Volume 8

CURRENT TOPICS IN BIOENERGETICS

Current Topics in Bioenergetics

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VOLUME 8
PHOTOSYNTHESIS: PART B

1978

ACADEMIC PRESS
NEW YORK SAN FRANCISCO LONDON

A Subsidiary of Harcourt Brace Jovanovich, Publishers

ACADEMIC PRESS, INC.
111 Fifth Avenue, New York, New York 10003

United Kingdom Edition published by
ACADEMIC PRESS, INC. (LONDON) LTD.
24/28 Oval Road, London NW1 7DX

LIBRARY OF CONGRESS CATALOG CARD NUMBER: 66–28678

ISBN 0–12–152508–2

PRINTED IN THE UNITED STATES OF AMERICA

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Preface

Photosynthesis has always been a fruitful field for investigation, not only because of its inherent importance, but also because it is an integrated biological process which has traditionally attracted the interest of the physicist, the chemist, and the biologist. The major advances in our understanding of the process include contributions from each of the disciplines: the fundamental study by Priestley of the gases involved, the quantum requirement experiments of Warburg and Emerson, Arnold's research into the size of the photosynthetic unit, the pathway of carbon dioxide fixation by Calvin's group, Van Niel's elegant work on comparative photosynthesis, and so on.

The articles contained in this volume and the preceding Volume 7 reflect this same broad approach to the modern study of photosynthesis. The experimental sophistication has extended markedly to permit picosecond measurements of the primary photophysical and photochemical reactions, the study of individual polypeptide components of the chlorophyll–protein reaction center complexes, detection of the individual complexes and their arrangement in the photosynthetic membrane, and the measurement of proton and other ion movement across the membrane as a function of the photosynthetic electron transfer reactions.

In reviewing the manuscripts for Volumes 7 and 8, it became evident that we were no longer dealing with black boxes with alphabets in them, but with specific molecules, their function, and their interaction with other specific molecules. One gains the impression that the major areas of investigation of photosynthesis have been defined and we are in a "mopping up" phase in which we supply those final, critical pieces of the puzzle that tie everything together. It is obvious that we are moving quickly on all fronts, just as in most areas of biology, and the pieces are quickly falling into place. We feel, however, that there are still a few surprises left before the picture is complete.

D. Rao Sanadi

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Electron Transport and Photophosphorylation

Alternate Fates of the Photochemical Reducing Power Generated in Photosynthesis: Hydrogen Production and Nitrogen Fixation

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I. Introduction

The impact of the successful oil embargo experienced by the United States in 1973 was sufficiently severe to cause an increased awareness of

and interest in alternate sources of energy, including biosolar energy conversion devices (NSF/NASA Report, 1972; NSF/RANN Report, 1972, 1973). Nature's primary process for the conversion of light energy to a biologically useful chemical energy form is photosynthesis. It is this process that provided energy for the production of the fossil fuel supply of the earth and continues to supply the yearly organic energy supply and reserves for the world's population. With the ever-increasing global population combined with the insatiable appetite for all energy forms in countries throughout the world, it has become paramount (1) to increase the productivity of agriculture (the Green Revolution) by manipulation of a variety of extrinsic and intrinsic factors known to influence photosynthesis, (2) to explore the possibilities of increasing the inherent efficiency of the basic mechanism of photosynthesis, and (3) to investigate the possibilities of utilizing the primary stable photoreductant 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 item (3) above in terms of the *in vivo* capacity of three major groupings of photosynthetic organisms—the green algae, the blue-green algae, and the photosynthetic bacteria—to perform the two light-dependent processes of hydrogen production and nitrogen fixation; both reactions represent natural processes for utilization of the energy provided by either the generation of the primary photoreductant of photosynthesis or ATP, or both. Furthermore, the rate-limiting aspects of the nitrogen-fixing reaction sequence represent potential points of attack for improving the yield of photosynthesis under conditions where the availability of reduced nitrogen is a major limiting factor for plant productivity.

II. Nature of the Primary Photoreductant

In the process of photosynthesis the existence of two photosystems allows for one of them to generate a strong "photooxidant," which is responsible for the photolysis of water, and the other to generate a strong "photoreductant," which reduces the primary electron acceptor (Clayton, 1965). In one sense the primary photoreductant is equivalent to the reduced state of some component of the reaction center of PS I, i.e., P700 (see the chapter by Ke in Volume 7 of this series). Because of the short lifetime of such intermediates, it is biochemically more pertinent to describe the primary photoreductant in terms of the first stable reductant generated by the photoactivation of P700. The chemical candidates for this particular function have changed continually during the past 20 years as new components of the chloroplast have been

discovered. As concerns reactions in which hydrogen gas is formed or the photochemistry is linked to nitrogen fixation, two or three chloroplast constituents require attention: these are NADP, ferredoxin, and a "bound" ferredoxin. Through observations on the light-induced (EPR) signal at 77°K it was deduced that a bound ferredoxin functions as this primary electron acceptor in PS I (Bearden and Malkin, 1975; see also the chapter by Ke in Volume 7 of this series for a discussion of P430 and bound ferredoxin). Bolton and colleagues (see Bolton and Warden, 1976) are of the opinion that some substance other than bound ferredoxin fulfills the role of the primary acceptor of PS I; this substance is recognized only by its *g* components of 1.75 and 2.07. Since Ke (1973) observed that the kinetics of appearance and disappearance of the oxidized forms P700 and of the bound ferredoxin were identical at low temperature, more credence is currently afforded to the interpretation that bound ferredoxin is the primary acceptor in PS I of green plants and algae. It is unlikely, however, that bound ferredoxin reacts directly in photohydrogen production or in the light-driven nitrogen fixation of blue-green algae and photosynthetic bacteria. In the case of the photosynthetic bacteria, the primary acceptor is viewed as being both iron and ubiquinone (Bolton and Warden, 1976). From an energetic viewpoint it is assumed that if it is not the bound form then it is certainly the free form of ferredoxin that provides the reduction potential sufficient to produce hydrogen gas in concert with the conventional hydrogenase of the green algae. For hydrogen evolution and/or nitrogen fixation by the blue-green algae and the photosynthetic bacteria, there is the additional requirement, perhaps exclusive, for the provision of ATP by the photosynthetic machinery.

Although it has been recognized for some time that reduced ferredoxin and the $NADP^+$ oxidoreductase system cooperate in the formation of reduced NADP, and that the reduced NADP is then utilized in specific reductive steps in the Calvin cycle, it is questionable whether this system participates directly in either hydrogen evolution or nitrogen fixation (Arnon and Yoch, 1974).

III. Hydrogen Production by Green Algae

In continuation of studies on the anaerobic metabolism of the green algae *Scenedesmus obliquus*, Gaffron and Rubin (1942) observed that thoroughly adapted cells when illuminated in the absence of both hydrogen and carbon dioxide produced hydrogen gas. This phenomenon, since termed photohydrogen production, was separable from a dark fermentative hydrogen metabolism through the action of dinitrophenol

(DNP); this substance caused complete inhibition of the dark hydrogen production and apparent stimulation of photohydrogen evolution (Gaffron and Rubin, 1942; Gaffron, 1944). They recognized that this form of metabolism for a normal aerobic-type organism was unique, that it was dependent upon the adaptable hydrogenase of *Scenedesmus*, and that it was most likely representative of an anaerobic photooxidation of some unknown intermediate formed in fermentation. Because of the esoteric nature of the problem, of the apparent miniscule rate of hydrogen evolution, and of the inability of anaerobically adapted cells to show a sustained production of hydrogen, only limited additional studies have been conducted, and these principally by subsequent students of Gaffron.

It is now known that the reactions catalyzed by anaerobically adapted algal cells that possess an adaptable hydrogenase include the following:

Light-dependent reactions

1. Photosynthesis: $CO_2 + 2H_2O + \text{light} \longrightarrow (CH_2O) + O_2 + H_2O$
2. Photoreduction: $CO_2 + 2H_2 + \text{light} \longrightarrow (CH_2O) + H_2O$
3. H_2 photoproduction: $XH_2 + \text{light} \xrightarrow[H_2ase]{} X + H_2$

Dark reactions

4. Oxy-hydrogen reaction: $2H_2 + O_2 \xrightarrow{H_2ase} 2H_2O$
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: $RH_2 + \frac{1}{2}O_2 \xrightarrow{H_2ase} R + H_2O$

The increased complexity of the metabolism associated with activation of the hydrogenase system, as summarized above, must be evaluated and understood in order to explain the many factors that influence photohydrogen production. The prevention of reactions 1, 2, and 6, by dinitrophenol inhibition, for example, would greatly simplify the reaction sequences.

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 studied in a restricted few species, notably *Scenedesmus obliquus*, strain D_3, it is now known (Kessler, 1974) that numerous additional species of algae have similar anaerobic physiology. Recently, we have examined over 100 species of algae from ten classes of algae for their capacity for photoreduction and photohydrogen evolution. Practically all the species possessing an adaptable hydrogenase were members of the class Chlorophyceae and within the orders Volvacales or Chlorococcales. The majority of species having the