Photosynthesis Research **80:** 157–163, 2004.

Minireview

My daily constitutional in Martinsried

James P. Allen
Department of Chemistry and Biochemistry, Arizona State University, Tempe, AZ 85287-1604, USA; (e-mail: jallen@asu.edu; fax: +1-480-965-2747)

Received 31 March 2003; accepted in revised form 14 April 2003

Key words: bacterial photosynthesis, Johann Deisenhofer, George Feher, Robert Huber, Hartmut Michel, reaction center, Douglas Rees, X-ray diffraction

Abstract

The three-dimensional structures of bacterial reaction centers have served as the framework for much of our understanding of anoxygenic photosynthesis. A key step in the determination of the structure of the reaction center from *Rhodobacter sphaeroides* was the use the molecular replacement technique. For this technique, we made use of two sets of data. First, X-ray diffraction data had been measured from crystals of the reaction center from *R. sphaeroides* by our research group in California, led by George Feher and Douglas Rees. The second data set consisted of the coordinates of the three-dimensional structure of the reaction center from *Rhodopseudomonas* (now *Blastochloris*) *viridis*, which had been solved in the pioneering efforts of a group in Martinsried, led by Johann Deisenhofer, Robert Huber and Hartmut Michel. The collaborative efforts of these two groups to determine the structure of the reaction center from *R. sphaeroides* is described.

Abbreviation: EPR – electron paramagnetic resonance

Introduction

The summer of 1982 was an eventful time for me. I had just graduated from the University of Illinois at Urbana and headed out to La Jolla, California, to perform postdoctoral studies in the laboratory of George Feher. Little did I know that the field of photosynthesis was undergoing developments that would lead to an explosion of new ideas that now serve as a critical basis for our current understanding of photosynthesis. For an engaging story of the entire history of research on bacterial reaction centers, see Feher (1998). Below I recount these events from my humble perspective of postdoctoral fellow.

Isolation of reaction centers

The first reports of the isolation of the bacterial reaction center in 1968 described a large complex that upon illumination produced changes in the electron paramagnetic resonance (EPR) and optical spectra (Parson 1968; Reed and Clayton 1968; McElroy et al. 1969). In a very short time, the addition of the detergent lauryl dimethyl amine oxide to cell extracts of the bacterium *Rhodopseudomas sphaeroides*, that was later renamed *Rhodobacter sphaeroides*, was found to produce a small complex that retained full activity (Clayton and Wang 1971; Feher et al. 1971; Clayton 2002). This small complex consisted of three protein subunits that were named the L, M, and H subunits according to their apparent molecular weights, as determined by sodium-dodecyl sulfate polyacrylamide gel electrophoresis. The protein also contained a number of cofactors; four bacteriochlorophylls, two bacteriopheophytins, two ubiquinones, and one non-heme iron. These original isolations were from a blue-green strain named R-26 and so did not contain another cofactor, a carotenoid, that is usually present in wild-type strains. The availability of an isolated

complex provided the means for detailed characterization of the functional properties by a large number of spectroscopic techniques (Feher 1998).

Crystallization of reaction centers

Spectroscopic studies of the reaction center had led to a basic understanding of many functional features by 1982 (Parson 2003). For example, the electron transfer characteristics had been determined for the two quinones that serve as electron acceptors, most notably by George Feher, Mel Okamura and their colleagues (Figure 1). However, a detailed understanding was not possible due to the lack of the three-dimensional structure of the reaction center. Although the structures of thousands of proteins had been determined by protein crystallography, the reaction center had never been crystallized. One critical reason for the lack of crystals was the required presence of the detergent lauryl dimethyl amine oxide for the isolation of the protein. The reaction center is embedded in the cell membrane, and the detergent is required to replace the lipids normally surrounding the surface of the protein. Although these detergent molecules do not interfere with the spectroscopic measurements, the presence of the detergents demands that a protein–detergent complex be crystallized rather than a simple protein. For crystallization, the complex can be viewed as having a well-defined protein and cofactor structure with a disordered belt of detergents around the center of the protein. Thus, the only ordered regions of the surface are the protein loops emerging from each end. In order to produce crystals it is necessary to create specific interactions between these protein–detergent complexes involving specific atoms of the protein loops. At that time, all efforts to crystallize protein–detergent complexes had been unsuccessful, and many viewed it to be essentially impossible. This dire view of the crystallization of membrane proteins came to an abrupt end in 1980 with reports of the crystallization of bacteriorhodopsin (Michel and Oesterhelt 1980) and the outer membrane porin (Garavito and Rosenbush 1980). This was soon followed by the crystallization of the reaction center from *Rhodopseudomonas viridis*, later renamed *Blastochloris viridis* (Michel 1982).

Figure 1. A photograph of George Feher (rightmost), Mel Okamura (middle) Ed Abresch, who is a research scientist (second from right), Marco Flores (leftmost) and Govindjee, one of the editors of this special volume of Photosynthesis Research (second from left). The photograph was taken at the University of California, San Diego, in the laboratory where most of the EPR experiments were performed on the bacterial reaction centers.

Where do I fit into all of this?

My original decision to move to La Jolla was based upon a desire to learn new biophysical techniques. As a graduate student at Urbana, Illinois, I worked in the laboratory of Dr Harvey Stapleton on the characterization of proteins using EPR. For most proteins, an understanding of the EPR spectra in terms of ligand field theory had been developed. Our work involved the use of pulsed, rather than continuous, microwaves to generate signals that then decayed with rates that were highly temperature dependent. For proteins, this temperature dependence was very unusual but could be interpreted in terms of 'fractals,' or equivalently the irregular fashion by which proteins fold (Stapleton et al. 1980; Alexander and Orbach 1982; Allen et al. 1982; Wagner et al. 1985). From these EPR studies I had known of Professor Feher and had been excited by his new project of studying the crystallization process of proteins (Kam et al. 1978). Therefore, in La Jolla, I began biophysical measurements on various proteins, such as myoglobin, that were intended to elucidate the mechanism by which proteins crystallize.

The report of the crystallization of the reaction center from *B. viridis* led us to attempt some crystallization trials of the reaction center from *R. sphaeroides* that was being characterized in Feher's laboratory. In a very short time our efforts were successful and presented at the Biophysical Society Meeting in February 1983. The crystals were small but very reproducible and our work was published in 1984 (Allen and Feher 1984). However, for the determination of the structure using X-ray diffraction it was necessary to improve on the size of the crystals. Since our results were so promising I redirected my efforts exclusively towards the crystallization of the reaction center, and the

original project was put aside until a new postdoctoral fellow, Steve Durbin, picked it up again (Durbin and Feher 1986). The attempts to improve on the crystallization were at times frustrating, but the crystals did become larger and the X-ray diffraction could be measured. This led us to collaborate with Doug Rees who was at the University of California, Los Angeles, and later moved to the California Institute of Technology (Pasadena, California).

Strategies for structure determination

By 1985 we were able to measure a complete diffraction data set from the crystals but additional information was required to determine the structure. The diffraction data from a protein crystal is not sufficient because only the intensity of each diffraction point can be measured and information concerning the phase of each diffraction point is lost. This obstacle can be overcome by modifying the protein biochemically using heavy metals and determining the changes in the diffraction due to the addition of the metal. Indeed, this approach had been successfully used to determine the structure of the cofactors and protein backbone for the reaction center from *B. viridis* (Deisenhofer et al. 1984, 1985). We realized that it would probably take a few years to perform the heavy metal studies and determine the structure. However, the availability of the structure of the reaction center from *B. viridis* provided us with a different option. We could in principle make use of that structure in order to determine the phases by using what is termed a molecular replacement approach.

The efforts to determine the structure of the reaction center from *B. viridis* were lead by Johann Deisenhofer and Hartmut Michel who worked with Robert Huber, the director of project; the experiments were performed at the Max Planck Institute in Martinsried, Germany. Our group in California and the group in Martinsried decided that we would try the molecular replacement approach and have one person from our group go to Martinsried. Doug Rees would have been the logical person to go but he had various obligations that prevented him from making the trip. So it was decided that I would go to Martinsried even though I had never performed such an analysis before.

Molecular replacement approach

I flew to Germany in July 1985. On my first day there, I met Huber, Deisenhofer and Michel for the first time and started work immediately. The molecular replacement approach required that the diffraction data that I had brought be analyzed using the structure of the reaction center from *B. viridis*. The assumption of the approach, that the two reaction centers were structurally very similar, was unproven and so the feasibility was uncertain. Since the diffraction data had already been obtained, the work involved exclusively computer analysis using the crystallographic package PROTEIN that had been developed in Martinsried. To make the reaction center from *B. viridis* more closely resemble the reaction center from *R. sphaeroides*, the tetraheme subunit that is present in *B. viridis* but not *R. sphaeroides* was removed from the structural model. In addition, the non-conserved amino acid residues from the L and M subunits were truncated to alanine. The amino acid residues of the H subunit were all alanines, as explained below.

The molecular replacement approach would work if we could rotate and translate the structural model such that it was at the exact position that the reaction center from *R. sphaeroides* occupied in the crystals. Simply summarized, when this was the case the diffraction simulated from the structural model would closely resemble the true diffraction pattern. So the approach involves looking for correlations that are optimized when the model is correctly positioned. Due to the complexity of proteins, rotating and translating the search model was far beyond the reach of the computers at that time and the problem was actually broken down into steps that involve 'Patterson maps.'

The next several weeks consisted of me going into the Institute and working on adjusting the various parameters of the programs and seeing what the results were. Most of the time I worked with Hans Deisenhofer and Robert Huber who together had solved many protein structures. Hartmut Michel was primarily involved in the crystallization and characterization of the reaction center and would occasionally stop by to check on our progress. Since this was essentially a 'winner take all' approach and we did not know at that time if our work would be successful, we would analyze the previous day's result and make adjustments accordingly.

Our daily constitutional

My most pleasant memory of that time was our daily constitutionals. We would start at the Institute around

8 A.M. and work until about noon. The crystallographers, including Hans Deisenhofer and the other group members that were working on various projects, such as Jim Remington and Jim Pflugrath, would all go to the Mensa and have lunch together. After lunch we would go for a constitutional around the large forested area that was immediately adjacent to the Institute. These times provided an opportunity for me to have non-scientific discussions with the group while having a nice meal and a walk outside. The topics were far ranging and helped relieve the ever-present question of success from my mind.

After approximately three weeks we had indications that the approach was working but still no clear answer. So George Feher and I made the decision that I would stay for about another week for one final push. I made my best efforts to work on the problem and late one day I stayed until the program was finished so that I could analyze the output before leaving. For the first time I found what appeared to be the correlation that we had been looking for (technically in the translation peaks of the different Harker sections). Since it was late no one else was around and I simply left my notebook with the summary of the results open on my desk. The next day I found 'We got it!' written on the page by Robert Huber. The approach was successful and we now could solve the structure of the reaction center from *R. sphaeroides*.

Back to La Jolla, California

I returned to La Jolla shortly thereafter and we started to rebuild the structure. Although the major hurdle was overcome, there was still much work that needed to be done; this was performed by myself, a graduate student, Todd Yeates, and another postdoctoral fellow, Hiromi Komiya. The molecular replacement work was reported at the Biophysical Society Meeting in February 1986 and published soon thereafter (Allen et al. 1986). Another group, based at Argonne National Laboratories, obtained the coordinates of the reaction center from *B. viridis* and also presented a molecular replacement solution that same year (Chang et al. 1986). This competition motivated us to complete the rebuilding and refinement as quickly as possible and we were able to present the structure at the Biophysical Society Meeting in February 1987 and report the structure in a series of three manuscripts (Allen et al. 1987a, b; Yeates et al. 1987).

Gene sequencing, the structure and beyond

The X-ray diffraction data were sufficient to determine the structure of the cofactors and protein backbone of the reaction centers, but completion of the structures with the positions of the amino acid side chains required that the genes be sequenced. The efforts of George Feher and Lisa Steiner lead to the determination of the first 25–28 residues forming the amino-terminus of each protein subunit for *R. sphaeroides* (Sutton et al. 1982). These protein sequences provided the basis for the construction of oligonucleotide probes that were used by JoAnn Williams, a graduate student in George Feher's laboratory, to sequence the genes encoding the L, M, and later H subunits (Williams et al. 1983, 1984, 1986). With the availability of these sequences, we were able to position the amino acid side chains in the electron density maps and complete the full three-dimensional structure by 1988 (reviewed in Feher et al. 1989). At the same time the sequences of the protein subunits of the reaction center from *B. viridis* were completed (Michel et al. 1986) and the three-dimensional struc-

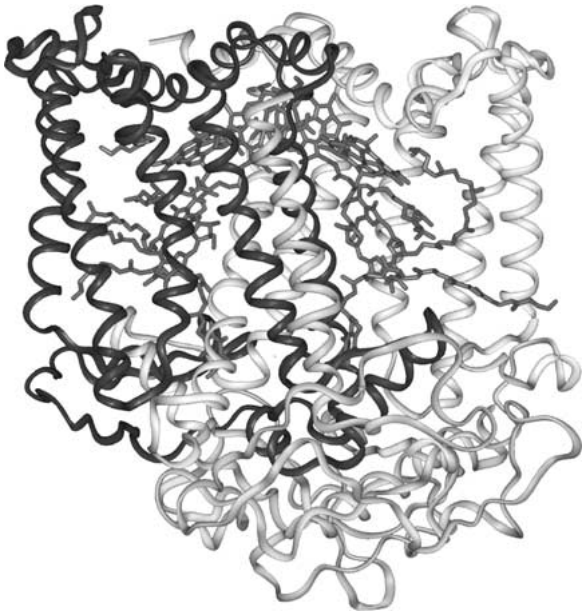

Figure 2. The three-dimensional structure of the reaction center from *R. sphaeroides*. Shown are the backbones of the L (light shade), M (dark shade), and H (medium shade) subunits as well as the cofactors (intermediate shade). The approximate two-fold symmetry axis of the protein is aligned in the plane of the paper with the cytoplasmic side of the membrane at the top. For a color version of this figure, see color section in the front of the issue.

Figure 3. A photograph of our group taken at Arizona State University outside of the laboratories where we perform our research. *From left to right:* myself, JoAnn Williams, Ana Camara-Artigas, a visiting Professor from the University of Almeria in Spain, Alayna Goetsch, an undergraduate who is now a PhD student at Michigan State University, Greg Uyeda, a graduate student, Ana Julia Naravez, who is now a postdoctoral fellow in Sweden and Uma Swamy. In the tree are graduate students Matt Rosenow and Alex Smith.

ture, including the amino acid side chains, of the reaction center from *B. viridis* was finished (Deisenhofer and Michel 1989). The group at Argonne continued to work on the structure determination and presented their results in 1991 (Chang et al. 1991).

The determination of the structure of the reaction center is an example of a rewarding scientific investigation that also yields unexpected and beautiful results (see Figure 2 for the *R. sphaeroides* reaction center; see color section in the front of the issue for a color version of this figure). The L and M subunits were found to each have five transmembrane helices; this result was the first clear demonstration of the usefulness of hydropathy analysis for integral membrane proteins. These two subunits are related to each other by a central two-fold symmetry axis. Surprisingly, the cofactors were found to form two branches that are also related by the same two-fold axis; only one of which is an active participant in light-induced electron transfer. One pleasing outcome of the symmetry was the presence of two closely interacting bacteriochlorophylls that serve as the primary electron donor as predicted from some of the first spectroscopic measurements of the reaction center (Feher 1998). The H subunit of the reaction center has a single transmembrane helix and largely forms an extracellular domain that protects the quinones and non-heme iron.

For their pioneering efforts to determine the structure of the reaction center from *B. viridis*, which was the first structure of a membrane protein, Deisenhofer, Michel and Huber (of the Martinsried group) were rewarded with a Nobel Prize in 1988 (Deisenhofer and Michel 1989). The availability of the two structures finally allowed researchers in photosynthesis to ask questions concerning how the protein structure influenced the properties of the cofactors at a molecular level. Theoreticians could finally perform calculations of the molecular orbitals of the bacteriochlorophylls, electron transfer rates, and protonation states on side chains near the quinones. These calculations provided a platform for experimental testing of the factors that determined the various electron transfer rates. A rewarding approach for these experiments was to investigate the effects of mutations, with the reaction center from *R. sphaeroides* being the preferred system. Mutations that interfered with the function of the reaction center could still be expressed in *R. sphaeroides* under non-photosynthetic conditions, but *B. viridis* would not tolerate these growth conditions and so it was difficult to use for mutagenesis. Such efforts are still ongoing to understand different functional aspects, such as why only one branch of cofactors serves as carriers for the light-induced transfer of electrons.

Research at Arizona State University

After completing the structural work in La Jolla, both JoAnn Williams and I accepted positions at the Photosynthesis Center at Arizona State University and started our independent research efforts (Figure 3). We continue to use the structure to address one of the major remaining questions concerning photosynthesis: how Photosystem II is capable of converting water into molecular oxygen in a four electron–proton process. Our efforts have focussed on manipulating the bacterial reaction center such that it gains the functional features of Photosystem II (Kalman et al. 1999). Meanwhile there are research groups throughout the world that are at a similar point described in this short essay, namely diligently worked in growing crystals of Photosystem II, with low-resolution electron density maps hinting at the excitement to come (Zouni et al. 2001).

Acknowledgments

Our current work on the bacterial reaction center is supported by grant No. MCB 0131764 from the United States National Science Foundation. This manuscript was edited by J. Thomas Beatty.

References

Alexander S and Orbach R (1982) Density of states on fractals: fractons. J Phys Lett 43: L625–L631

Allen JP and Feher G (1984) Crystallization of reaction center from *Rhodopseudomonas sphaeroides*: preliminary characterization. Proc Natl Acad Sci USA 81: 4795–4799

Allen JP, Colvin JT, Stinson DG, Flynn CP and Stapleton HJ (1982) Protein conformation from electron spin relaxation data. Biophys J 38: 299–310

Allen JP, Feher G, Yeates TO, Rees DC, Deisenhofer J, Michel H and Huber R (1986) Structural homology of reaction centers from *R. sphaeroides* and *R. viridis* as determined by X-ray diffraction. Proc Natl Acad Sci USA 83: 8589–8593

Allen JP, Feher G, Yeates TO, Komiya H and Rees DC (1987a) Structure of the reaction center from *Rhodobacter sphaeroides* R-26: the cofactors. Proc Natl Acad Sci USA 84: 5730–5734

Allen JP, Feher G, Yeates TO, Komiya H and Rees DC (1987b) Structure of the reaction center from *Rhodobacter sphaeroides* R-26: the protein subunits. Proc Natl Acad Sci USA 84: 6162–6166

Chang CH, Tiede D, Tang J, Smith U, Norris J and Schiffer M (1986) Structure of Rhodopseudomonas sphaeroides R-26 reaction center. FEBS Lett 205: 82–86

Chang CH, El-Kabbani O, Tiede D, Norris J and Schiffer M (1991) Structure of the membrane-bound protein photosynthetic reaction center from *Rhodobacter sphaeroides*. Biochemistry 30: 5352–5360

Clayton RK (2002) Research on photosynthetic reaction centers from 1932–1987. Photosynth Res 73: 63–71

Clayton RK and Wang RT (1971) Photochemical reaction centers from *Rhodopseudomonas sphaeroides*. Meth Enzymol 23: 696–704

Deisenhofer J and Michel H (1989) The photosynthetic reaction center from the purple bacterium *Rhodospeudomonas viridis*. Science 245: 1463–1473

Deisenhofer J, Epp O, Miki K, Huber R and Michel H (1984) X-ray structure analysis of a membrane protein complex: electron density map at 3 Å resolution of the chromophores of the photosynthetic reaction center from *Rhodopseudomonas viridis*. J Mol Biol 180: 385–398

Deisenhofer J, Epp O, Miki K, Huber R and Michel H (1985) Structure of the protein subunits in the photosynthetic reaction centre of *Rhodopseudomonas viridis* at 3 Å resolution. Nature 318: 618–624

Durbin SD and Feher G (1986) Crystal growth of lysozyme as a model for protein crystallization. J Cryst Growth 76: 583–592

Feher G (1998) Three decades of research in bacterial photosynthesis and the road leading to it: a personal account. Photosynth Res 55: 1–40

Feher G, Okamura MY, Raymond JA and Steiner LA (1971) Subunit structure of reaction centers from *Rhodopseudomonas sphaeroide*s. Biophys J 11: 38a (abstract)

Feher G, Allen JP, Okamura MY and Rees DC (1989) Structure and function of bacterial reaction centers. Nature 339: 111–116

Garavito RM and Rosenbush JP (1980) Three dimensional crystals of an integral membrane protein: an initial X-ray analysis. J Cell Biol 86: 327–329

Kalman L, LoBrutto R, Allen JP and Williams JC (1999) Modified reaction centres oxidize tyrosine in reactions that mirror Photosystem II. Nature 402: 696–699

Kam Z, Shore HB and Feher G (1978) On the crystallization of proteins. J Mol Biol 123: 539–555

McElroy JD, Feher G and Mauzerall DC (1969) On the nature of the free radical formed by electron spin resonance. Biochim Biophys Acta 79: 430–432

Michel H (1982) Three-dimensional crystals of a membrane protein complex: the photosynthetic reaction center from *Rhodospeudomonas viridis*. J Mol Biol 158: 567–572

Michel H and Oesterhelt D (1980) Three-dimensional crystals of membrane proteins: bacteriorhodopsin. Proc Natl Acad Sci USA 77: 1283–1285

Michel H, Weyer KA, Gruenberg H, Dunger I, Oesterhelt D and Lottspeich F (1986) The 'light' and 'medium' subunits of the photosynthetic reaction centre from *Rhodopseudomonas viridis*. EMBO J 5: 1149–1158

Parson WW (1968) The role of P870 in bacterial photosynthesis. Biochim Biophys Acta 153: 248–259

Parson WW (2003) Electron donors and acceptors in the initial steps of photosynthesis in purple bacteria: a personal account. Photosynth Res 76: 81–92

Reed DW and Clayton RK (1968) Isolation of a reaction center fraction from *Rhodopseudomonas sphaeroides*. Biochem Biophys Res Commun 30: 471–475

Stapleton HJ, Allen JP, Flynn CP, Stinson DG and Kurtz SR (1980) Fractal form of proteins Phys Rev Lett 45: 1456–1459

Sutton MR, Rosen D, Feher G and Steiner L (1982) Amino-terminal sequence of the L, M, and H subunits of RCs from photosynthetic bacterium *Rhodospeudomonas sphaeroides* R-26. Biochemistry 16: 3842–3849

Wagner CG, Colvin JT, Allen JP and Stapleton HJ (1985) Fractal models of protein structure, dynamics, and magnetic relaxation. J Am Chem Soc 107: 5589–5594

Williams JC, Steiner LA, Feher G, Ogden RC, Simon ML and Feher G (1983) Primary structure of the M subunit of the reaction center from *Rhodopseudomonas sphaeroides*. Proc Natl Acad Sci USA 80: 6505–6509

Williams JC, Steiner LA, Feher G and Simon ML (1984) Primary structure of the L subunit of the reaction center from *Rhodopseudomonas sphaeroides*. Proc Natl Acad Sci USA 81: 7303–7307

Williams JC, Steiner LA and Feher G (1986) Primary structure of the reaction center from *Rhodopseudomonas sphaeroides*. Proteins 1: 312–325

Yeates TO, Komiya H, Rees DC, Allen JP and Feher G (1987) Structure of the reaction center from *Rhodobacter sphaeroides* R-26: membrane-protein interactions. Proc Natl Acad Sci USA 84: 6438–6442

Zouni A, Witt HT, Kern J, Fromme P, Krauss N, Saenger W and Orth P (2001) Crystal structure of Photosystem II from *Synchococcus elongatus* at 3.8 Å resolution. Nature 409: 739–742