + 5 % (v/v) air, although no appreciable increase of dry weight occurred (Fig. 1). A cell-free preparation having hydrogenase activity was obtained from the organisms after incubation, by breaking them in a Hughes press (Hughes, 1951). No such active preparation was obtained from the organisms before incubation in the hydrogen + air mixture.

Fig. 1

Fig. 2

Fig. 1. The time course of the development of hydrogenase activity on incubation in 95 % (v/v) H_2 + 5 % (v/v) air. Heterotrophically grown organisms were suspended in $m/30$ phosphate buffer (pH 7.2) and incubated during continuous passage of a stream of 95 % (v/v) H_2 + 5 % (v/v) air. Samples were removed at intervals and tested for hydrogenase activity. —▲—, Incubation flask 1; —●—, incubation flask 2.

Fig. 2. The effect of the composition of the gas phase during incubation on the development of hydrogenase activity. Heterotrophically grown organisms were suspended in $m/30$ Sørensen phosphate buffer (pH 7.2) and tested for hydrogenase activity after 24 hr. incubation in: (1) —●—, 95 % (v/v) H_2 + 5 % (v/v) air; (2) —x—x—, 100 % (v/v) H_2 ; (3) —▲—▲—, 95 % (v/v) N_2 + 5 % (v/v) air. Equivalent dry weight of organism in each Warburg vessel = 0.65 mg.

Less hydrogenase activity developed when the organisms were incubated in 90 % (v/v) H_2 + 10 % (v/v) air and none at all when the gas phase lacked either hydrogen or oxygen (Fig. 2). The development of hydrogenase activity was inhibited when either 2,4-dinitrophenol (Fig. 3) or chloramphenicol (Fig. 4) was present in the incubation flasks; neither of these substances, at the concentrations used, had any effect on hydrogenase activity itself.

The development of hydrogenase activity is therefore dependent on the presence of both hydrogen and oxygen during the incubation period. Since the development of activity was inhibited by 2:4-dinitrophenol and by chloramphenicol (an inhibitor of protein synthesis; Wisseman, Smadel, Hahn & Hopps, 1954), it seems reasonable to conclude that some enzyme synthesis is necessary. Presumably the energy for the synthesis comes from the oxy-hydrogen reaction catalysed by the bacteria.

Fig. 3

Fig. 4

Fig. 3. The effect of 2:4-dinitrophenol on the development of hydrogenase activity. Heterotrophically grown organisms suspended in $m/30$ Sørensen phosphate buffer (pH 7.2) were incubated for 24 hr. at 25° under an atmosphere of 95% (v/v) H_2 + 5% (v/v) air and in the presence of 2:4-dinitrophenol at different concentrations. The organisms were then assayed for hydrogenase activity. Equivalent dry weight of organism in each Warburg flask during assay = 0.85 mg.

Fig. 4. The effect of chloramphenicol on the development of hydrogenase activity. Heterotrophically grown organisms suspended in $m/30$ Sørensen phosphate buffer (pH 7.2) were incubated for 24 hr. at 25° under an atmosphere of 95% (v/v) H_2 + 5% (v/v) air in the presence of different concentrations of chloramphenicol. The organisms were then assayed for hydrogenase activity. Equivalent dry weight of organism in each Warburg flask during assay = 0.85 mg. Chloramphenicol concentration during incubation: —●—, 0 $\mu\text{g./ml.}$; —▲—, 1 $\mu\text{g./ml.}$; —▼—, 2 $\mu\text{g./ml.}$; —x—, 3, 5 or 10 $\mu\text{g./ml.}$

The effect of acetate and lactate on the development of hydrogenase activity

This was investigated in a number of experiments, and the effect of the addition of an inorganic source of nitrogen was also studied. In the earlier experiments, ammonium nitrate was added since *Hydrogenomonas facilis* grows autotrophically in a medium containing this (Schatz & Bovell, 1952). The results obtained were erratic, however, and the amount of growth (increase in turbidity) often poor. Analysis showed that nitrite accumulated in the medium when ammonium nitrate was present and further experiments showed that 10^{-3}M -nitrite inhibited hydrogenase activity. Later experiments were therefore carried out with 0.1% (w/v) ammonium sulphate as nitrogen source.

The organisms multiplied when incubated in phosphate buffer in the presence of sodium acetate or sodium lactate (0.1 or 0.2%), and growth was

much increased by the addition of ammonium sulphate. The addition of sodium acetate or lactate, however, stopped the development of hydrogenase activity unless ammonium sulphate was also present (Fig. 5). Clearly, hydrogenase formation is not always depressed by the addition of an organic

Fig. 5. The effect of lactate on the development of hydrogenase activity. Heterotrophically grown organisms suspended in $M/30$ Sørensen phosphate buffer (pH 7.2) were incubated under an atmosphere of 95 % (v/v) H_2 + 5 % (v/v) air. Sodium lactate (final concentration 0.2 % (w/v)) was added to one flask of each pair after 8 hr. incubation. Samples were withdrawn at intervals and assayed for hydrogenase activity. The incubation flasks were refilled with the H_2 + air mixture each time samples were removed. —●—●—, control; —▼—▼—, lactate added. (a) Ammonium sulphate not added; (b) 0.1 % (w/v) ammonium sulphate present in both incubation flasks.

substrate. The situation resembles others in which the relative activity of an adaptive enzyme falls markedly under conditions of nitrogen deficiency (Virtanen & De Ley, 1948).

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