SHORT COMMUNICATIONS

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Hydrogenase activity in cell-free preparation of Chlorella

In previous investigations[1–3] we reported that glucose-grown cells of Chlorella required no dark adaptation period for carrying out various hydrogenase-mediated reactions. Among them are photoreduction, oxyhydrogen reaction and nitrite reduction.

Hydrogenase (H_2:acceptor oxidoreductase) has been assayed in several ways[4]. In this laboratory KESSLER's method[5], using nitrite as the acceptor for hydrogen consumption in the dark, is commonly employed to measure hydrogenase activity in the intact cells. This method has several obvious limitations, one of the foremost being the rate of penetration of hydrogen acceptors into the cells. This paper describes hydrogenase activity in cell-free homogenates of Chlorella using various substrates as acceptors. Hydrogenase activity of the intact cells with the same acceptors is included for comparison.

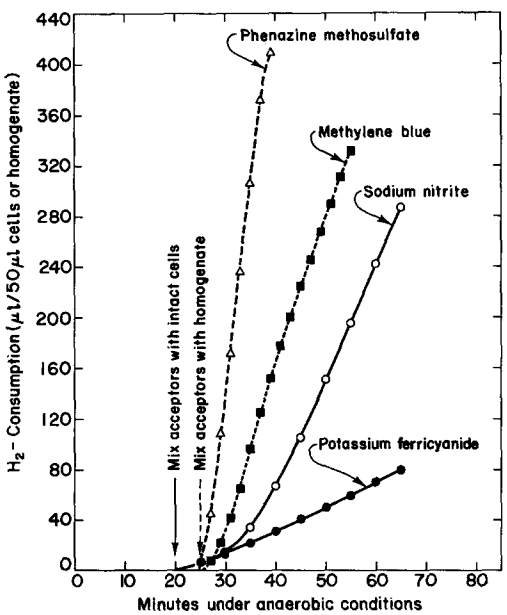

Fig. 1. Time course of hydrogen consumption by the intact cells and cell-free homogenate of glucose-grown Chlorella. For hydrogenase assay in the intact cells, 0.4 ml of 0.05 M acceptor (0.1 M in the case of the viologens) was placed in one sidearm of each Warburg vessel, 0.3 ml of 10% KOH in the other sidearm, a small pyrogallol crystal in the through, and 50 µl of cells suspended in 1.8 ml of phosphate buffer (pH 6.4) in the main compartment. Vessels were flushed with H_2 at zero min and the reaction was begun at 20 min including 10 min for H_2-flushing and another 10 min for mixing KOH with pyrogallol and temperature equilibration. In cell-free homogenate, hydrogenase was assayed as described in the text. Twenty-five min of anaerobic conditions comprised the preparation of the homogenate under N_2 and equilibration in vessels under H_2. Broken line, homogenate; solid line, intact cells.

Cells of *Chlorella pyrenoidosa*, Sorokin's strain, were grown on a glucose medium and harvested as described previously[3]. Cells were suspended in 0.1 M phosphate buffer (pH 6.4) to make a concentration of 500 μl per ml by packed cell volume, and were assayed immediately for hydrogenase activity according to the routine procedure[3] or treated as in the following. An appropriate amount of the algal suspension was mixed with a half volume of glass beads in a stainless-steel capsule pre-chilled in ice-water. After 5 min flushing with N_2 the capsule was covered tightly and shaken in a Nossal shaker[6] in the cold room at 4°. Four 15-sec shaking periods were performed and the capsule was chilled in ice-water for 1 min between each shaking.

The resulting homogenate was stored in a flask under N_2. The homogenate was transferred with a hypodermic syringe and an exact amount of 0.1 ml was injected into the sidearm of a Warburg vessel through a rubber cap. Vessels were usually flushed with H_2 for 10 min in advance, with 2.0 ml of the same buffer containing 20 μmoles of the acceptor in the main compartment, 0.3 ml of 10% KOH in the other sidearm and a small pyrogallol crystal in the trough. Pyrogallol and alkaline solution were mixed before the injection of the homogenate.

After the vessels were shaken in a water bath at 25° for about 5 min, the homogenate was tipped into the main compartment to start the reaction. A total time of approximately 25 min elapsed between the onset of anaerobic conditions in the whole cell suspension and the tipping of the homogenate from the sidearm. In some cases, readings were recorded at 2-min intervals when the reaction rates were tremendously high.

Some of the reaction rates in both the intact and disintegrated cells are shown in Fig. 1. The results obtained from these two different situations are summarized and compared in Table I. In many instances, hydrogenase activity increased from less than

TABLE I

HYDROGENASE ACTIVITY IN INTACT AND DISINTEGRATED CELLS OF CHLORELLA IN PRESENCE OF VARIOUS ACCEPTORS

In both intact cells and the homogenate 20 μmoles of the acceptor (40 μmoles in the case of viologens) was provided, and hydrogenase assay was carried out in 0.1 M phosphate buffer (pH 6.4) at 25°.

Acceptors	*Rate of H_2-uptake (μl/10 min)*		*E_0' (mV)*
	Intact cells	*Homogenate*	
Methyl viologen	8	11	−446
Benzyl viologen	11	41	−359
NAD+	14	33	−320
NADP+	12	12	−320
FMN	7	26	−220
FAD	19	23	−220
Methylene blue	15	158	+ 11
Phenazine methosulfate	37	359	+ 80
2,6-Dichlorophenolindophenol	12	24	+217
p-Quinone	6	2	+279
Potassium ferricyanide	19	115	+429
NO_3^-	7	0	
NO_2^-	92	0	
NH_2OH–HCl	16	3	
SO_3^{2-}	8	0	

one- to nearly ten-fold, over the whole cell preparation, depending on the substrates used.

Among various substrates examined phenazine methosulfate which served as a fair electron acceptor in the intact cells caused the most rapid hydrogen uptake in cell-free preparation, over 90% of 20 μmoles provided being reduced in 15 min. It seems evident that the enhancement of hydrogenase activity by phenazine methosulfate, methylene blue and other acceptors was due to the elimination of the cell membrane as a barrier to the penetration of substrates into the intact cells. On the other hand, nitrite which was the best hydrogen acceptor in the intact cells failed to bring about hydrogen consumption in the homogenate. It is probable that other enzymes and cofactors participating in nitrite-reducing system were separated during the manipulation.

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1,4-Diamino-2-butanon (2-ketoputrescine) as strong and short acting competitive inhibitor of diamine oxidase

Diamine oxidase (diamine:O_2 oxidoreductase (deaminating), EC 1.4.3.6) a Cu^{2+} and pyridoxal phosphate containing enzyme, is inhibited by metal chelating agents or carbonyl reagents such as cyanide, semicarbazide, hydroxylamine and some hydrazine derivatives[1]. In the course of our study on the oxidative deamination of 2-hydroxyputrescine and 2-hydroxycadaverine[2] we have found that the corresponding ketones, 1,4-diamino-2-butanon and 1,5-diamino-2-pentanon, possess a powerful blocking activity toward plant and animal diamine oxidase. In this paper some results of this action are given.

1,4-Diamino-2-butanon and 1,5-diamino-2-pentanon were synthetized as dihydrochlorides[3] and partially purified pea seedling and pig kidney diamine oxidase was used as in our previous paper[2]. The enzyme activity was determined by modified spectrophotometric method of HOLMSTEDT, LARSSON AND THAM (*cf.* ref. 2), unless otherwise stated. In general, freshly prepared substrate and inhibitor solutions were