Biological Solar Energy Conversion

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Preface

A conference on Biological Solar Energy Conversion was held at the Rosenstiel School of Marine and Atmospheric Science, University of Miami, on November 15-18, 1976, under the sponsorship of the United States-Japan Cooperative Science Program, U.S. National Science Foundation, and the Japanese Society for Promotion of Science. This volume consists of the formal papers presented during the conference.

Solar energy has now been duly recognized as our largest and ultimate nonfossil, nonnuclear energy resource. Mankind is totally dependent on this energy supply for his continued existence. Unfortunately, man has not yet learned to harness solar energy on a scale commensurate with his ever increasing energy requirements. On the other hand, the enormous magnitude of the solar radiation that reaches the land surfaces of the earth is so much greater than any of the foreseeable needs that it represents an inviting technical target. In planning this conference, we attempted to bring together a group of scientists who have made significant observations concerned with various aspects of Biological Solar Energy Conversion. The excellent exchange of information and ideas among the participants will surely further our understanding of these biological processes and lead hopefully to future practical application for the benefit of mankind.

We thank most sincerely Dr. William W. Hay, Dean, Rosenstiel School of Marine and Atmospheric Science, University of Miami, for his support and encouragement of this conference. It is a pleasure to acknowledge the kindness of Dr. Harris Stewart, Director, Atlantic Oceanographic and Meteorological Laboratories, NOAA, for providing space in his institute for the conference sessions. We express also our sincere gratitude to those participants who willingly served as Chairmen for the various sessions. The smooth running of the conference was due in great measure to the untiring efforts of the students and assistants in Dr. A. Mitsui's laboratory. Ms. Diana Rosner and Ms. JoAnn Radway of the University of Miami provided excellent editorial help. Lastly we acknowledge partial support for the conference from the U.S. Government Energy Research and Development Agency.

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SECTION I. Capture and Utilization of Solar Energy

PHOTOHYDROGEN PRODUCTION IN GREEN ALGAE: WATER SERVES AS THE PRIMARY SUBSTRATE FOR HYDROGEN AND OXYGEN PRODUCTION

by

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I. INTRODUCTION

The impact of the successful oil embargo as experienced by the United States in 1973 was sufficiently severe to cause an increased awareness of and interest in possible alternate sources of energy, including the bio-solar energy conversion device of photosynthesis (NSF/NASA Report, 1972; NSF/RANN Report, 1973a; NSF/RANN Report, 1973b; NSF/RANN Report, 1975). Nature's primary process for the conversion of light energy to a biologically useful chemical form is photosynthesis. Obviously it is the method which provided energy for the development of the earth's fossil fuel supply and continues to provide the yearly organic energy supply and reserves for the global population. Because of an ever-increasing demand for all forms of energy throughout the world it has become necessary to consider the possibilities of (1) increasing the productivity of agriculture (the so-called Green Revolution), (2) exploring the potential of increasing the basic effeciency of the fundamental mechanism of photosynthesis and (3) attempting to devise alternate mechanisms for the utilization of the primary stable photo-reductant generated in photosynthesis in a way more direct than that represented by the mechanism for carbon dioxide assimilation in green plant photosynthesis. It is the primary purpose of this article to examine the last item listed above in terms of the in vivo capacity of the green algae to perform the light-dependent process of hydrogen production; this reaction represents a natural process for utilization of the energy provided by the generation of the primary photoreductant of photosynthesis. If assumed that water serves as the electron donor for this reaction then the photoevolution of hydrogen should be comparable in magnitude to that of carbon dioxide assimilation or oxygen evolution.

II. HYDROGEN PRODUCTION BY GREEN ALGAE

During studies on the anaerobic metabolism of the green alga, Scenedesmus obliquus, Gaffron and Rubin (1942) noted that thoroughly adapted cells in the absence of both hydrogen and carbon dioxide produced hydrogen gas when illuminated. This phenomenon, since termed photohydrogen production, was separable from a much slower dark fermentative hydrogen metabolism through the action of dinitrophenol; this inhibitor caused complete suppresion of the dark hydrogen evolution and apparent stimulation of photohydrogen evolution (Gaffron and Rubin, 1942; Gaffron, 1944). They recognized that this form of metabolism was unique, that it was dependent upon the adaptable hydrogenase of Scenedesmus and that it was most likely representative of an anerobic photooxidation of some unknown intermediate formed in fermentation. Because of the esoteric nature of the problem, because of the apparent miniscule rates of hydrogen evolution and because of the inability of anerobically adapted cells to demonstrate a sustained production of hydrogen only limited additional studies were conducted during the intervening years and principally by subsequent student of Gaffron. The recent focusing of attention upon the potential of green algae to produce hydrogen photochemically has revived interest in this area as well as in the similar phenomenon, but not identical, in nitrogen fixing blue-green algae and photosynthetic bacteria. With the renewed interest it has been possible to ask additional questions about the precise mechanism for photohydrogen formation and to determine with some precision the role of the photosystems and the nature of the electron donor.

It is now apparent that the reactions catalyzed by anaerobically adapted algal cells which possess an adaptable hydrogenase are more numerous than originally suspected. These reactions include the following:

Light-Dependent Reactions:

1. Photosynthesis:

$$CO_2 + 2H_2O + \text{light} \longrightarrow (CH_2O) + O_2 + H_2O$$

2. Photoreduction:

$$CO_2 + 2H_2 + \text{light} \xrightarrow[H_2\text{ase}]{} (CH_2O) + H_2O$$

3. H_2 Photoproduction:

$$2H_2O + \text{light} \xrightarrow[H_2\text{ase}]{} 2H_2 + O_2$$

Dark Reactions:

4. Oxy-hydrogen Reactions:
$2H_2 + O_2 \xrightarrow[H_2ase]{} 2H_2O$

5. Dark CO_2 Fixation:
$CO_2 + 2H_2 + \text{energy} \xrightarrow[H_2ase]{} (CH_2O) + H_2O$

6. H_2 Production: $RH_2 \xrightarrow[H_2ase]{} R + H_2$

7. H_2 Uptake: $R + H_2 \xrightarrow[H_2ase]{} RH_2$

8. Respiration: $RH_2 + \frac{1}{2}O_2 \longrightarrow R + H_2O$

The increased complexity of the general "anaerobic" metabolism of algal cells resulting from the activation of the hydrogenase system, as summarized above, must be thoroughly evaluated and understood in order to explain the many factors influencing photohydrogen production. The inhibition of reactions 1 and 3, for example by dinitrophenol would markedly simplify the subsequent reaction sequence. Similarly the presence of DCMU, a known inhibitor of PS-II activity and of oxygen evolution would allow only reaction 2 to proceed in adapted cells.

Although the presence of an adaptable hydrogenase and the ability of algal cells to perform photoreduction and the several associated reactions listed above was originally analyzed for in a restricted few species, notably Scenesdesmus obliquus, strain D_3, it is now recognized that numerous additional species of algae possess an adaptable hydrogenase and an anaerobic physiology comparable to Scenedesmus (Kessler, 1974). Recently, we have examined over 100 additional species from ten classes of algae for their capacity for photoreduction and photohydrogen production. Nearly all of the species possessing an adaptable hydrogenase were members of the class Chlorophyceae and within either the order Volvocales or Chlorococcales. The majority of species having the potential for hydrogen photoproduction were found in the latter order and included a number of species of Chlorella. However, only two of these, Kirchneriella lunaris and Coelastrum proboscideum had a capacity for photohydrogen production greater than that observed for Scenedesmus (Table 1). In Table I we have summarized the observed rates of hydrogen and oxygen evolution noted in some of the species studied. These values were obtained with the two electrode system as described by Jones and Bishop, 1976; this methodology allowed for an assessment of the immediate rates of hydrogen metabolism and, consequently, only those values are presented in the data of Table I. It is important to stress that these values do not represent sustained rates and, furthermore, are not necessarily maximized values for hydrogen evolution. Typical traces demonstrating the nearly simultaneous production

TABLE I
Rates of Hydrogen and Oxygen Evolution in Anaerobically Adapted Cultures of Various Algal Species Containing Hydrogenase

Species	umoles H_2 / ml cells/ hr	umoles H_2 / mg Chl/ hr	umoles O_2 / ml cells/ hr	umoles O_2 / mg Chl/ hr
Scenedesmus obliquus (heterotrophic)	208.1	48.1	116.6	27.0
Scenedesmus obliquus (autotrophic)	128.8	20.2	134.1	15.9
Kirchneriella lunaris	204.5	32.1	89.0	14.0
Selenastrum sp.	75.2	11.8	112.5	17.6
Coelastrum proboscideum	156.4	24.6	145.8	22.9
Ankistrodesmus braunii	70.8	11.1	135.2	21.6
Ankistrodesmus falcatus	1.7	0.2	19.4	3.0
Chlorella vacuolata 211-8a	96.0	15.1	64.2	10.1
Chlorella vacuolata 211-8c	8.8	1.4	50.0	7.8
Chlorella protothecoides 211-8d	104.4	16.4	103.0	16.2
Chlorella vulgaris 211-11c	18.1	2.8	43.2	6.8
Chlorella sorokiniana 211-11d	92.4	14.5	184.2	28.9
Chlorella sorokiniana 211-11k	47.4	7.4	40.1	6.3
Chlorella sorokiniana 211-32	59.5	9.2	51.6	8.1
Chlorella sorokiniana 211-33	39.3	6.2	34.4	5.4
Chlorella sorokiniana 211-34	55.4	8.7	61.6	9.6
Chlorella fusca 343	14.2	2.2	60.6	9.5

All rates were determined during the initial one minute of illumination with corrections made for the subsequent dark uptake of either hydrogen or oxygen. All measurements made with 10 ul PCV resuspended in 1 ml of phosphate buffer, pH = 6.5. Temperature = 25 C. Adaptation time = 4 hours.

of hydrogen and oxygen in Scenedesmus and several Chlorella species are presented in Figures 1 and 2 respectively. From these traces it is obvious that the production and accumulation of oxygen in the reaction cell leads to a rapid inactivation of the hydrogen photoevolving capacity of all of the algae when the experiments are performed under either high light intensity or for prolonged periods of time.

A. The Role of PS-I in Photohydrogen Production.

Since the early 1960's the basic mechanism of photosynthesis has been recognized to require two photosystems, photosystem I (PS-I) and photosystem II (PS-II), which are functionally connected by an intersystem electron transport system. This formulation in which water serves as the electron donor and $NADP^+$ as the terminal electron acceptor for these two photosystems represents the most generally accepted mechanism for photosysthesis of green plants. The experimental findings culminating in the elaboration of this formulation have had extensive reviewing (Boardman, N. K., 1970; Bishop, N.I., 1971a; Avron, M., 1967; Bishop, N.I., 1973; Brown, J.S., 1973; Trebst, A., 1974; Cheniae, G.M., 1970) and formulations representing this mechanism, i.e., the so-called Z-scheme of photosynthesis, occur in practically all general biology, botany and biochemistry textbooks.

The anaerobic, DCMU insensitive, assimilation of carbon dioxide and hydrogen gas by adapted cells of Scenedesmus, the process termed photoreduction (Gaffron, 1940), was shown to be an exclusive PS-I type reaction by Bishop and Gaffron (1962). In this reaction H_2 gas, through the intervention of the alga's hydrogenase system, serves as the source of electron for the photoreduction of carbon dioxide and is, in a qualified sense, similar to the process of carbon dioxide assimilation in bacterial photosynthesis. The initial attempts to determine if photohydrogen production were also a PS-I catalyzed reaction revealed that both photosystems were apparently involved (Bishop and Gaffron, 1963). Since both photoreduction and photohydrogen production require similar circumstances for their activation and function and, furthermore, appear to compete for the primary photoreductant generated by PS-I, the apparent participation of PS-II activity for hydrogen photoproduction seemed anamolous. However, if water serves as the electron donor for this reaction, as was originally suggested by Gaffron and Rubin, then the participation of both photosystems would be mandatory. The possibility of water serving as the substrate for hydrogen formation has been, and continues to be, a point of controversy. Additional lines of evidence confirming the requirement of at least PS-II, if not water photolysis, in hydrogen evolution will be discussed later.

The clearest lines of evidence demonstrating the absolute requirement for PS-I in photohydrogen evolution was obtained by mutational studies with Scenedesmus. Data summarizing the consequence of the loss of either P-700, cytochrome f or cytochrome