H_2 METABOLISM IN PHOTOSYNTHETIC ORGANISMS[1]

II. LIGHT-DEPENDENT H_2 EVOLUTION BY PREPARATIONS FROM CHLAMYDOMONAS, SCENEDESMUS AND SPINACH

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SUMMARY

Light-dependent H_2 evolution from dithiothreitol as electron donor was observed with cell-free preparations of anaerobically adapted Chlamydomonas reinhardii, Scenedesmus obliquus and from spinach chloroplasts mixed with Chlamydomonas hydrogenase. NADH substituted for dithiothreitol as electron donor only in the Chlamydomonas preparation. Dibromothymoquinone, an antagonist of plastoquinone, selectively inhibited H_2 photoevolution from NADH. These results are interpreted as indicating that 3-(3,4-dichlorophenyl)-1, 1-dimethyl urea insensitive H_2 photoevolution by algae containing hydrogenase is due to the capability of NADH to reduce plastoquinone in the electron transport chain, and to evolve H_2 by a low redox potential carrier of photosystem I.

Cell-free preparations of hydrogenase have been isolated from anaerobically adapted green and blue green algae (1, 2) and from bacteria (3). Of these preparations, light-dependent H_2 evolution was shown only by Abeles (4) with a cell-free preparation of Chlamydomonas eugametos from reduced pyridine nucleotides. Coupling of spinach chloroplasts to a clostridial hydrogenase was reported first by Arnon et al (5) with ascorbate-dichlorophenolindophenol as electron donor. Recently Benemann et al (6) showed light-dependent H_2 evolution from water with a chloroplast-ferredoxin-clostridial hydrogenase system.

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Abbreviations
DBMIB: dibromothymoquinone, DCMU: 3-(3-4-dichlorophenyl)-1,1-dimethyl urea, CHl: chlorophyll

We have now obtained cell-free hydrogenase preparations from Chlamydomonas reinhardii, and from Scenedesmus obliquus. These preparations and spinach chloroplasts coupled to Chlamydomonas hydrogenase could photochemically evolve H_2 from dithiothreitol in the presence of DCMU. Our results confirm in vivo studies with Chlamydomonas (7) and suggest that a NADH-plastoquinone system is involved in H_2 photoevolution by this alga.

MATERIALS AND METHODS

Chlamydomonas reinhardii and Scenedesmus obliquus were obtained from the Indiana Culture Collection and were grown in a Tris, acetate, phosphate medium containing per liter: 14.3 mg K_2HPO_4; 7.2 mg KH_2PO_4; 2.4 g Tris; 1ml glacial acetic acid; 0.4 g NH_4Cl; 50 mg $CaCl_2 \cdot 2H_2O$; 100 mg $MgSO_4 \cdot 7H_2O$; 1ml of trace element solution; made up to 1.0 liter with distilled water. Trace elements contained the following: 50 g Na_2EDTA; 22 g $ZnSO_4 \cdot 3H_2O$; 11.4 g H_3BO_3; 5.0 g $MnCl_2 \cdot 4H_2O$; 4.9 g $FeSO_4 \cdot 7H_2O$; 1.6 g $CoCl_2 \cdot 6H_2O$; 1.57 g $CuSO_4 \cdot 5H_2O$; 1.1 g $(NH_4)_6 Mo_7O_{24} \cdot 4H_2O$ in a total volume of 1 liter. The final pH after autoclaving was 7.3. Cultures were grown with slow shaking at 22°C under white fluorescent lamps with an intensity of about 200 ft.c.

Chloroplast-containing cell-free hydrogenase preparations were prepared in the following manner. Chlamydomonas cells were harvested by centrifugation and resuspended in 0.125M sucrose, 25mM Na, K phosphate, pH 7.3; 2mM $MgCl_2$; 2mM KCl and 10 μM DCMU at a final concentration of 0.4mg Chl/ml. Scenedesmus cells were harvested by centrifugation and resuspended in 25mM Na, K phosphate, pH 7.3; 2mM $MgCl_2$; 2mM KCl and 10 μM DCMU. Algal suspensions were flushed with N_2 or H_2 and adapted for 1 hr at room temperature in the dark (2). After adaptation Chlamydomonas cells were sonicated with a Branson sonic power sonifer for 30 sec under H_2 at 0°C. In order to break Scenedesmus, the cells were first frozen in liquid N_2 for 12 hr, thawed, and then were sonicated in the same manner. Sonicated Scenedesmus and Chlamydomonas were then transfered by syringe into anaerobic plastic tubes and were centrifuged for 10 min at 500xg to remove whole cells. Of the 500xg supernatant fluid, 2.8 ml were routinely used for light-dependent hydrogenase assays. The chloroplast-free hydrogenase preparation was prepared similarly from a Chlamydomonas suspension in 25mM Na, K phosphate, pH 7.3, 2mM $MgCl_2$; 2mM KCl and 10 μM DCMU. After sonication this preparation was centrifuged for 30 min at 30,000xg and 2.8 ml of the supernatant fluid were used for assays with spinach chloroplasts. Intact spinach chloroplasts were prepared according to Avron and Gibbs (8) and were osmotically broken upon dilution in the assay reaction mixture.

H_2 evolution was measured manometrically in a Warburg apparatus with illumination from below by 40W lamps with a light intensity of 11 watts/meter^2 at 20°C. All manipulation were carried out under conditions of strict anaerobicity.

RESULTS AND DISCUSSION

Cell-free preparations of anaerobically adapted Chlamydomonas and Scenedesmus photoevolved H_2 from dithiothreitol in the presence of DCMU in a reaction that was dependent on photosystem I (Table I). H_2 photoevolution was dependent on the presence of both the chloroplasts preparation and the hydrogenase fraction.

TABLE I

ASSAYS FOR H_2 PHOTOEVOLUTION AND DARK H_2 EVOLUTION BY CELL-FREE PREPARATIONS OF CHLAMYDOMONAS, SCENEDESMUS AND SPINACH CHLOROPLASTS

A suspension of Chlamydomonas rehinardii cells containing about 0.4 mg Chl/ml in 0.125M sucrose, 25mM Na, K phosphate, pH 7.3; 2mM $MgCl_2$; 2mM KCl and 10 μM DCMU was flushed with N_2 or H_2 and adapted for 1 hr at room temperature in the dark. After adaptation the cells were sonicated for 30 sec under H_2 at 0°C. Suspensions of Scenedesmus obliquus cells containing about 0.4 mg Chl/ml in 25mM Na, K phosphate, pH 7.3; 2mM $MgCl_2$, 2mM KCl and 10 μM DCMU were flushed with N_2 or H_2 and adapted for 1 hr at room temperature in the dark. After adaptation the cells were first frozen in liquid N_2 for 12 hr, then thawed and sonicated for 30 sec under H_2 at 0°C. Sonicated Chlamydomonas and Scenedesmus were then transfered by syringe into anaerobic plastic tubes and centrifuged for 10 min at 500xg to remove whole cells. Chloroplast-free hydrogenase preparation was prepared by centrifugation at 30,000xg for 1 hr. Of the 500xg extract or 30,000xg supernatant 2.8 ml were injected through a serum stopper into the Warburg flask. The complete reaction mixture (3ml) for the light assay contained: 12mM dithiothreitol and 2.8 ml of the 500xg extract, or 0.2 ml spinach chloroplasts containing 0.5 mg Chl with 2.8 ml of Chlamydomonas hydrogenase preparation. The complete reaction mixture (3ml) for the dark assay contained: 20mM dithionite, 5mM methyl viologen and 2.8 ml of the 500xg supernatant, or 0.2 ml spinach chloroplasts containing 0.5mg Chl with 2.8 ml Chlamydomonas hydrogenase. H_2 evolution was assayed at 20°C.

Changes in Reaction Mixture	H_2 Evolution		
	Chlamydomonas 500xg extract	Scenedesmus 500xg extract	Spinach chloroplasts plus Chlamydomonas 30,000xg extract
		μl H_2/mg Chl·hr	
Light dependent reaction			
Complete	45	80	97
minus dithiothreitol	0	5	0
minus chloroplasts (30,000xg pellet)	0	0	0
minus hydrogenase (30,000xg supernatant) plus chloroplasts (30,000xg pellet)	0	0	0
Dark reaction			
Complete	220	600	254
minus dithionite	0	0	0
minus methyl viologen	70	120	80
minus chloroplasts (30,000xg pellet)	220	600	250
minus hydrogenase (30,000xg supernatant) plus chloroplasts (30,000xg pellet)	0	0	0

Mixing spinach chloroplasts with Chlamydomonas hydrogenase resulted in a similar production of H_2 from dithiothreitol in the light.

The algal preparations evolved H_2 from dithionite in the dark (Table I).

Chloroplasts were not required for the dark activity and H_2 evolution was stimulated with reduced methyl viologen. These findings are consistent with the observations by Abeles (4) and Ward (9, 10).

Photooxidation of water or a flow of reductant from carbon metabolism under anaerobic conditions into photosystem I of the photosynthetic electron transport chain were suggested previously to be the major source of H_2 in Scenedesmus and Chlamydomonas, respectively (7, 11). We attempted to test these proposals using the in vitro assay described in Table I with NADH substituting for dithiothreitol as electron donor. We observed a NADH dependent H_2 photoevolution with cell-free preparation of Chlamydomonas; about 20% stimulation with cell-free preparation of Scenedesmus and no activity with Chlamydomonas hydrogenase mixed with spinach chloroplasts (Fig. 1). No significant NADH oxidation was

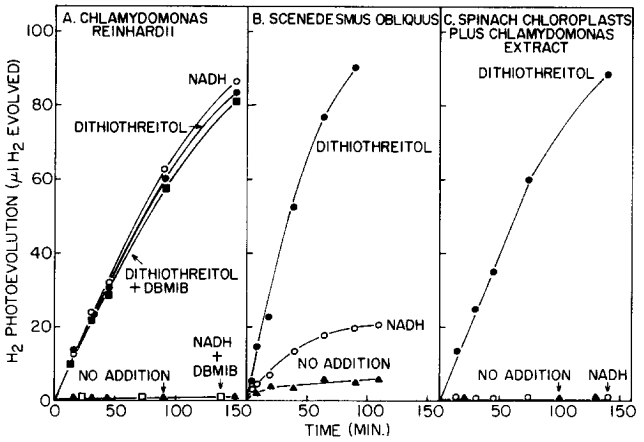

Fig. 1. Effect of dithiothreitol, reduced pyridine nucleotide and DBMIB on stimulation of H_2 photoevolution from cell-free preparations of Chlamydomonas, Scenedesmus and spinach chloroplasts mixed with Chlamydomonas extract.

The reaction mixture (3ml) A contained: 0.125M sucrose; 25mM Na, K phosphate, pH 7.3; 2mM $MgCl_2$; 2mM KCl; 10 μM DCMU; cell-free hydrogenase from Chlamydomonas containing 1.2 mg Chl with 8mM NADH, 8mM dithiothreitol or 10 μM DBMIB, where indicated. The reaction mixture (3ml) B contained: 25mM Na, K phosphate, pH 7.3; 2mM $MgCl_2$; 2mM KCl; 10 μM DCMU; 8mM dithiothreitol or 8mM NADH, where indicated and cell-free hydrogenase from Scenedesmus containing 1.2 mg Chl. The reaction mixture (3ml) C contained: 25mM Na, K phosphate, pH 7.3; 2mM $MgCl_2$; 2mM KCl, 10 μM DCMU, 8mM NADH or dithiothreitol were indicated; spinach chloroplasts containing 1.0 mg Chl and chloroplast-free hydrogenase from Chlamydomonas saturating for H_2 evolution with reduced methyl viologen. ▲-▲, no addition; O-O, NADH; ●-●, dithiothreitol; ■-■, dithiothreitol + DBMIB; □ - □, NADH + DBMIB.

observed spectrophotometrically in the dark. It seems, therefore, that photoevolution of H_2 in Chlamydomonas involves a mechanism different from that in Scenedesmus at the level of the photosynthetic electron transport chain. This was in agreement with our observation of insensitivity of H_2 photoevolution in intact Chlamydomonas to DCMU and about 80% sensitivity in Scenedesmus (not shown).

Figure 1, A presents the results of studies with a photosynthetic electron transport inhibitor in an attempt to define the site of reduction on the oxidizing side of photosystem I in Chlamydomonas. DBMIB, an inhibitor of the oxidation of reduced plastoquinone (12), inhibited H_2 photoevolution with NADH at 10 uM. Similarly, DBMIB at 10 μM was found to inhibit H_2 photoevolution in intact Chlamydomonas. DBMIB at this concentration had no effect on H_2 photoevolution with dithiothreitol. It is concluded, therefore, that H_2 photoevolution by intact Chlamydomonas is due to the reduction of plastoquinone in chloroplasts with NADH formed by fermentation of cellular substances (13).

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