

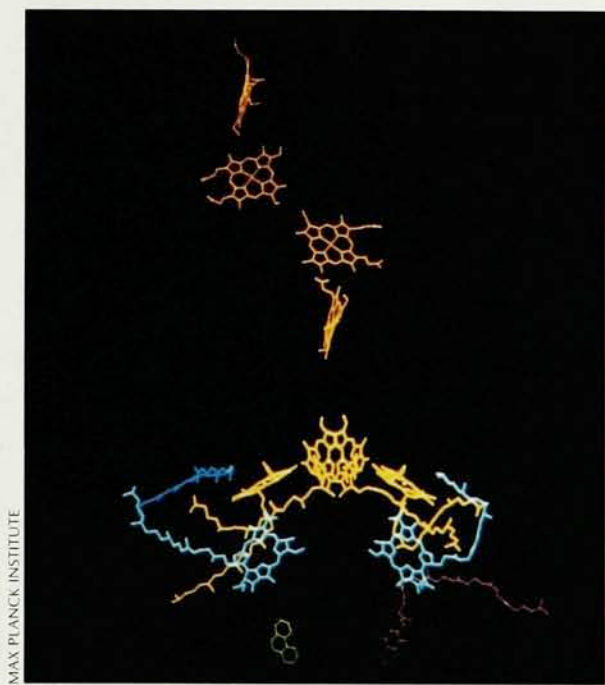
NOBEL CHEMISTS SHED LIGHT ON KEY STRUCTURE IN PHOTOSYNTHESIS

In December the 1988 Nobel Prize in Chemistry was presented to three scientists who delineated the physical structure of a protein-pigment complex that resides in a cell membrane and plays a critical role in photosynthesis. The three are Johann Deisenhofer of the Howard Hughes Medical Institute Laboratories at the University of Texas Southwestern Medical Center in Dallas, Texas; Robert Huber of the Max Planck Institute for Biochemistry in Martinsried, West Germany; and Hartmut Michel of the Max Planck Institute for Biophysics in Frankfurt, West Germany. Before their work, many had felt that crystallizing and subsequently resolving the structure of a membrane-bound protein would not be possible. The prize not only honors the researchers for this feat but also indirectly recognizes the key role played by this protein in nature's process for converting sunlight into energy.

Photosynthesis begins when chlorophyll absorbs a photon and initiates the transfer of charge from a donor to an acceptor. Eventually this charge separation drives the production of the high-energy compound adenosine triphosphate, or ATP. The minimum complex of proteins and pigments that can accomplish the charge separation is called the reaction center. Extensive studies over the past few decades have revealed much about the electronic behavior of the reaction center, but the 1988 Nobelists were the first to determine the structural arrangements of atoms within it.

Crystallizing the reaction center

The reaction center has been difficult to study, partly because it sits in the middle of a cell membrane, with its ends protruding into the aqueous solution on either side. Extracting it from its surroundings is particularly daunting because those parts of the protein embedded in the lipid membrane are hydrophobic, or insoluble in water. In the early 1980s Michel and



Structure of the reaction center of *Rh. viridis* includes the two chains of photosynthetically active elements shown at left in the computer-generated display of interatomic bonds. Protein subunits (not shown) support the chain. Electrons are transferred down each chain from bacteriochlorophyll molecules (yellow) to bacteriopheophytins (light blue) and then to quinones (purple). A single iron atom (yellow dot) sits between the chains. At the top of the image are four hemoglobin-like molecules (gold).

others were trying to crystallize membrane-bound proteins and wash them out of their membranes with a variety of detergents. Detergents, like the membrane proteins, are amphiphilic, meaning that different parts have different solubilities. Thus some ends of the detergents can bind to the protein's hydrophobic surfaces while the other ends can interact with the aqueous solution. Once the protein is encased in the detergents, which replace the membrane, the remaining complex including the detergents can be purified and crystallized.

Together with Dieter Oesterhelt (Max Planck Institute for Biochemistry), Michel experimented with smaller detergents than had been tried previously and with even smaller amphiphilic molecules. He and Oesterhelt crystallized the bacterial membrane protein bacteriorhodopsin, and at about the same time, Michael Garavito (now at the University of Chicago) and Jurg Rosenbusch

(University of Basel, Switzerland) did the same for the membrane protein porin. Neither group, however, has yet been able to determine the structure of these proteins at atomic resolution. Michel then tried to crystallize the reaction center from the purple bacterium *Rhodospirillum rubrum*. In 1982 he succeeded in producing large, well-ordered crystals.¹

Michel then teamed up with the crystallographic group at the Max Planck Institute at Martinsried. Deisenhofer was working there at the time, and Huber directs the group. Over the years, Huber's group has developed sophisticated tools and computer software to facilitate the x-ray diffraction analysis of structures as complex as the reaction center, with its several thousand atoms. In their work, Michel and Deisenhofer were helped first by Kunio Miki (now at Osaka University, Japan) and then by Otto Epp (Max Planck Institute). Two years after receiving the crystal,



Hartmut Michel



Johann Deisenhofer



Robert Huber

the Max Planck team solved the structure,² and in 1985 they gave a full description³ of the reaction center with a resolution of 3 angstroms. Thus *Rh. viridis* became the first membrane protein whose structure was solved at atomic resolution.

Although Deisenhofer, Michel and Huber delineated the reaction center only for *Rh. viridis*, the basic architecture of the center is shared by the reaction centers of other bacteria. As a result, the work on *Rh. viridis* gave a tremendous head start toward the elucidation of the structure of the reaction center of its close cousin, *Rhodospirillum rubrum*. Two groups, one led by George Feher and Douglas Rees at the University of California at San Diego, and the other led by James Norris and Marianne Schiffer at Argonne National Laboratory, solved that structure in 1986. The *Rh. sphaeroides* complex has been intensely studied for nearly 20 years, and the new knowledge of the spatial relations among its atoms greatly enriches this field. Furthermore, investigators can learn much by comparing the two reaction centers. The work on photosynthesis in bacteria is expected to enhance the understanding of green plant systems as well.

Earlier work

In 1968 Dan W. Reed and Roderick K. Clayton (Cornell University) used detergents to extract from *Rh. sphaeroides* a complex capable of charge separation, although the high molecular weight of their sample suggested it included extraneous material. Two years later Feher used another detergent to extract a much smaller active reaction center unit from the same bacteria. The ability to extract such small units enabled Clayton, Feher and many others to begin extensive studies of the reaction center. These earlier investigators discovered much

about the spatial organization and electronic structure of the complex.

The earlier studies found that the reaction center of *Rh. sphaeroides* is made up of three protein subunits and six pigments. The protein subunits form a kind of scaffolding that positions the other components, known as cofactors, so that electrons can be efficiently transferred across the photosynthetic membrane. (See the figure of *Rh. viridis* on page 17.) Numerous studies indicated that the electron transfer proceeded from one donor D1 through a chain of acceptors: an intermediate acceptor A1 and then primary and secondary acceptors A2 and A3. Over the years spectroscopic evidence has identified the donor and acceptors with particular cofactors: D1 with a pair of pigments known as bacteriochlorophylls, A1 with bacteriopheophytins and A2 and A3 with quinones. Two of the three protein subunits, designated L and M, appeared to be associated with the chain of donor and acceptor components. Labeling techniques enabled investigators to infer that the reaction center spans the membrane, suggesting that the charge separation occurs across it.

The most impressive aspect of the reaction center is the speed with which it separates the charge. The electron transfer from D1 to A1 takes only about 10^{-12} sec and the subsequent transfer to A2 takes about 10^{-10} sec. These rates are faster by several orders of magnitude than those for charge recombination. They explain why nearly every photon results in a stabilized, charge-separated species. The energy conversion efficiency is only 30–40%.

The protein's structure

The work of Deisenhofer, Huber and Michel largely confirmed the components and organization predicted by

the earlier studies. The reaction center of the bacteria *Rh. viridis* is made up of four protein subunits. As the studies of *Rh. sphaeroides* showed, however, only two of these—L and M—are associated with the cofactors. The L and M subunits possess five membrane-spanning helices each, and together they have an approximate twofold symmetry. Along the subunits are arrayed two chains of pigment molecules, which are postulated to form the path for the electron transfer. The crystallographic studies showed that the two chains are nearly identical, with the pair of chlorophyll molecules near one end and one quinone acceptor near the other end (the other quinone, now known to be present, was not apparent in this first crystal). Near the quinones is a single iron atom in the high-spin ferrous state. One surprise and continuing mystery is that the electron transfer occurs preferentially along only one of these two chains.

Two specific structures are now available to aid ongoing studies of the reaction center. One challenge is to explain the picosecond electron transfer from D1 to A1, which is now observed over large distances (across ten intervening atoms). Researchers are trying to identify what factors in the structure affect the observed rates. They are further investigating why the forward electron transfer rates are so much faster than the recombination rates.

—BARBARA GOSS LEVI

References

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