THE ELECTRON CARRIER SPECIFICITIES OF THE HYDROGENASES OF DIFFERENT ORIGINS

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The enzyme hydrogenase catalyzes the reaction:

$$H_2 + \text{Acceptor} \rightleftarrows \text{Reduced Acceptor}$$

Various electron carriers (redox dyes) serve as acceptors and the enzyme activity is measured by the forward and reverse reactions in the presence of the carriers. Ishimoto, Yagi and Shiraki (1957) reported that cytochrome c_3 showed the function of the carrier in the reverse reaction catalyzed by a crude hydrogenase preparation from Desulfovibrio desulfuricans. Mortenson et al. (1962), on the other hand, concluded that ferredoxin was the natural carrier in the reaction catalyzed by a crude enzyme preparation from Clostridium pasteurianum.

In this paper, evidence is presented to prove that these two hydrogenases are different in their electron carrier specificities. A purified hydrogenase preparation of D. desulfuricans catalyzed the evolution of hydrogen in the presence of ferrocytochrome c_3 or reduced methyl viologen and not of reduced ferredoxin, while the clostridial hydrogenase catalyzed the hydrogen evolution in the presence of reduced ferredoxin which could not be substituted by reduced cytochrome c_3 or methyl viologen.

MATERIALS AND METHODS

Organisms.----- Desulfovibrio desulfuricans was cultured as described by Ishimoto et al. (1954). Clostridium pasteurianum W5 was cultured in a nitrogen-free medium with gaseous nitrogen as the nitrogen source (Carnahan et al., 1960).

Electron Carriers.----- Cytochrome c_3 was isolated from the sonicate of D. desulfuricans by an Amberlite XE 64 (ammonium type) column (Ishimoto et al., 1957).

The cytochrome c_3 solution thus obtained was added with ammonium sulfate (up to 90 % saturation) and the precipitated contaminants were removed by centrifugation. The supernatant was dialyzed and the cytochrome was adsorbed on the above column, eluted with 0.1 N ammonia and concentrated by freeze-drying. Ferredoxin was prepared from Cl. pasteurianum as described by Mortenson (1964).

Assay of Hydrogenase.----- The rate of hydrogen evolution in the presence of a reduced carrier was measured as described by Tamiya et al. (1955). A reaction mixture containing an appropriate amount of hydrogenase preparation and carrier (6×10^{-4} M methyl viologen for D. desulfuricans' enzyme or 2×10^{-5} M ferredoxin for clostridial enzyme, unless otherwise stated) in 0.020 M phosphate buffer, pH 7.0 (3.0 ml), was placed in the main compartment of a Warburg vessel. Center well contained alkaline pyrogallol (0.2 ml). The gas phase was nitrogen. Solid anhydrous $Na_2S_2O_4$ (2.4 mg) was added from the side arm and the rate of hydrogen evolution determined. Activity of the enzyme was tentatively expressed as Q_{H_2} (μliters of H_2 evolved per hour per unit absorbance of enzyme solution at 230 mμ).

RESULTS

Partial Purification of Hydrogenase of D. desulfuricans.----- The bacterial sonicate which had been deprived of cytochrome c_3 by an Amberlite XE column (see Materials and Methods) was treated with DEAE-cellulose (Mortenson et al., 1962) to remove any ferredoxin-type compound, and used as the crude enzyme.

The crude enzyme (50 ml) was added with 0.4 g streptomycin sulfate to precipitate the bacterial nucleic acids. The enzyme was precipitated from the supernatant solution by ammonium sulfate (between 30 and 90 % saturation), dissolved in distilled water (4 ml), applied to a Sephadex G-200 column (3 × 85 cm) equilibrated with 0.05 M Tris-HCl buffer, pH 7.3, containing 0.2 M NaCl and chromatographed with the same buffer. The activity was eluted in two separate peaks (the first peak, from 170 to 240 ml; the second peak, from 300 to 370 ml). The latter was collected and the enzyme was precipitated with ammonium sulfate (between 30 and 90 % satu-

ration), dissolved again in distilled water (4.0 ml), and rechromatographed on the same column under the identical conditions. From the active fractions, the enzyme was collected by ammonium sulfate precipitation, dissolved in distilled water (4.0 ml) and dialyzed overnight against distilled water. The supernatant of the dialyzed solution was used as the partially purified hydrogenase preparation.

Partial Purification of Hydrogenase of Cl. pasteurianum.----- The precipitate obtained by the addition of acetone to the Cl. pasteurianum extract (Mortenson, 1964)

TABLE I Summary of Purification Steps

Step	Desulfovibrio desulfuricans			Clostridium pasteurianum		
	Total OD_{230}	Total Activity (Recovery)	Specific Activity QH_2	Total OD_{230}	Total Activity (Recovery)	Specific Activity QH_2
Crude extract	5250	182500 (100%)	35	2300	27200 (100%)	12
Super. after streptomycin treatment	3480	97500 (53%)	28			
First $(NH_4)_2SO_4$ precipitate	1380	47000 (26%)	34	1415	22600 (83%)	16
Active freactions from the first chromatography	370	16800 (9%)	45	824	21100 (78%)	26
Second $(NH_4)_2SO_4$ precipitate	200	14300 (8%)	72	442	18600 (69%)	42
Active fractions from the second chromatography	77	7450 (4%)	97	165	11600 (43%)	70
Third $(NH_4)_2SO_4$ ppt., dialyzed	17	4000 (2%)	240	123	8700 (32%)	71

was lyophilized and extracted with 20 times amount of 0.2 M phosphate buffer, pH 7.0, and the extract was used as the crude hydrogenase preparation.

The enzyme was purified by a similar procedure except that the streptomycin treatment was omitted and that chromatography on Sephadex G-100 column (3 × 45 cm) was performed instead of G-200. In this case, the activity was eluted between

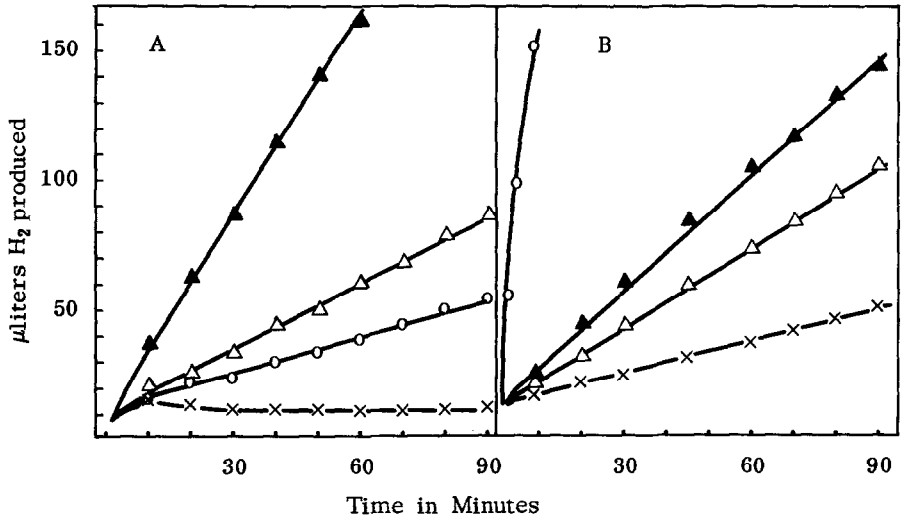

Fig. 1. Evolution of hydrogen by the crude enzyme preparations. The main compartment of a Warburg vessel contained the enzyme (A: 1.0 mg N of Desulfovibrio enzyme; B: 0.9 mg N of clostridial enzyme) and 2×10^{-5} M each electron carrier (△: cytochrome c_3, ▲: methyl viologen, o: ferredoxin or ×: control without addition) in 0.040 M phosphate buffer, pH 7.0, in a total volume of 3.0 ml. The gas phase was nitrogen. The center well contained alkaline pyrogallol (0.2 ml). Solid anhydrous $Na_2S_2O_4$ (2.4 mg) was added and the rate of hydrogen evolution was followed at 30°.

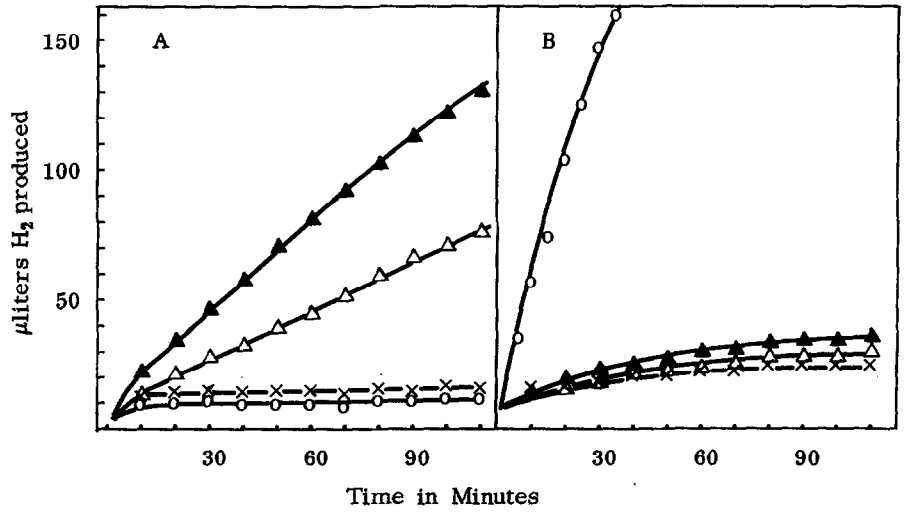

Fig. 2. Evolution of hydrogen by the partially purified preparations. The conditions are the same as Fig. 1 except that the main compartment contained the purified enzyme (A: 3.3 OD_{280} units of Desulfovibrio enzyme; B: 5.6 OD_{280} units of clostridial enzyme) and 2×10^{-5} M electron carrier in 0.020 M phosphate buffer, pH 7.0.

130 and 190 ml.

The purification procedures and the results are summarized in Table I.

Hydrogen Evolution by the Crude and the Partially Purified Enzyme Preparations.----- The hydrogen evolution in the presence of methyl viologen, cytochrome c_3 or ferredoxin by the enzyme preparations are illustrated in Figures 1 and 2.

DISCUSSION

The experimental evidence presented in this paper shows that hydrogenases from Clostridium pasteurianum and Desulfovibrio desulfuricans are different entities. While the hydrogenase of Cl. pasteurianum [EC 1.12.1.1, Hydrogen : ferredoxin oxidoreductase] catalyzes the evolution of hydrogen gas in the presence of reduced ferredoxin, the hydrogenase of D. desulfuricans catalyzes the production of hydrogen in the presence of ferrocytochrome c_3 which is not replaced by the reduced ferredoxin. We propose that the hydrogenase of Desulfovibrio desulfuricans be classified in EC 1.12.2 group, and called "Hydrogen : ferricytochrome c_3 oxidoreductase."

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