Light-Induced Structural Changes in the Primary Processes of Photosynthesis: Watching an Enzyme in Action**

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Understanding enzyme function at a molecular level is one of the important goals of biochemistry. Moreover, since enzymes often catalyze chemical reactions that are difficult to perform in vitro, one could hope to "learn from nature" and thus find a way to improve the corresponding syntheses.

One of the dilemmas of studying enzyme function is that the reactions usually involve structural and conformational changes of the protein. However, the most accessible state of the enzyme is usually the ground state, whose structure can often be determined by X-ray crystallography or, more recently, by NMR spectroscopy. Conformational changes are harder to determine; therefore, studies of enzymes usually involve a combination of ground-state structural information with spectroscopic evidence for the changes involved in enzyme turnover. This approach is indirect with respect to the conformational changes, and whether a molecular model of function can be derived, and how accurate it is, depends on the quality of the interpretation of the spectroscopic evidence. Structural information on active intermediates in enzyme action would be a more direct and thus more reliable approach towards understanding function, but is often difficult to obtain experimentally.

In a recent publication by the groups of Feher and Rees,[1] it was demonstrated how such structural changes can be determined directly by crystallographic methods. They investigated the primary processes of photosynthesis, that is, the reactions which allow plants and bacteria to convert sunlight into electrical and ultimately chemical energy with high efficiency.

In photosynthetic bacteria the primary processes take place in the reaction center (RC), which is a membrane protein whose ultimate task is to use light energy to pump electrons and protons across the photosynthetic membrane (Figure 1). This establishes the charge and concentration gradients that are required to drive the synthesis of energy-rich intermediates during the "dark reactions". Electron transfer (ET) and proton transfer reactions within the RC are performed by the cofactors embedded in the protein. Their arrangement is depicted in Figure 1.

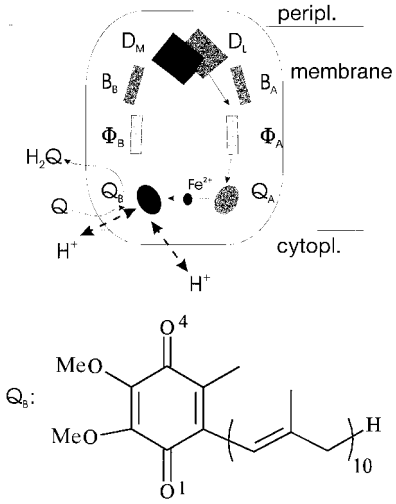

Figure 1. Schematic representation of the reaction center of a photosynthetic bacterium (in analogy to the structure of *Rhodobacter sphaeroides*)[5] in the photosynthetic membrane, which separates the periplasmic (peripl.) from the cytoplasmic compartments (cytopl.) of the cell. The cofactors are the primary electron donor (a dimer of D_L and D_M, two bacteriochlorophyll *a* (BChl*a*) molecules in *Rb. sphaeroides*), the accessory BChl*a* molecules B_A and B_B, the bacteriopheophytins Φ_A and Φ_B, and the quinones Q_A and Q_B. (The structural formula of Q_B, ubiquinone 10, is given.) Reduction of Q_B by two electrons requires the uptake of two protons from the cytoplasmic side of the membrane. The dashed arrows indicate the pathways for proton transfer through protonable amino acid residues and well-ordered water molecules. For details of the location of pathways see reference [1].

In reference [1] the structural changes accompanying these primary processes (light reactions) are determined by low-temperature X-ray crystallography on a light-induced intermediate occurring during the light reactions. This gives new insight into how the efficient transformation of light into electrical energy is achieved, and helps to solve some of the questions that are currently being heavily debated. Since the primary processes of photosynthesis have been the focus of research interest of a number of groups over a few decades, the results of Feher and Rees et al. can be compared to a large body of spectroscopic data. This makes it a good model for comparing the direct X-ray crystallographic analysis with the more indirect spectroscopic studies.[2]

To better appreciate the experimental task involved in determining the structure of the light-induced intermediate, it should be recalled that the combination of hydrophobic and hydrophilic surfaces of membrane proteins made them notorious for being difficult to crystallize. Indeed, the first protein of this class for which the X-ray structure was determined was an RC of a photosynthetic bacterium—a feat for which the Nobel prize was awarded to Michel, Deisenhofer, and Huber in 1988[3]—and even to date there are less then ten membrane proteins for which an X-ray structure is

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available. In the study described,[1] low-temperature X-ray crystallography[4] made it possible to trap the intermediate, and it significantly improved the resolution of the ground-state structure (to 2.2 Å, which is higher than in any RC structure available to date).[5] This allowed, for example, determination of the position of a larger number of ordered water molecules than found previously.[5] These water molecules are considered to be involved in proton-transfer pathways. The term pathway refers to a chain of water molecules and protonable amino acid residues which conduct protons through the unpolar protein interior in a bucket-brigade fashion. Crystallography on the light-induced intermediate additionally requires that a significant fraction of the RCs in the crystal can be converted into and trapped in the excited state, as any superposition of states would make it impossible to solve the structure. Feher and Rees et al. achieved a yield of 90 % charge-separated state by continuous illumination of the crystal and subsequent freezing.

One of the most important results obtained is that substantial motions accompany the primary processes, which is (perhaps) surprising since these reactions are essentially just ET or proton transfer reactions (i.e., processes which are generally not assumed to involve large-scale structural changes). The chemical reactions performed by the RC are a sequence of electron and proton translocations which follow the electronic excitation of the primary electron donor D (a bacteriochlorophyll dimer) into its excited singlet state (D*). The ubiquinones Q_A and Q_B (ubiquinone 10 (UQ_{10}) for *Rhodobacter sphaeroides*) in Equation (1) act as sequential electron acceptors as shown in Figure 1.

$$DQ_AQ_B \xrightarrow{h\nu} D^*Q_AQ_B \rightarrow D^{\bullet+}Q_A^{\bullet-}Q_B \rightarrow D^{\bullet+}Q_AQ_B^{\bullet-} \quad (1)$$

In the RC the reaction is completed by a second charge-separation event, which transfers a second electron onto Q_B. The concomitant uptake of two protons converts Q_B into the hydroquinone H_2Q_B, which leaves the RC and is subsequently replaced by another (oxidized) UQ_{10}. The protons taken up originate from the cytoplasmic side of the membrane (Figure 1). Proton depletion on that side of the membrane constitutes part of the proton gradient required to drive the dark reactions.[6] As such, the reduction and protonation of Q_B is the process that transmits the electrical energy to chemical energy by coupling ET to the chemical follow-up reactions.

The light-induced intermediate investigated by Stowell et al.[1] is $D^{\bullet+}Q_AQ_B^{\bullet-}$, and the most pronounced structural changes are observed in the vicinity of Q_B (see Figure 2). Amongst the questions discussed[1] we will focus on only two: the origin of the difference in binding strengths for the redox and protonation states of Q_B (Q_B, $Q_B^{\bullet-}$, and H_2Q_B), and ET from Q_A to Q_B. For optimum function the binding strength is required to increase in the order $H_2Q_B < Q_B < Q_B^{\bullet-}$, as this allows the RC to replace H_2Q_B by UQ_{10} and to bind $Q_B^{\bullet-}$ most strongly. This prevents the semiquinone radical, an intermediate of potentially harmful reactivity, from being released prematurely.

Transfer of the first electron from Q_A to Q_B [Eq. (1)], which proceeds on the microsecond timescale at room temperature, is blocked at low temperature (below 90 K). Freezing RCs under illumination, however, partially restores the ET. A light-induced structural change was suggested to be the origin of this temperature effect;[7] however, a mode of motion responsible could not be determined.

A comparison of the X-ray structures[1] of the "dark" state before light excitation and of the light-induced charge-separated state $D^{\bullet+}Q_AQ_B^{\bullet-}$ ("light" structure) indicated that the following changes occur upon reduction of Q_B. Quinone Q_B moves by 4.5 Å inside the binding pocket, and the quinone ring rotates by 180° about the isoprenoid chain (Figure 2; Q_{B1} is the position of Q_B in the dark, and Q_{B2} that in the light structure). The movement results in visible changes of the hydrogen bonding to the quinone oxygen atoms O1 and O4, allowing Q_B to form a different set of hydrogen bonds after moving to Q_{B2}. It also brings Q_B much closer to the Fe^{2+} ion in the RC and shortens the distance to Q_A, which should make ET from Q_A to Q_B more favorable. Feher and Rees et al. therefore suggest that prior to reduction Q_B has to move to the position corresponding to Q_{B2} to enable ET. The motion required explains why ET from Q_A to Q_B is inhibited at low temperatures: The starting position of Q_B (Q_{B1}) is not suitable for ET, and at low temperatures the motion of Q_B to the new site in the protein pocket is inhibited.

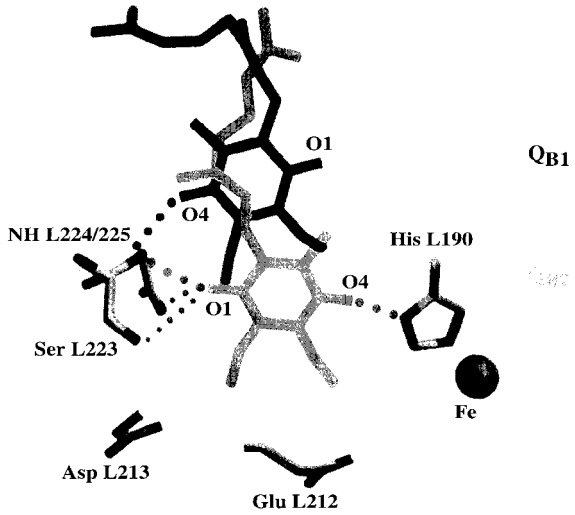

Figure 2. The different positions of the secondary electron acceptor Q_B in the dark (Q_{B1}) and light structures (Q_{B2}) of the reaction center. Selected amino acid residues of the protein with their sequence numbers are shown (L represents the protein subunit L of the RC which is made up of the subunits L, M, and H), and hydrogen bonds are indicated by dotted lines. See text for further details.

According to these results, the differences in binding affinities of the different functional redox/protonation states of Q_B are due to changes in hydrogen bonds: In the Q_{B1} position the quinone can form only one hydrogen bond to the O4 oxygen, whereas in the Q_{B2} position it can form one short hydrogen bond to O4 and three hydrogen bonds to O1 (Figure 2). These additional hydrogen bonds should supply the extra binding energy to firmly anchor $Q_B^{\bullet-}$ in the RC. The fully protonated H_2Q_B cannot form any hydrogen bonds and can therefore be easily replaced by an oxidized UQ_{10} from the quinone pool in the membrane.

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The large-scale motions observed by Feher and Rees et al. suggest that interpretations of the mechanism of quinone reduction must be problematic when based on the dark structure alone. The authors also suggest that some of the differences in the position of Q_B found in earlier crystal structures[5, 8] may reflect partial reduction of Q_B. The fact that the motions are far beyond the type that could be followed in molecular dynamics simulations underlines the necessity to determine such changes by experiments.

Beyond the immediate interest which these findings will have for the understanding of the primary processes of photosynthesis, they give some new ideas about how large structural changes during the active cycle of an enzyme can be, even if its main task is just to shuffle electrons. It shows how binding affinities of a substrate can be adapted to the function by allowing the substrate to move between different sites, rather than by changing the protein conformation. Ultimately, the beauty of seeing the protein in action and appreciating that it has surprises in store that were not suspected before suggests that the approach taken by Feher and Rees et al. will be a reference point for future investigations.

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