Additional Updated Report on MX-78 (Submitted August 28 2005).

Photosynthetic organisms thrive by generating chemiosmotic potential across their biological membranes. One such bacterium is *Rhodobacter sphaeroides* in which the light-driven reaction takes place in a membrane-bound protein complex called the reaction centre (RC). When a photon is absorbed by the special pair (P) in the RC an electron transfer reaction is initiated across the membrane which ultimately leads to the reduction of a ubiquinone molecule (Q_B). The ubiquinone molecule diffuses in the membrane to the cytochrome bc_1 complex which oxidises it in two steps on the other side of the membrane. This so called redox loop is responsible for the active translocation of protons across the membrane. As a consequence a proton gradient is maintained and the energy stored in this gradient is harvested to propel vitally important reactions. One such example is the synthesis of ATP, the universal energy currency of the cell, which is primarily synthetised by the ATPsynthase utilising the chemiosmotic potential of H^+ ions.

In reaction centres light induced structural changes were first predicted for the $P^+Q_B^-$ state based on kinetic evidence. For example the electron transfer from static ubiquinone (Q_A) to Q_B was much faster in frozen samples when the reaction centres were illuminated prior freezing(*1*). Stowell *et al*. in 1997 compared the illuminated and dark-adapted structure of the reaction centres. The crystals were illuminated before freezing and during data collection with a wide bandpath tungsten light source. In the illuminated crystals, trapped in the $P^+Q_B^-$ state, the head group of the secondary quinone has moved ~5 Å and undergone a 180° rotation compared to the dark-adapted structure (*2*). However, no change in the protein matrix could be detected in the charge-separated structure. Similarly kinetic and spectrophotometric experiments led to the observation that conformational changes might be associated with the $P^+Q_A^-$ state (3,4). To extend our knowledge about the reaction centre catalytic mechanism, we aimed to observe structural changes associated with this earlier state.

In order to trap the $P^+Q_A^-$ intermediate the electron transfer from Q_A to Q_B has to be inhibited. This was accomplished in two ways: firstly the crystals were cryo-cooled to 100 K at which the electron is not transported beyond Q_A unless the crystals were frozen in saturating light conditions and secondly an inhibitor, terbutryn was used to completely replace the Q_B ubiquinone. The crystals were illuminated with an 840 nm infrared laser where the light was propagated along an optical fibre, which was mounted on the goniometer head. This set-up allows the uniform illumination of the crystals from one orientation without interfering with the data collection. Two strategies were used for trapping: the first case (protocol A) only one crystal was used for collecting the illuminated and non-illuminated dataset. For this experiment, an optical shutter was placed in front of the laser diode such that, for each crystal orientation, X-ray diffraction data were first collected during simultaneous illumination, immediately followed by the same frame being collected without illumination. For the second strategy (protocol B) only one fully illuminated dataset was collected from a crystal and this data was subsequently compared to one or more ground state datasets. The ground state crystals, in this case, were not illuminated at all prior or during data collection.

To detect changes that occur upon the illumination, difference Fourier maps were calculated (Figure 1). The maps were then subjected to statistical analysis. Protocol A did not produce reproducible features in the difference Fourier maps that were calculated between illuminated and non-illuminated states from six crystals. However, using protocol B yielded significantly more reproducible peaks than would be expected for a random case, suggesting that a reproducible conformational change occurs which does not relax within the timeframe of the experiment (minutes) at low temperature. These conformational changes were primarily centered on the Q_A site in the cytoplasmic H-subunit. We hypothesized that the conformational changes play a role in extending the lifetime of the charge separated state in order to protect the reaction centre from futile charge recombination reaction under intense illumination. Our results were published recently in Nature Structural and Molecular Biology. (5)

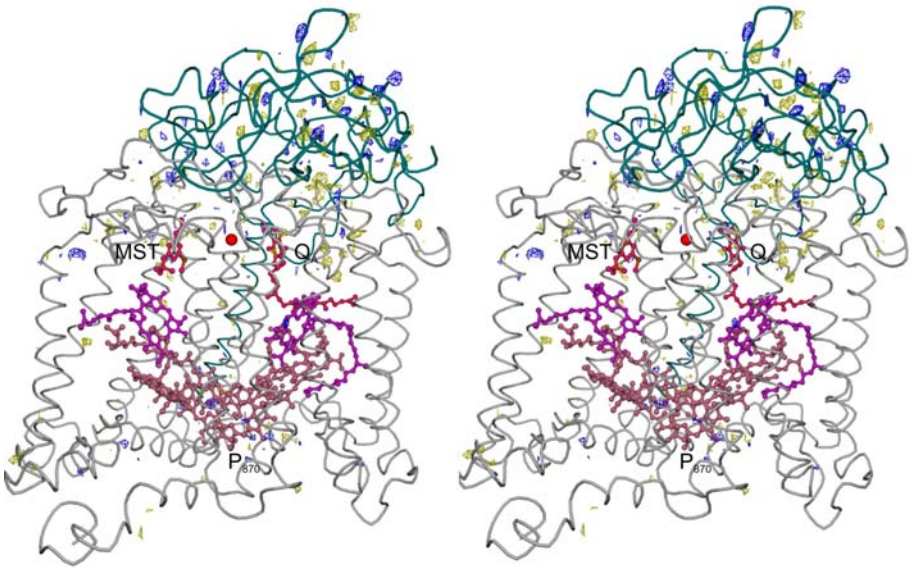

Figure 1 Light induced structural changes in the $P^+Q_A^-$ intermediate of RC. $(F_i\text{-}F_g)\cdot\exp(i\Phi_g)$ difference Fourier electron density map recovered when a RC crystal was illuminated with bright light (λ = 840 nm) at 100 K (yellow, negative electron density changes; blue, positive electron density changes). This map was contoured at 3.1 σ.

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