



Minireview

The isolated Photosystem II reaction center: first attempts to directly measure the kinetics of primary charge separation

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Received 15 August 2002; accepted in revised form 17 December 2002

Key words: charge separation, femtosecond spectroscopy, Govindjee, Scott Greenfield, Photosystem II, reaction center, Michael Seibert, Michael Wasielewski

Abstract

Direct measurements of the intrinsic rate of primary charge separation in the isolated Photosystem II (PS II) reaction center complex had to await the availability of suitable, stabilized reaction center materials as well as sophisticated femtosecond transient absorption spectroscopic techniques. The events that led to the first direct measurements of the primary charge separation act in PS II and discussions of the results thereafter are chronicled in this brief historical review.

Abbreviations: ANL – Argonne National Laboratory; Chl – chlorophyll; NREL – National Renewable Energy Laboratory; PS I – Photosystem I; PS II – Photosystem II; RC – reaction center

The Photosystem II reaction center material

The mid-1980s were interesting times for those of us interested in photosynthesis because the first integral membrane protein complex, the bacterial reaction center from *Rhodospseudomonas viridis*, had just been crystallized (Michel 1982). Shortly thereafter, Deisenhofer et al. (1985) solved the X-ray structure of the complex; and Hartmut Michel, Johann Deisenhofer, and Robert Huber shared the 1988 Nobel Prize in Chemistry for this important advance in membrane biophysics. (See Govindjee and Krogmann, 2002, for a listing of all Nobel Prizes related to photosynthesis research.) However at that point, the structure of the Photosystem II (PS II) reaction center (RC) was unknown, and in fact the proteins that constitute the PS II RC were not as yet correctly identified. The dogma at the time suggested that CP47 (chlorophyll protein complex of 47 kDa) was the RC, but this point was being debated since evidence suggesting sequence homologies between the L and M subunits

of the bacterial reaction center and the *psbA* (the herbicide-binding or Q_B protein, D1) and *psbD* (D2) gene products of PS II was appearing. Several groups in 1985 suggested that D1 and D2 constituted the PS II RC, and a folding model for the purported PS II RC was proposed (Trebst 1986) and verified (Sayre et al. 1986). Around the same time, Jim Metz, while he was at the National Renewable Energy Laboratory (NREL), was examining the LF-1 (processing) mutant of the green alga *Scenedesmus obliquus*. This PS II mutant, from Norman Bishop's laboratory, had normal RC and reducing-side function, but it was deficient in donor-side O₂ evolution and bound decreased amounts of Mn compared to wild-type (WT) cells. Jim found that it was affected in a 34-kDa, PS II core protein that bound azidoatrazine (Metz et al. 1985) and later showed conclusively by Western blots that the affected protein was D1 (Metz et al. 1986). This was the first biochemical evidence demonstrating that D1 had both donor- and acceptor-side function and strongly supported the camp that thought D1, and not CP47, was a

part of the PS II RC. Although this conclusion was not stated *per se* in the published version of the paper, the issue became moot as the result of Kimiyuki Satoh's exciting announcement at the VIIth International Photosynthesis Congress in Providence, RI, that he and his student (O. Nanba) had successfully isolated the PS II RC complex and that it indeed contained both the D1 and D2 proteins (see Satoh, this issue).

Satoh was very gracious at the Congress and fully described details about the isolation procedure to anyone who asked. One of us (MS) went back to Colorado after the meeting and spent many hours in a cold room trying to reproduce the results. With some effort, purified PS II RC complex came off the Toyopearl 650S column, exactly as Satoh had said. However, the red absorption peak of the material right off the column was at 676 nm and not 673 nm as Nanba and Satoh had reported in their landmark paper (Nanba and Satoh 1987). We thought this result was curious and spent a lot of time trying to characterize the material spectrally. It became apparent that the RC complex as originally isolated, exhibited rapid blue-shifting of the red peak in both its absorption and fluorescence spectra. We suspected that the reason for this was an inherent instability of the complex and shipped a paper off to a leading fast-publishing journal to warn our colleagues that the RC material was labile and lost primary photochemical activity very rapidly if exposed to air under room light and temperature conditions. Unfortunately, despite the spectroscopic characterization of the material and its labile nature, the paper was rejected because it was categorized as a 'methods' paper. Fortunately, *Plant Physiology* saw the results as being relevant for those who wanted to use the new RC material, and our paper was published (Seibert et al. 1988) after some delay. For this and a follow-up article (McTavish et al. 1989), Rafael Pico-rel spent a lot of time, while he was at NREL, helping to develop the techniques that are now widely used to stabilize isolated PS II RC materials for spectroscopy (i.e., substitution of dodecyl maltoside for Triton X-100 and the use of an enzymatic oxygen-scrubbing system to prevent photo-oxidative damage).

Transient absorption spectroscopy

At around the same period of time, the Illinois contingent (Govindjee and Mike Wasielewski) had begun a collaboration to examine the primary photochemistry of Photosystem I (PS I) (Wasielewski et al. 1987). At

that time accurate determination of the time-resolved absorption spectra of the intermediates involved in the primary charge separation within PS I was a difficult problem because the most stable protein complex still contained about 40 Chls per P700. This made it difficult to separate the relatively small absorption changes due to $P700^+-A_0^-$ from the spectral changes due to formation of excited states within the antenna pigments. The ultrafast laser apparatus in the laboratory of one of us at Argonne (MW) in the late 1980s consisted of an actively mode-locked Ar ion laser that synchronously pumped a dye laser with a gain dye/saturable absorber mixture to produce 500 fs pulses. These pulses were amplified with a four-stage dye amplifier, pumped at 10 Hz by a frequency-doubled Nd-YAG laser to give pulses of about 500 fs duration with 1 mJ of energy. A large fraction of this energy was used to generate a white light continuum in water or CCl_4 to produce a spectroscopic probe pulse of comparable duration to the yellow pump pulse.

The initial collaboration

At the time that the above-mentioned *Plant Physiology* paper was accepted, Govindjee, with some hesitancy, revealed to us that he had reviewed the manuscript. He had already started collaboration with one of us (MW) with PS II RC preparations that were prepared by him and his student, Hyunsuk Shim, at Urbana, Illinois. These preparations seemed to become inactive and lose photochemical activity. Govindjee's aim was to propose a three-way collaboration between Argonne National Laboratory (ANL), NREL and the University of Illinois in an attempt to use ANL's ultrafast transient absorption equipment to measure primary charge separation kinetics of isolated, active PS II RC material. We were, of course, interested, and those phone calls started a long series of events in the US and the rest of the world that led to great improvements in the understanding of the early photophysical and photochemical events in PS II.

Following a lot of preparation, we all first met at ANL in the early summer of 1988. The Seibert lab isolated the PS II complex and employed the newly discovered procedures developed at NREL to improve the stability of the material; the Wasielewski lab had state-of-the-art equipment ready; and Govindjee brought his extensive experience, keen insights, knowledge of the literature, and passion for Japanese food. In looking back over the period, the outings

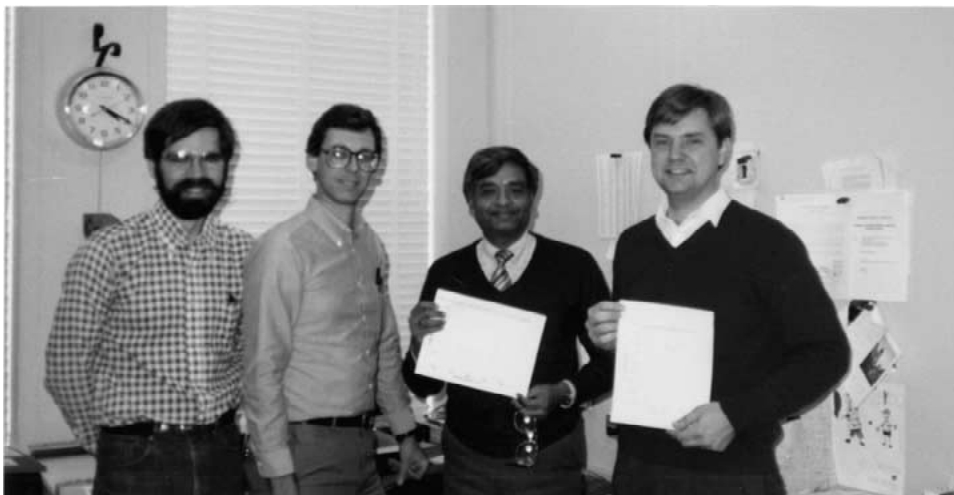


Figure 1. A photograph of (left to right) Doug Johnson, Mike Seibert, Govindjee, and Mike Wasielewski at Argonne National Laboratory during the summer of 1988 with the results of the first direct measurements of primary charge-separation kinetics in isolated Photosystem II reaction center complexes. Photo by Walter Svec.

to the Yokohama restaurant in neighboring Westmont, Illinois, during these collaborative runs may have been the cement that kept this work going. In any case the reports addressing the first direct measurements of the primary charge-separation kinetics in isolated spinach PS II RC complex at 277 K (Wasielewski et al. 1989a) and at 7 K (Wasielewski et al. 1989b) appeared within the first year of the troika collaboration. Figure 1 shows a 1988 photograph of the participants in this work (Doug Johnson, the authors and Govindjee). Concurrent 4 K hole-burning experiments with the same RC material in Gerry Small's laboratory at Iowa State University (Jankowiak et al. 1989) provided credence for the lifetimes observed at ANL. This complementary technique, which is carried out in the frequency domain, also yields lifetime information. Furthermore, Alfred Holzwarth's group in Mülheim, Germany, used complementary ultrafast fluorescence techniques to estimate lifetimes close to those observed at ANL (Roelofs et al. 1991).

In a similar time frame, the research groups of Jim Barber and the late Sir George Porter (1920–2002; for a photograph of Porter, see Brody 2002) in London, UK, principally through the efforts of David Klug and James Durrant, were engaged in similar ultrafast transient absorption experiments (Durrant et al. 1992; Hastings et al. 1992) as was Hans van Gorkom's group at Leiden, The Netherlands (Schelvis et al. 1994). A great many lively discussions arose over those years about the exact rate of primary charge separation that

engaged everything from the technical details of the ultrafast experiments to the quality and treatment of the sensitive PS II RC samples. Ultimately, it is our view that over a period of time the actual data being generated by all groups converged to the point where they were essentially identical. The devil was and still may be in the details of the interpretation. Many of the technical aspects of this story, along with the contributions of a number of other groups, are presented in recent reviews (Dekker and van Grondelle 2000; Diner and Rappaport 2002) and will not be repeated in this short historical report. However, the essential experimental observation was that the transient absorption kinetics at many wavelengths, especially in the 545 nm bleach region that is partially due to the formation of $P680^+-Pheo^-$, are multi-phasic. Imposing an exponential fit onto these data yields short, medium, and long-lived components, having approximately 3, 20, and between 50–100 ps duration (Greenfield et al. 1996). Our group along with several others argued strongly for assigning the 3-ps component to the primary charge separation, whereas the British group favored the 20-ps component. Modeling energy and electron transfer within this system proved to be complex because the energy levels of all of the chlorophylls and pheophytins within the RC are very similar. Thus, it was difficult to separate the actual primary charge separation time from the time for energy equilibration among the ensemble of pigment molecules.

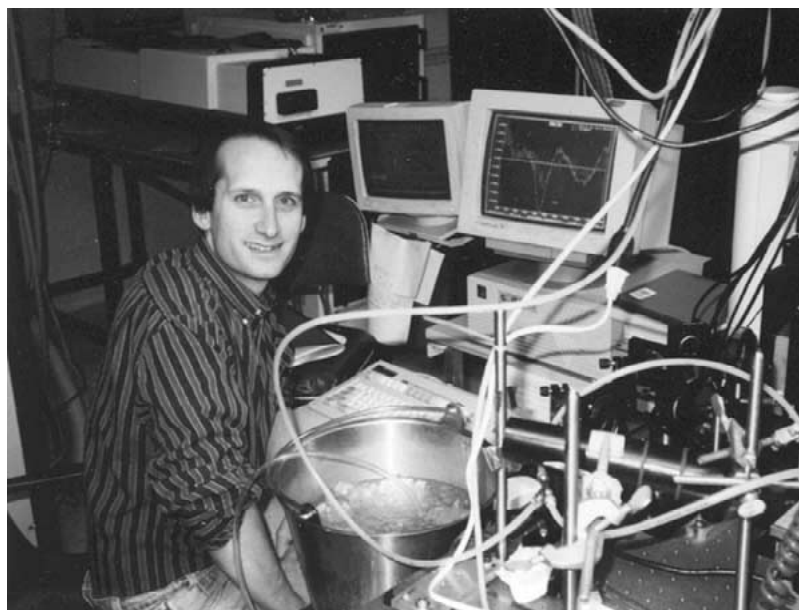


Figure 2. A photograph of Scott Greenfield, taken in 1997, with the instrument that led to the work published by him, the authors and Govindjee in 1996 and thereafter. Photo by Govindjee.

The more recent past

As time progressed the laser technology improved with (1) the advent of Nd-YAG regenerative amplifiers that permitted the generation of amplified pulses at 1-kHz repetition rates and (2) the use of dispersion compensated mode-locked dye lasers that dropped pulse durations to about 75 fs and total instrument response to about 100 fs. Using the improved signal-to-noise that this technology provided, it was possible to explore the linear dichroism of the transient absorption changes with PS II (Wiederrecht et al. 1994). However, the dye laser technology was short-lived, and rightly so, given the difficulties of keeping this type of laser system running well on a day-to-day basis. It was supplanted by Ti-sapphire technology, which has become the standard method used for generating stable, amplified laser pulses. MW constructed his first such system at ANL in early 1993 and the trio (Wasielewski, Seibert and Govindjee), in collaboration with an outstanding research associate Scott Greenfield, applied it immediately to the study of PS II. An important feature of this system, developed in 1994, was the design and construction of an optical parametric amplifier that gave us transform limited tunable laser pulses of about 150 fs duration throughout the visible spectrum (Greenfield and Wasielewski 1995). Figure 2 shows a 1997 photograph of Scott

Greenfield in front of the Ti-sapphire system at ANL. This new capability allowed us to take a careful look at the excitation wavelength dependence of the PS II energy and electron transfer dynamics. Ultimately this technology has allowed us to rapidly acquire sufficient signal-to-noise on these sensitive samples, so that degradation during the experiments is virtually eliminated. Furthermore, the enhanced signal-to-noise has permitted the use of weak excitation pulse energies to minimize the effects of multi-photon absorption and to analyze small transient spectral changes with more confidence. This resulted in new ways to analyze the data that clarify some of the issues that were difficult to ascertain when we first began in the late 1980s (Greenfield and Wasielewski 1996; Greenfield et al. 1996). For example, a long-lived component in the transient spectra results in an overall shift in the optical absorption baseline due to excited states of the RC pigments. Removal of this background results in an 8 ps time constant for charge separation at 277 K (Greenfield et al. 1997), which is reduced slightly to 5 ps at 7 K (Greenfield et al. 1999). Müller et al. (1996) also reported an 8.9 ps component at 277 K using combined global analysis but attributed it to slow energy transfer from an external chlorophyll. The 5 ps (7 K) number agrees very well with recent data obtained from photochemical hole burning experiments at 4 K (Jankowiak et al. 2003). Dekker and van Grondelle (2000) sug-

gested that the 8 ps time is for an expanded radical pair formation, whereas the actual primary charge separation may occur in equal to or less than 2 ps. Furthermore, they suggested that the 20 ps time may be due to energy transfer from the Chl molecules (on D1 and D2) to RC Chl molecules, and the 50 ps time may arise from protein relaxation. These concepts need to be tested with various site-directed mutants.

The future

After nearly 15 years of both scientific and personal enjoyment, our three-way collaboration has expanded to include Dick Sayre's group at the Ohio State University. Our work with Dick has added the new dimension of studying PS II RCs from green algae with site-directed mutations in the D1 and D2 RC polypeptides. This work is currently beginning to yield its first results (Wang et al. 2002). A few years ago the Wasielewski group moved to Northwestern University, where the ultrafast spectroscopy is now being carried out. In the meantime, Govindjee became Professor Emeritus on July 21, 1999, and our trips to the Japanese restaurant ended. We are confident that as laser technology improves and as other RC preparations become available, we will be able to obtain a better understanding of the complexities of energy and electron transfer within RCs from both plants and algae. The first publication of an X-ray crystal structure for a PS II core complex (Zouni et al. 2001) will be very useful in this endeavor. We look forward to the future because the journey thus far has been tremendously satisfying.

Acknowledgments

Work performed at NREL was supported by the Division of Energy Biosciences, Office of Science, U.S. Department of Energy (MS), and that at ANL and Northwestern University was supported by the Division of Chemical Sciences, Office of Science, U.S. Department of Energy (MRW). We especially thank Govindjee, who has been with us since the beginning of our journey, as well as all of our other collaborators who participated in the journey from 1988–2002. The latter include Hyunsuk Shim, Rafael Picorel, Doug Johnson, Chris Preston, Gary Wiederrecht, Scott Greenfield, Dave Gosztola, Ryan Hayes, Alex Gusev, Jun Wang, Xiong Ling, and Dick Sayre.

Finally, we want to acknowledge the close relationship of the ultrafast work with the complementary hole-burning studies spearheaded so ably by our colleagues Gerry Small, Ryszard Jankowiak, and their associates at Iowa State University. This paper was edited by Govindjee.

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