PHOTOCHEMISTRY AND ENZYMOLOGY OF PHOTOSYNTHESIS

PROGRESS REPORT

MASTER

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Bessel Kok, Charles F. Fowler, Richard J. Radmer and Bruno Velthuys

MARTIN MARIETTA CORPORATION
Martin Marietta Laboratories → 950 634
1450 South Rolling Road
Baltimore, Maryland 21227

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I. O₂ EVOLUTION STUDIES

Our study of H₂O₂-oxidation by the water splitting enzyme of the chloroplast, described in our previous report, has been reviewed by your office, and is in print at *Biochimica Biophysica Acta*.

Additional observations on O₂ evolution and photosystem II were reported in a paper that was previously reviewed by your office. Its introduction reads:

"With the hope of gaining more insight into the nature of the water-oxidizing enzyme and its various redox-states ('S-states') we have tried to induce and then study anomalies in the oxygen flash-yield pattern. We have pretreated material with many chemicals, in particular oxidants and reductants, but unfortunately, have been able to produce only a few such anomalies. Moreover, in most cases, these turned out to be only apparent; the S-enzyme itself was not affected, but other system II components, instead, which could be oxidized by the S-enzyme in competition with water or which influenced the working of system II in some other way.

Last year, at the Congress on Photobiology, we discussed some of these effects. On that occasion, we briefly mentioned an anomalous double hit which correlated with a specific oxidizing condition (a pH-dependent midpoint potential of 400 mv). Here, we describe this phenomenon more extensively and present recent data which show that in addition to the S states S₀-S₄, the O₂ precursor enzyme system can also occur in a true 'S₋₁' state."

A controversial point concerned the oscillation of the flash yield of protons liberated from water. As was described in previous reports and publications, the oscillatory pattern of the O₂ yield differs considerably from that of the H⁺ yield. The linear sequence of water oxidation proposed earlier in

this laboratory implies that in essence, each trapping centre evolves an O_2 molecule every fourth flash (in terms of electron equivalents we can write the flash yield sequences as 0,0,0,4). Measurements of proton yields in our laboratory revealed the sequence 1,0,1,2. Recently, Junge et al () used a dye method to detect pH changes. To us, their results seemed rather featureless, but according to the authors, they differed substantially from ours, and pointed to a sequence 0,1,1,2.

To settle this difference, we have reinvestigated this subject, using the same dye technique as Junge et al.. However, a higher sensitivity allowed us to avoid signal averaging and to use a simpler protocol and more fully deactivated chloroplasts. As a result, our pH yield oscillation was relatively more pronounced and amenable to analysis. It clearly confirmed our previous pH yield data and the mechanism 1,0,1,2 derived from it. We intend to publish this material shortly.

II. MASS SPECTROMETER STUDIES OF O_2 AND CO_2 EXCHANGE IN PHOTOSYNTHESIS

A manuscript describing the techniques developed in this laboratory (a membrane-inlet system sampling the liquid phase, and a rapid data acquisition and processing system to scan various masses) has been reviewed by your office and submitted to "Methods in Enzymology."

A paper concerning the competition between O_2 and CO_2 for the reductant of photosynthesis has been reviewed by your office and submitted to "Plant Physiology." Its abstract reads:

"A mass spectrometer with a membrane inlet was used to monitor light-driven O_2 evolution, O_2 uptake and CO_2 uptake in suspensions of algae. We observed the following:

- (1) The rate of O_2 uptake, which, in the presence of iodoacetamide, replaces the uptake of CO_2 , showed a distinct plateau (V_{max}) beyond 30% O_2 and was half-maximal at 8% O_2 . We concluded that this light-driven O_2 uptake process, which does not involve carbon compounds, is saturated at lower O_2 concentrations than are photore-spiration and glycolate formation.
- (2) In the absence of inhibitor, O_2 evolution was relatively unaffected by the presence or absence of CO_2 . During the course of CO_2 depletion, electron flow to CO_2 was replaced by an equivalent flow to O_2 .
- (3) There was a distinct delay between the cessation of CO_2 uptake and the increase in O_2 uptake. We ascribe this delay to the transient utilization of another electron acceptor -- possibly bicarbonate or another bound form of CO_2 .

In this reporting period, we have improved our capability to measure the gases taken up and evolved after single (5 μ s) flashes. We have begun to study the simultaneous observation of uptake and release O_2 under this illumination pattern. In another study with this apparatus, we made the curious observation that if hydroxylamine instead of water is the electron source of photosynthesis, one observes the evolution of N_2 instead of O_2 ! Finally, in this program we have begun to combine observations of O_2 and CO_2 rates with measurements of the yield of fluorescence emission. The two measurements are eminently compatible and we hope that the second measurement will aid in the interpretation of the first.

III. PROTON AND ELECTRON EXCHANGE ASSOCIATED WITH THE REACTIONS BETWEEN PHOTOSYSTEM I AND PHOTOSYSTEM II.

A few years ago, it was reported from this laboratory that the ratio H^+/e^- in isolated chloroplasts is 3 or 4, (depending upon conditions). These values were considerably higher than the ratio $H^+/e^- = 2$, which was observed previously in this and other laboratories. This new high value made better sense considering the H^+/ATP values that have been observed in recent years in chloroplasts and mitochondria. Last year a paper was published in which our conclusions were contested (Saphon and Crofts, *Z. Naturf.* **32C** 810, 1977). We have been able to disprove their criticism, and thus gain confidence in our own numbers. The manuscript for a paper describing this work will be submitted soon.

Entirely independent measurements concerning this topic were made by Velthuis, who studied the electrochromic absorption shift at 515 nm. His observation proved the existence of an energy conserving site in the reaction chain between the photoacts, and thus indirectly confirmed our high H^+/e^- ratio. The paper reporting these results has been reviewed by your office and submitted to "Biochimica Biophysica Acta." Its summary reads as follows:

"Isolated spinach chloroplasts that were preilluminated in the absence of electron acceptors showed a slow phase in the flash-induced 515-nm absorption increase. This observation, combined with earlier evidence (Bouges-Bocquet (1977) *Biochim. Biophys. Acta* 462, 371-379), indicates that besides the two electrogenic primary photoacts, a third electrogenic reaction across the membrane occurs, which is associated with electron transport between Photosystems II and I. This implies an H^+/e^- ratio of at least 3."

IV. OBSERVATIONS OF A LONG-LIVED EXCITED STATE OF P700

We have made more detailed observations of a short-lived charge separation in photosystem I. From spectral analyses, we conclude that a true electron transfer is involved and not the formation of the triplet state of P700. A first draft of a manuscript reporting these results for publication has been written; an improved version will be mailed to your office soon.

During this reporting period, a very similar observation was reported from the laboratory of P. Mathis (Paris). To our opinion, we have made a sufficient number of additional observations to warrant another publication on the subject. For instance, the transient disappears when the iron sulfur protein is removed from the system I particles. This might imply that the "early" electron acceptor involved in the transient is a ferredoxin -- like the "secondary" acceptor P430.

Status of Publications

- "Photosynthetic Oxygen Evolution from Hydrogen Peroxide," B.R. Velthuys and B. Kok, Biochim. Biophys. Acta. In press.
- "Measurement of the Oxygen Cycle: The Mass Spectrometric Analysis of Gases Dissolved in a Liquid Base," R. Radmer and O. Ollinger. In press.
- "Observations on the O₂ Evolution System," B.R. Velthuys and B. Kok. Proc. Fourth Internat. Cong. on Photosynthesis (1977)
- "Evidence for a Complete H⁺-Transporting Loop in the Chain Connecting Photosystems II and I," B.R. Velthuys. Biochim. Biophys. Acta. Submitted 3/78.
- "Kinetics and Apparent K_m of Oxygen Cycle under Conditions of Limiting CO₂ Fixation," Plant Physiol. In press.