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ABSTRACT

Work during the past reporting period can be separated into four main topics. Highlights include:

(1) Oxygen Evolution. Under some conditions we have observed the apparent transition of S_1 to S_2 in the dark. Redox titrations showed that this transition has a midpoint potential of 400 mV at pH 7, and a pH dependence of 60 mV per pH unit.

(2) Proton Transport. The proton uptake and release that apparently accompany the reduction and oxidation of plastoquinone were found to oscillate with a period of two in flashing light. Proton release associated with the O_2 -evolving system was found to oscillate with a period of four in flashing light; however, only one-half of the protons seemed to be liberated in the final O_2 -evolving step. We found that three to four protons per electron were transported from the outside to the inside of the thylakoid, which suggests that at each coupling site one electron transports two protons simultaneously.

(3) Acid-Base Induced Fluorescence Changes. Further evidence was obtained for a base-induced backflow of electrons to the System II acceptor Q. A subsequent fluorescence transient, which could not be readily rationalized in the context of standard models, was also observed.

(4) Mass Spectrometer Studies. Using whole algae, we observed that molecular O_2 directly competes with CO_2 for photosynthetically generated reducing power via a process unrelated to carbon metabolism. In a study using CO_2 -depleted chloroplasts, we found that bi carbonate was not the immediate source of O_2 in the bicarbonate-dependent Hill reaction.

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INTRODUCTION

During the past reporting period our group has investigated a number of facets of green plant photosynthesis, including:

(1) Polarographic studies of the oxygen evolution process have been continued. Experiments were again centered on attempts to influence the oxygen precursor states by externally-added chemical oxidants.

(2) Our continued studies of light-driven proton transfer in chloroplasts showed that proton uptake and release, which presumably accompanies the reduction and oxidation of quinone, has a strong oscillation with a period of 2. The yields and kinetics of the proton release in the O_2 evolution process were also reevaluated.

(3) Haim Hardt, who joined us for a post-doctoral tenure, has continued some of his earlier studies of a transient increase of the fluorescence yield induced by an acid-base transition.

(4) A fast mass spectrometric gas analysis system was used to monitor transient gas exchange in illuminated algae. A rapid O_2 uptake unrelated to carbon metabolism was observed.

The results of this work are described in the following sections. Two reviews were also completed: one concerned the energy transfer between bacteriochlorophyll and reaction center chlorophyll; the other, the efficiency of photosynthesis. In addition, we have collected some of our thoughts (or rather speculations) on the topic of photosynthetic productivity; a draft of the manuscript is enclosed (Enclosure 1). Also enclosed is a draft of another manuscript in preparation (Enclosure 2). The other listed publications and manuscripts have been previously forwarded to your office.

OXYGEN EVOLUTION

Our effort in this area has been on a relatively low level. Still, one study has yielded interesting new results. As was reported earlier, ⁽¹⁾ the yield of the second flash given after a long dark period appeared to be higher than normal in the presence of ferricyanide. Further analyses showed that this increase of Y_2 was dependent upon the concentration of ferricyanide and became maximal and constant at concentrations ≥ 10 mM. At this concentration Y_2 approached the value of the steady state yield (Y_{ss}), as if 1/4 of the traps were oxidized to the " S_2 " state in the dark.

We have since attempted to perform a redox titration of this oxidation. Chloroplasts samples were stored in the dark in sucrose buffer without added oxidants. After sufficient time to allow complete deactivation, they were exposed for a few minutes to mixtures of ferro- and ferricyanide of measured redox potential. The samples were then placed in the polarograph and a sequence of O_2 flashyields was measured. The results are shown in Fig. 1, in which the value of Y_2 is plotted as a function of the redox potential of the medium at 3 different pH values. These results are consistent with a one equivalent oxidation-reduction step with a midpoint potential of 400 mv at pH 7, and a pH dependence of 60 mv per pH unit (see insert Fig. 1). Our present interpretation is that we view the oxidation reduction of a semiquinone-type intermediate. No such intermediate has been reported to occur in the chloroplast.

Since we don't believe that the midpoint potential of the S_2 precursor state could be as low as 400 mv (see ref 1), we have tried to decide between this and an alternate hypothesis: viz. that the high Y_2 is due to a "double hit" phenomenon. Experiments to date point in the

Fig. 1. Dependence of Y_2 Upon Redoxpotential

latter direction. However, the experiment is technically quite difficult and, as described in the accompanying proposal, we plan to pursue this interesting phenomenon in greater detail.

In addition to the above effect, we have had occasional success in changing the oxygen precursor distribution in still more drastic and interesting ways. Under very strongly oxidizing conditions we have seen high concentrations of S_2 and at times even of S_3 . The difficulty still is that these observations have not been sufficiently reproducible to allow systematic studies.

PROTON TRANSPORT IN PHOTOSYNTHESIS

Our continued studies of light-driven proton transfer in chloroplasts concentrated on a new observation: viz. that proton uptake and release, which presumably accompany the reduction and oxidation of quinone, show a strong oscillation with a period of 2. These data therefore offer a direct observation of a) the pairing of electrons and protons in the proton uptake at the outside of the membrane, and b) the unpairing in the H^+ release at the inside of the thylakoid. A draft of a manuscript reporting this work is enclosed. Its summary reads as follows:

"Using dark adapted isolated chloroplasts and sequences of brief saturating flashes we studied the uptake and release of protons correlated with electron transport from Photosystem II to Photosystem I. The following observations and conclusions are reported:

1) Flash induced proton uptake shows a weak, damped binary oscillation, with maxima occurring after the 2nd, 4th, etc., flashes. The damping factor is comparable to that observed in the O_2 flash yield oscillation and therefore explained by misses in Photosystem II.

2) We correlate this phenomenon with the earlier reported binary oscillations of electron transport⁽²⁾ and conclude that both electrons and protons are transported in pairs between the photosystems.

3) After each flash (i. e., after each reduction of Q) two protons are taken up from the outside of the chloroplast.

4) These protons and the associated electrons are not released to the inside of the thylakoid until $4 H^+$ and $2e^-$ are accumulated. Subsequently, the two electrons are transferred to nonprotonated acceptors in Photosystem I (probably plastocyanin and cytochrome f) and the $4 H^+$ are released inside the thylakoid.

5) We conclude that an energy conserving step located between Q and P is being observed."

Using the same technique, we also made more quantitative determinations of the number of protons which are released in each of the four steps needed to liberate oxygen from water. The new data warrant a revision of our earlier hypothesis that all protons are liberated in the last of the four oxidation steps. Instead only 2 protons are liberated in this final reaction; the other two are released seemingly at random in the earlier oxidations which lead to state S_3 . A draft of a manuscript describing this work is in preparation. A summary of the results is:

"Using dark adapted isolated spinach chloroplast and sequences of short saturating flashes we made a quantitative study of the release of protons from the O_2 evolving system. The following observations are reported:

1) As was reported earlier, the proton yield shows a damped oscillation having a period of 4.

2) These oscillations are a linear combination of several components. One component has a periodicity identical to that of the O_2 flash yields. It amounts to an average release of $1/2 H^+/e^-$, or the simultaneous release of $2H^+$ with every O_2 released. Another release component oscillates with a period of two, with maxima on the 2nd, 4th, etc., flashes and amounts to $1/4 H^+/e^-$. This suggests that some of the S state transitions release more H^+ (or take up more OH^-) than others. Modification of the initial dark distribution of the O_2 precursor states by preflashes shows that the number of protons per electron released on the S_0 to S_1 and S_2 to S_3 is greater than on the S_1 to S_2 transition.

3) The results are interpreted by assuming that either 1 OH^-/e^- is taken up (or 1 H^+/e^- released) only during the S_0 to S_1 and S_2 to S_3 transitions. The transition S_4 to S_0 results in the release of 2 H^+/e^- concurrently with the release of O_2 ."

During this reporting period, a paper was written and published that described some of our earlier pH studies: we determined the number of protons per electron that are translocated from the outside to the inside of the thylakoid. Interestingly, this number proved to be 3-4 H^+ per electron, twice as high as formerly assumed.⁽³⁾ We postulated that at each of the two coupling sites, one electron passage translocates two protons simultaneously. The summary of the paper states:

"Using a rapid pH electrode, measurements were made of the flash-induced proton transport in isolated spinach chloroplasts. To calibrate the system, we assumed that in the presence of ferricyanide and in steady-state flashing light, each flash liberates from water one proton per reaction chain. We concluded that with both ferricyanide and methylviologen as acceptors two protons per electron are translocated by the electron transport chain connecting Photosystem II and I. With methylviologen, but not with ferricyanide, as an acceptor, two additional protons per electron are taken up due to Photosystem I activity. One of these latter protons is translocated to the inside of the thylakoid while the other is taken up in H_2O_2 formation. Assuming that the proton released during water splitting remains inside the thylakoid, we compute H^+/e^- ratios of 3 and 4 for ferricyanide and methylviologen, respectively.

In continuous light of low intensity, we obtained the same H^+/e^- ratios. However, with higher intensities where electron transport becomes rate limited by the internal pH, the H^+/e^- ratio approached 2 as a limit for both acceptors.

A working model is presented which includes two sites of proton translocation, one between the photoacts, the other connected to Photosystem I, each of which translocates two protons per electron. Each site presents a ≈ 30 ms diffusion barrier to proton passage which can be lowered by uncouplers to 6-10 ms. "

AN ACID BASE INDUCED REDUCTION OF Q IN CHLOROPLASTS

It is generally accepted that the fluorescence yield of chlorophyll a in isolated chloroplasts reflects the reduction state of Q, the primary electron acceptor in Photosystem II. Recently, it was found⁽⁴⁾ that by subjecting the chloroplasts to an acid-base transition the fluorescence yield undergoes a temporary increase. These authors suggested that the momentary ΔpH created across the thylakoid membrane induced a pulse of reverse electron flow towards Q, causing its temporary reduction.

In the present work we analyzed the kinetic components of the signal and tried to find how they relate to the electron transport through the photosystems. Figure 2 shows the detailed picture of a typical signal induced by the injection of a base (pH 9.0) into the chloroplast suspension (initially at pH 6.0). Note that the injection of base caused a fast rise (A) followed by a slow rise up to a maximum (B) and finally a slow decay (C) to a new steady state. The magnitude of the signal from the level in acid to the maximum corresponds to a reduction of about 20% of Q. Control experiments, in which a pH 8.9 $\rightarrow$ 5.7 transition was carried out, showed a fast drop rather than a rise in the fluorescence yield.

We were able to demonstrate that the rapid initial rise (A) of the base induced high fluorescence is due to a dark reaction: the effect is also observed if the base is added in the dark, before turning on the measuring beam.

By increasing the dark time between the preillumination and the addition of base (in the range 10 sec-10 min) we found a decrease of the

Fig. 2. Typical Acid-Base Induced Fluorescence Signal

effect that paralleled the dark reoxidation of the pool of secondary electron acceptors. This decrease was accelerated by a short illumination with far red light -- a well known procedure to oxidize the pool. It therefore was no surprise to find that ferricyanide abolished the acid-base signal.

Although at higher intensities of the measuring beam Q was more reduced in the acid stage (reflected in a higher level of fluorescence) the fraction of Q reduced rapidly by the addition of base was always the same (~20%).

The final decay phase (C) of the signal proved to be influenced by methylviologen. In the presence of this System I acceptor (but not in its absence) the decay phase became faster at higher intensities, obeying the "I x t rule." This observation relates the decay phase to an oxidation by System I.

The rise phase (B) also became faster at higher intensities. However, in this case, the dependence was not influenced by the presence of methylviologen; hence we conclude that a reduction of Q by System II is involved. 20 mM NH_2OH , which is known to inhibit the electron transport between H_2O and system II, does not affect the fluorescence change, suggesting that the effect is in the chain between the two photoacts.

Taken together, these observations seem to point to a base-induced flow of electrons to the photoreductant Q, at least in the "dark" part of the process (A). The fluorescence rise (B) driven by System II is not readily understood and additional studies are planned.

MASS SPECTROMETER STUDIES

Using an improved version of the mass spectrometer system described in Proposal #B175-14R, we have obtained significant new insight into 1) the fate of photosynthetic reducing power, and 2) the source of photosynthetically evolved oxygen.

We recently observed that molecular O_2 directly competes with CO_2 for photosynthetically generated reducing equivalents, and that O_2 reduction can occur at a rate comparable to the maximum photosynthetic rate. A report of this work, submitted to Plant Physiology for publication, is enclosed. Its summary states:

"A mass spectrometer with a membrane inlet system was used to directly monitor gaseous components in a suspension of algae. Using labeled oxygen, we observed that during the first 20 seconds of illumination after a dark period, when no net O_2 evolution or CO_2 uptake was observed, O_2 evolution was normal but completely compensated by O_2 uptake. Similarly, when CO_2 uptake was totally or partially inhibited by iodoacetamide, KCN, or FCCP, O_2 evolution proceeded at a high (near maximal) rate. Under all conditions, O_2 uptake balanced that fraction of the O_2 evolution which could not be accounted for by CO_2 uptake.

From these observations we concluded that O_2 and CO_2 are in direct competition for photosynthetically generated reducing power, with O_2 being the main electron acceptor during the induction process and under other conditions in which CO_2 reduction cannot keep pace with O_2 evolution. The high rate of the O_2 uptake reaction observed in the presence of Calvin cycle inhibitors suggests that a special high

capacity oxidase distinct from RuDP oxygenase exists in whole cells.

The rapid reduction of molecular O_2 after a period of darkness probably serves as a priming reaction for the photosynthetic apparatus. The high steady state rate of the oxygen cycle in the absence of CO_2 fixation suggests that the regulation of photosynthesis does not involve significant changes in the rate of photochemical electron transport."

In another study we determined the isotopic composition of O_2 evolved in the CO_2 -dependent Hill reaction. We found that even when ^{18}O -labeled bicarbonate was used to activate the Hill reaction, the evolved oxygen was unlabeled. Thus bicarbonate is not the immediate source of photosynthetically evolved oxygen. A reprint of this work, which appeared in Science, has been forwarded to your office.

PUBLICATIONS (1975-1976)

"Determination of H/e Ratios in Chloroplasts with Flashing Light," C. F. Fowler and B. Kok, Biochim. Biophys. Acta, 423, 510-523 (1976).

"Source of Photosynthetic Oxygen in Bicarbonate-Stimulated Hill Reaction," A. Stemler and R. Radmer, Science 190, 457-458 (1975).

"Energy Capture in Photosynthesis: Photosystem II," R. Radmer and B. Kok, Ann. Rev. of Biochemistry 44, 409 (1975).

"Prospects of Photosynthetic Energy Production," B. Kok, Science and Technology 35, 519-526 (1975).

"Electron Transport in Photosystem II," B. Kok, R. Radmer and C. Fowler, Proc. of Third International Congress on Photosynthesis, Elsevier Scientific Publishing Co., Amsterdam, p. 485-496 (1975).

"Oxygen Evolution in Photosynthesis," P. Joliot and B. Kok, In: Bioenergetics of Photosynthesis, Chapt. 8, Ed. Govindjee, Academic Press, N. Y., p. 387-412 (1975).

MANUSCRIPTS (in press, submitted for publication or in preparation)

"Mechanisms in Photosynthesis," Am. Chemical Society's Monograph Series, B. Kok and R. Radmer, in press.

"Photoreduction of O₂ Primes and Replaces CO₂ Assimilation," R. Radmer and B. Kok, submitted to Plant Physiology (1976).

"Photosynthesis: Limited Yields, Unlimited Dreams," B. Kok and R. Radmer, to be submitted to Bi-Science. *Removed*

"Energy Transfer Between Antenna Components and Reaction Centers," K. L. Zankel, Photosynthetic Bacteria, in press.

"Light Conversion Efficiency in Photosynthesis," R. Radmer and B. Kok, In: Encyclopedia of Plant Physiology, in press.

"Photosynthesis: The Path of Energy," B. Kok, Plant Biochemistry, Third Edition, Academic Press, J. Bonner and J. Varner, Eds., in press.

"Proton Translocation in Chloroplasts and its Relationship to Electron Transport Between the Photosystems," Charles F. Fowler, to be submitted to Biochim. Biophys. Acta *Removed*

"The Oscillation Behavior of Flash-Induced Proton Evolution in Chloroplasts and its Relationship to O₂ Evolution," Charles F. Fowler, to be submitted to Biochim. Biophys. Acta.

REFERENCES

1. Kok, B., Radmer, R., and Fowler, C.F. In: Proceedings of Third Internat. Congr. on Photosynthesis, Ed. M. Avron, Elsevier, Amsterdam, p. 485 (1975).
2. Bouges-Bocquet, B. Biochim. Biophys. Acta. 314, 250-256 (1973).
3. Fowler, C.F. and Kok, B. Biochim. Biophys. Acta. 423, 510-523 (1976).
4. Shahak, Y., Hardt, H. and Avron, M. FEBS Lett. 54, 151-154 (1975).