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Comparative Study of the Photochemistry of Chloroplast Membranes and Photosystem II Particles

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ABSTRACT

A comparative study of the photoreducing potentials of spinach thylakoid membranes and spinach photosystem II particles has been made. Hexachloroplatinate ions have been used as electron acceptors in a Hill-like assay for oxygen evolution measurements with both thylakoid membranes and photosystem II particles. However, unlike other Hill acceptors, such as ferricyanide, hexachloroplatinate can be fully reduced to metallic platinum that is catalytically active for hydrogen evolution. This is experimentally confirmed in the ability of chloroplast membranes to photoprecipitate platinum and photoproduce molecular hydrogen. Although similar experiments with photosystem II particles resulted in hexachloroplatinate-supported oxygen evolution, hydrogen evolution was not observed. Moreover, photosystem II particles coupled to ferredoxin and hydrogenase resulted in neither hydrogen nor oxygen evolution—a distinct contrast to the results obtained with chloroplast membranes.

Index Entries: Chloroplasts; photosystem II; oxygen; hydrogen; chloroplatinic acid; ferredoxin.

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ABBREVIATIONS

Mes [2-(*N*-morpholino)-ethanesulfonic acid];
DCIP 2,6 dichlorophenol-indephenol;
DCMU [3-(3,4-dichlorophenyl)-1, 1-dimethylurea];
DBMIB 2,5-Dibromo-3-methyl-β-isopropyl-*p*-benzoquinone;
TMPD N,N,N^1,N^1-tetramethyl-*p*-phenylene diamine;
CFH chloroplast, ferredoxin, hydrogenase

INTRODUCTION

As originally shown by Arnon et al., a mixture of chloroplasts, ferredoxin, and hydrogenase is capable of photoevolving molecular hydrogen when cysteine is used as the electron donor (*1*). The CFH system has been used for fundamental photosynthesis studies, as in the case of Arnon's work, and also for prototype studies in the field of renewable energy production (*2,3*). The Z scheme of photosynthesis is the accepted pathway whereby electrons are transported between the two photosystems from water to ferredoxin. Evidence consistent with this model comes from experiments in which the addition of DCMU (a photosystem II (PS II) inhibitor) to the CFH system abolished hydrogen production. Hydrogen is evolved, even in the presence of DCMU, when artificial electron donors to photosystem I (PS I), such as ascorbate and DCIP, are used (2). However, Arnon and his colleagues have found that the reduction of $NADP^+$ by an illuminated mixture of chloroplasts and ferredoxin was not totally abolished by DCMU. Furthermore, they determined that ferredoxin was fully reduced in the presence of 1 μM of DCMU, a concentration that severely inhibited electron flow from water. Thus, a restricted level of electron flow was able to keep ferredoxin reduced to a level optimal for cyclic photophosphorylation (*4*). They also found that, in the presence of 10 μM of DBMIB, an inhibitor of electron transfer between the two photosystems acting at the site of plastoquinone, ferredoxin could still be photoreduced by water, whereas little photoreduction of the bound iron-sulfur centers was observed. Arnon concluded that the photoreduction of ferredoxin by water and by the artificial ascorbate/DCIP donor system to photosystem I must occur by different mechanisms (*5*). He suggested that photosystem II can generate a reducing potential sufficient for ferredoxin reduction, a function hitherto ascribed to PS I in the Z scheme (*6*).

This paper describes the results of experiments to test the hypothesis of Arnon et al., in which photosynthetic water splitting was attempted using PS II particles coupled to hexachloroplatinate or to ferredoxin and hydrogenase (2FH system). The 2FH system was incapable of simultaneous photoevolution of hydrogen and oxygen. PS II coupled to chloroplatinic acid was capable of oxygen evolution on illumination, but no

hydrogen evolution was observed. These data suggest that PS II particles, unlike chloroplast membranes (*1,7*), cannot generate sufficient reducing potential to reduce ferredoxin.

MATERIALS AND METHODS

Materials

Spinach was purchased locally. Ferredoxin, type III from spinach, was obtained from Sigma. Cell extracts of hydrogenase were prepared from *Clostridium pasteurianum* (*8*). Chloroplatinic acid was purchased from Matthay Bishop, Inc., Malvern, PA.

Preparation of Photosystem II Particles

Chloroplasts were isolated by grinding spinach leaves, as described previously (*8*). The chloroplast suspension was strained through 2 layers of cheesecloth, followed by bench-top centrifugation for 3 min. The pellet was resuspended in a medium consisting of 0.33 *M* sorbitol, 2 m*M* EDTA, 10 m*M* $MgCl_2$, 1.0 m*M* $MnCl_2$ in 50 m*M* Hepes buffer, pH 7.6, and strained through 8 layers of cheesecloth, followed by bench-top centrifugation for 10 min. The pellet was finally resuspended in 50 m*M* Mes buffer, pH 6.0, containing 5 m*M* $MgCl_2$, 15 m*M* NaCl, and 1 m*M* sodium ascorbate. PS II was prepared from the osmotically ruptured chloroplasts by following the method described by Ghanotakis et al. (*9*).

Measurement of Hydrogen, Oxygen, and Chlorophyll

Measurements of the simultaneous hydrogen and oxygen production by the CFH, 2FH, and chloroplasts or PS II coupled to hexachloroplatinate were obtained with a specially constructed flow system that was continuously purged with helium (for details, *see* (*10*)). The system was calibrated with an electrolysis cell connected in tandem with the reaction cell, and the hydrogen and oxygen detection systems consisted of a combustible gas analyzer (Bio-Gas Detector Corp.) and a Hersh electrogalvinic cell, respectively (*11*). Chlorophyll analysis was performed according to Arnon's method (*12*). Also, the oxygen-evolving activity of chloroplasts and PS II particles was measured in a Clark electrode using artificial electron acceptors. PS I activity (hydrogen producing) present in PS II was monitored using the flow system by measuring hydrogen evolution produced in a reaction mixture consisting of 3 m*M* TMPD, 120 m*M* sodium ascorbate, 1 m*M* methyl-viologen, PS II, and 0.25 mL of the crude hydrogenase extract. All assays were conducted at 20°C.

Table 1
Comparison of the O_2-Evolving Activity of Chloroplasts and Photosystem II Particles[a]

Electron acceptor, final concentration m*M*	O_2-Evolution, μmol/mg chl/h	
	Chloroplasts	Photosystem II
Potassium ferricyanide (10) + *p*-benzoquinone (1.0)	81.2	132.3
Chloroplatinic acid (0.5)	19.8	22.5

[a]O_2 Evolution measured at 20°C in a Clark-electrode. Assay medium consisted of 1.9 mL, 50 m*M* Mes buffer, pH 6.0, containing 0.4 *M* sucrose, 5 m*M* $MgCl_2$, 0.15 *M* NaCl; 40 μL of electron acceptor solution in distilled water.

Light Sources and Filters

Reaction mixtures were illuminated at 20°C with a quartz iodide tungsten lamp (ANSI Code ELH). A plastic yellow filter was used to obtain light (>495 nm) for saturating illumination in the principal absorption bands of chlorophyll.

RESULTS AND DISCUSSION

Oxygen-Evolving Activity of Chloroplasts and PS II Particles

A comparison of the oxygen-evolving activity of chloroplasts and PSII particles determined in a Clark electrode is summarized in Table 1. Hexachloroplatinate also acts as a Hill electron acceptor, although the rate of O_2 evolution is much lower than with ferricyanide and *p*-benzoquinone. Its reduction, however, can lead to the precipitation of platinum onto thylakoid membranes that are then capable of photocatalytic hydrogen and oxygen evolution (*7*).

Photosystem I Activity of the Prepared Photosystem II Particles

There are several ways of determining whether a PSII preparation is largely devoid of PS I. Methods include freeze fracture electron microscopy, electron paramagnetic resonance, and low-temperature fluorescence. Also, PSII preparations with low PSI activity possess low methylviologen-mediated O_2 uptake compared to chloroplasts (*13*). In this study, the PS I activity of PS II particles was measured using ascorbate-reduced TMPD for the PS I-catalyzed photoreduction of methylviologen. The presence of hydrogenase reoxidized MV, resulting in the evolution of molecular hydrogen (*14*). No hydrogen evolution was observed using the PS II prepara-

tion. The sensitivity of the detection apparatus was such that a rate of hydrogen production of ~0.3 nmol min^{-1} could easily be detected. On the contrary, chloroplast membranes with a chlorophyll content of 2.7 mg were capable of catalyzing the photoreduction of MV with subsequent hydrogen evolution rate of 6.6 μmol/mg chL/h. Even though no hydrogen evolution was observed with the PS II particles, this is not conclusive evidence that PS I was totally absent. Although there may not be any PS I in the PS II preparation, the possibility exists that during the Triton extraction procedure used to prepare PS II, some PS I does remain, but it is nonfunctional in that it is incapable of participating in electron transfer reactions. The observation of photoproduction of hydrogen by the 2FH system and PS II coupled to chloroplatinic acid would therefore suggest that PS II is capable of generating sufficient reducing potential for reduction of ferredoxin.

A Comparison of the Ability of Chloroplasts and PS II Particles to Photoproduce Hydrogen and Oxygen when Coupled to Ferredoxin/Hydrogenase or Chloroplatinic Acid

It has been firmly established that the CFH system is capable of simultaneous photoproduction of hydrogen and oxygen (*8,10*). The reducing potential required for ferredoxin reduction in the CFH system (<—0.4 V) is generated by PS I. No photochemical evidence was obtained to indicate that PS II alone could reduce ferredoxin (as measured by hydrogen evolution). The reaction mixture for the CFH and 2FH systems in 50 m*M* Mes, pH 6.0, contained 0.4 *M* sucrose. A high stoichiometric excess of hydrogen over oxygen produced by the CFH system, under these experimental conditions (*15*), suggests that the bulk of hydrogen evolution is derived primarily from a photosystem I reaction in which reductant is oxidized by either P700 or a component of the electron transport chain. This interpretation of pathway of reductant flow is also consistent with the results of Neumann and Drechsler who demonstrated the photoreduction of ferredoxin with a variety of organic electron donors (*16*). The fact that neither hydrogen nor oxygen production was observed with the 2FH system is further evidence that either the PS II preparation is devoid of PS I or that any contaminating PS I is nonfunctional.

Illumination of a mixture of chloroplasts and chloroplatinic acid in sucrose medium, pH 6.0, resulted in an immediate transient gush of O_2 which settled to a steady state after 1 h and subsequently declined to zero after several hours of continuous illumination. Hydrogen evolution was observed ~90 min after the start of illumination (Fig. 1). Chloroplatinic acid acts as an electron acceptor, and, as it does so, platinum is precipitated onto the surface of the chloroplast membranes and then serves as the catalyst for hydrogen evolution. The delay observed before hydrogen

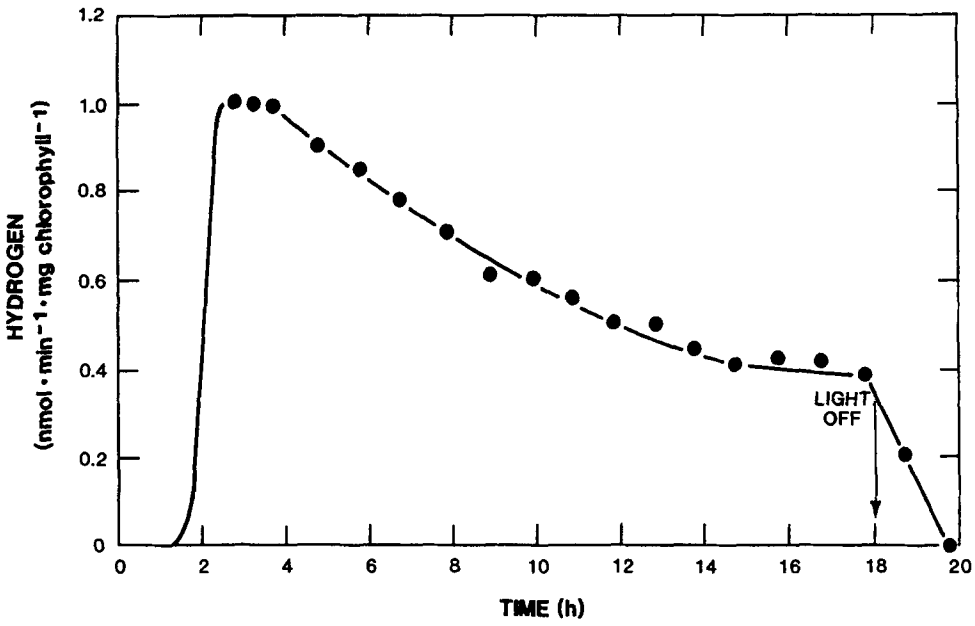

Fig. 1. Photoproduction of hydrogen by chloroplast membranes coupled to hexachloroplatinate ions. The reaction mixture consisted of 2.7 mg chlorophyll in 6.0 mL of the sucrose buffer, pH 6.0, containing chloroplatinic acid (~ 1.8 m*M*) The reaction was carried out at 20°C under saturating illumination.

production commences reflects the time required for sufficient catalyst to be precipitated onto the chloroplast membranes necessary for hydrogen production. Illumination of a mixture of PS II and chloroplatinic acid in medium at pH 7.5 resulted in a transient gush of oxygen (36 nmol min^{-1} mg^{-1} chl), but no hydrogen evolution was observed. Colloidal platinum was also precipitated directly onto PS II particles (7). The resulting PS II colloidal platinum composition was also incapable of photoproduction of hydrogen. It was also established that Triton X-100-containing PS II particles did not affect the photoproduction by a mixture of EDTA, proflavine, and methyl viologen.

The most likely explanation for these experimental results is that, as predicted by the conventional Z-scheme of photosynthesis, photosystem II is incapable of generating a reducing potential sufficiently negative to evolve hydrogen from water in a platinum-catalyzed reaction. Although explanations other than the thermodynamic limit of the reducing potential of photosystem II can be invoked to explain the lack of hydrogen evolution by photosystem II particles, it is felt that these are less likely in view of the positive role of hexachloroplatinate ions in serving as Hill-like electron acceptors and its well-known reductive chemistry in forming zero valent platinum with high catalytic activity in hydrogen evolution.

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