Photosynthesis Research **73:** 83–86, 2002.

Minireview

The two-electron gate in photosynthetic bacteria

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Received 4 July 2001; accepted in revised form 29 November 2001

Key words: Rod Clayton, photosynthetic bacteria, primary and secondary electron acceptors, quinone, two-electron gate, André Verméglio, Colin Wraight

Abstract

This paper gives a historical and personal account of the author's work in Rod Clayton's laboratory, when he observed the first evidence of the two-electron gate in bacterial reaction center. Colin Wraight had independently discovered this phenomenon at the same time. The high similarity between the acceptor side of Photosystem II (PS II) and of bacterial reaction centers was one of the first proofs for a profound homology between these two photosystems.

In Rod Clayton's laboratory, Cornell University, Ithaca, New York

When I arrived in October 1975 as a postdoc in Rod Clayton's laboratory at Cornell University, Ithaca, New York, it was generally admitted that the photochemistry of the photosynthetic reaction center of anoxygenic bacteria and of Photosystem (PS) I of plants or algae was very similar. This was essentially based on the occurrence of a light-induced cyclic electron transfer under anaerobic conditions in both cases. I was, however convinced, for my part, of a great similarity between the photochemistry of reaction center of purple bacteria and of PS II. This was based on several observations reported in the literature and from those obtained during my PhD thesis on the photochemistry of PS II at low temperature under the guidance of Paul Mathis in Saclay (France). For example, photooxidation of a cytochrome could be observed at low temperature for both systems and the secondary electron acceptors were quinone molecules in both cases. Hans van Gorkom (University of Leiden, The Netherlands) had conducted the most convincing experiment. Hans had performed detailed measurements of light-induced absorbance changes on PS II particles. In particular, he had clearly demonstrated that a bound molecule of plastoquinone was acting as primary electron acceptor in these particles (van Gorkom 1974). On the other hand, there was clear evidence that the primary electron acceptor of bacterial reaction centers (bRC) was an ubiquinone molecule (George Feher et al. 1972; Mel Okamura et al. 1975). Another similarity between the PS II and the bRC was that the formation of the semiquinone anion was accompanied by small absorbance band shifts. These changes were observed around 545 nm and in the red (685 nm, PS II) or far-red part (760 nm, bRC) of the spectrum. The 545 nm band shift observed upon reduction of the primary acceptor of PS II was originally discovered by David Knaff and Daniel Arnon (1969) and designated C550. The changes in isolated bRC were observed by Rod Clayton and S.C. Straley (1972) and attributed to absorption band shift of a bacteriopheophytin molecule upon reduction of the primary electron acceptor. By analogy, van Gorkom (1974) attributed the changes detected in PS II particles to an electrostatic influence of the semiquinone on a molecule of pheophytin.[1] Based on this high homology of the acceptor side of PS II and bRC, I decided to investigate carefully the light-induced absorption changes linked to photoreduction of the primary electron acceptor in isolated bRC. In an initial experiment, I wanted to determ-

ine the optimal concentration of an artificial electron donor necessary to rapidly re-reduce the primary electron donor after its photo-oxidation. These conditions were expected to stabilize the photoreduced electron acceptor. For this experiment, I used an apparatus built by Rod Clayton in the laboratory to measure light-induced absorption changes from the UV to the near infrared. In order to saturate the photochemistry of isolated reaction centers, Rod Clayton was utilizing a Xenon flash coupled to a large capacitor (few microfarads, 30 cm high, 20 cm long, 5 cm thick) charged at high voltage. I recorded the first absorption change at 450 nm, a wavelength where the formation of both the photooxidized primary electron donor (P^+) and the semireduced quinone were expected to give positive signals. I indeed observed a very fast increase due to the charge separation between electron donor and acceptor followed by a rapid decay due to P^+ (P being the RC bChl) reduction by the artificial electron donor (diaminodurene, DAD). Then a positive signal, stable for tens of seconds, was present. The signal was so stable in the dark that I thought at first that it was an offset of the baseline level induced by some electronic artifact during the discharge of the capacitor.[2] I repeated the experiment after one minute of dark adaptation and I observed again a rapid absorbance increase due to the photo-oxidation of the primary electron donor P followed by its re-reduction and a stable *negative* signal. I again believed this was due to artifactual changes in the baseline level. To overcome that problem I decided to give a series of 10 flashes 1 second apart. To my surprise, I observed a beautiful oscillation for the stable positive signal, with a periodicity of two flashes (Figure 1).

I quickly realized that these changes were not due to electronic artifacts but to the operation of a two-electron gate on the acceptor side of bRC. After each odd flash, the stable signal corresponded to the formation of a semireduced ubiquinone ($UQ^{\cdot -}$), which has a characteristic band at 450 nm, while after each even flash this characteristic band disappeared due to the formation of ubiquinol (UQH_2). A similar phenomenon had been discovered independently few years earlier by Bernadette Bouges-Bocquet and Bruno Velthuys (and Jan Amesz) for the PS II. Bouges-Bocquet (1973) had observed that electrons were available to PS I only after even-numbered photoreactions. Velthuys and Amesz (1974) had found that the re-reduction of the primary electron acceptor induced by the addition of 3-(3,4-dichlorophenyl)-1,1 dimethylurea (DCMU) in the dark (an inhibitor of the electron transfer between primary and secondary electron acceptors of PS II) or of sodium dithionite was more important after odd flashes than after even ones. I repeated the quinone oscillation experiment several times, varying the time between flashes and the amount of exogenous quinones. To clearly demonstrate that the stable species formed after each odd flash was a semiubiquinone, I needed to measure light-induced absorbance changes from the UV to the near infrared. This experiment was highly material-consuming, and I ran out of purified reaction centers. I therefore started the purification of a new batch. To my disappointment, I was not able to observe any quinone oscillations with this new preparation. I rapidly noticed that the two batches of RCs had not been prepared according to the same purification procedure. I had purified the second batch of RCs using ammonium sulfate precipitation, while I had used a diethylaminoethanol (DEAE) column to purify the first batch. The latter procedure was used for looking at linear dichroism spectra of RCs oriented on microscope slides, and involved brushing and drying. Jacques Breton in Saclay had successfully used this technique to orient chloroplasts or various photosynthetic membrane fragments (Breton et al. 1973). This purification method produced RCs that are less pure than those obtained from the ammonium sulfate precipitation procedure, but the RCs could be solubilized in the presence of a lower detergent concentration because they retained some lipids. This property was

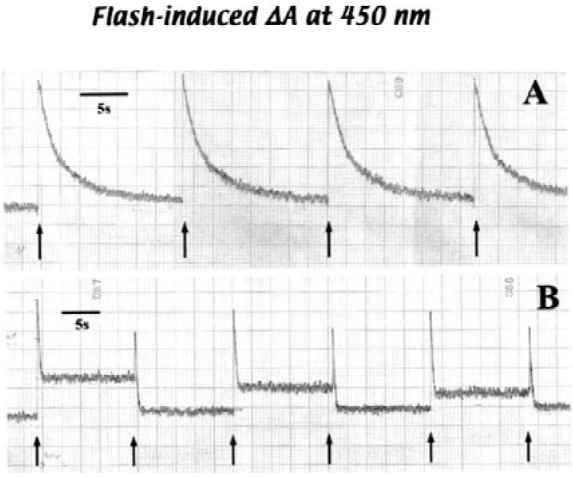

Figure 1. Original recording of the light-induced absorbance changes observed at 450 nm for a suspension of reaction centers isolated from *Rhodobacter sphaeroides* R26. (A) In the absence of electron donor. Excitation flashes were 15 s apart. The changes correspond to the formation of $P^+ Q_B^-$ followed by the back reaction. (B) In the presence of an articial electron donor. Excitation flashes spaced by 10 s. The stable change after each odd flash corresponds to the formation of the semireduced ubiquinone ($Q_B^{\cdot -}$). This signal disappears after each even flash due to the formation of an ubiquinol molecule (Q_BH_2) which does not absorb at this wavelength.

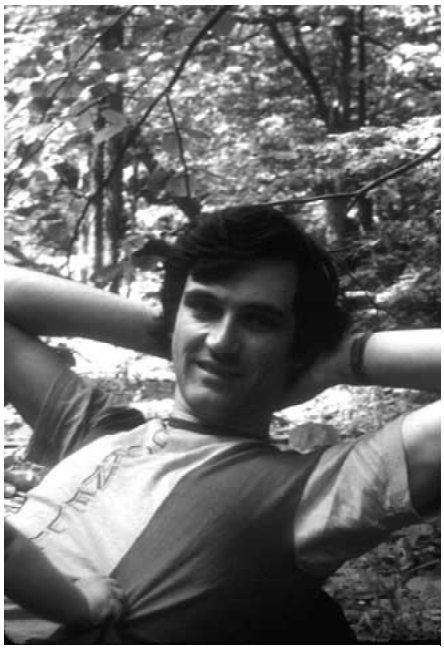

Figure 2. The author (André Verméglio), 1976.

Figure 3. Colin A. Wraight, 1999. Photograph provided by Govindjee.

then used by Rod Clayton to avoid a high concentration of detergent – when drying RCs on microscope slides, for example. I thus started a new preparation using the ammonium precipitation, and checked at each step of the procedure the occurrence of the quinone oscillatory phenomenon. It appeared that precipitation by the ammonium sulfate was the damaging step. Since the ammonium purification was the 'classical' procedure used by the different laboratories involved in the studies of RCs, this may explain why the oscillatory phenomenon had not been observed previously. This also means that I was lucky enough to use DEAE RCs for the first experiment. The reason why the purification method affects the quinone oscillations is still not fully clear to me.

Independent observation by Colin Wraight and back to back publication of our papers

A few months later, John Olson organized a symposium on photosynthesis at the Brookhaven National Laboratory. On this occasion I met Colin Wraight, who had observed independently the same phenomenon while working at the University of California in Santa Barbara. I remember comparing our results in the back of a station wagon while going to a restaurant. We had obtained exactly the same results. So we decided to send our papers to *Biochimica et Biophysica Acta* simultaneously and requested the editorial board to publish our two articles in the same issue (Wraight 1977; Verméglio 1977). Figures 2 and 3 show photographs of André Verméglio and Colin Wraight. Photographs of Rod Clayton are shown in his paper (this issue).

This story is a typical example of the mutual progress in studies of PS II and bRCs. The two-electron gate was first discovered in PS II, from strong but indirect evidence, by Bernadette Bouges-Bocques and Bruno Velthuys and Jan Amesz. The extension of this mechanism to bRCs not only highlighted the similarity between both systems, but also provided direct spectral identification of the formation of the semiquinone and opened the way to a number of exciting studies at the molecular level.

Mechanistic understanding of the two-electron gate

One surprise of our results was that the difference absorption spectrum linked to the formation of the semireduced ubiquinone indicated that this molecule was not protonated. Thanks to the combination

of molecular biology and elucidation of the three-dimensional structure, the key amino acids involved in these processes have now been discovered. Research by the groups headed by Colin Wraight and George Feher (Eiji Takahashi and Wraight 1992; S.H. Rongey et al. 1993) has made it clear that the pK of neighboring amino acids is changed upon the formation of the anionic semiquinone, which leads to proton uptake stabilizing the semiquinone. Recent work of Jerome Lavergne et al. (1999) in my laboratory has shown that, at variance with isolated bRCs, formation of the protonated semiquinone ($Q_BH^{\cdot}$) could be observed in bacterial membranes, with an apparent pK of about 6. The reason for the different behavior of membrane-embedded and detergent-solubilized bRCs is under investigation.

Since the discovery of the high similarity between the acceptor side of PS II and bacterial reaction centers, a number of findings (amino acid sequence, primary photochemistry, and crystallographic data on bRC (see J. Deisenhofer and H. Michel 1989) and on PS II (Athina Zouni et al. 2001) have highlighted the profound homology between these two photosystems and provided a new picture of the evolution of the photosynthesis.

In conclusion, the epistemological lessons of this story was that what looks like odd artifacts may deserve careful examination, and poorly purified preparations may give good results. The other great lesson I 'received' during this work was the generosity of Rod Clayton, who refused to cosign this work, and thus allowed me to flourish as an independent scientist sooner than later.

Acknowledgments

I wish to express my gratitude to Dr Roderick K. Clayton for his generous encouragement during my sojourn in his laboratory and Dr Jerome Lavergne for a critical reading of the manuscript.

Notes

[1] It was shown a few years later by several laboratories that a (bacterio)pheophtytin molecule acts as the real primary electron acceptor in PS II or bacterial reaction center (see Bacon Ke 2001)

[2] The discharge of the Xenon flash capacitor was making a tremendous (acoustic) noise.

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