Direct evidence of structural changes affecting electron transfer rate from Q_A to Q_B in photosynthetic bacterial reaction center proteins probed by Fe^{2+} binding site using XAFS

Lin X. Chen*, Lisa M. Utschig*, Philip D. Laible,† Sandra L. Schlesselman*, Deborah K. Hanson†, and David M. Tiede*
*Chemistry Division, Argonne National Laboratory, Argonne, IL 60439 USA
†Biosciences Division, Argonne National Laboratory, Argonne, IL 60439 USA

Introduction

The primary reactions of solar energy conversion in photosynthetic bacteria are accomplished via photoinduced electron transfer through a series of pigments in reaction center (RC) proteins. An electron is transferred from the photoexcited state of the special pair P* to a bacteriopheophytin H_A, then to ubiquinones Q_A and Q_B (Figure 1) [1]. In this study, the RC protein structure in the Q_A/Q_B region was examined by x-ray absorption fine structure (XAFS) using an intrinsic non-heme Fe^{2+} site as a probe. XAFS spectra at Fe K-edge for the Fe^{2+} site in RC proteins at different purification stages were measured. Two XAFS studies on the Fe site of the bacterial photosynthetic RC proteins were conducted in the early 1980s [2, 3]. Since then, several crystal structures of RCs have been published. However, the structural accuracy around the Fe site obtained from the x-ray crystal structures of RC is limited due to the

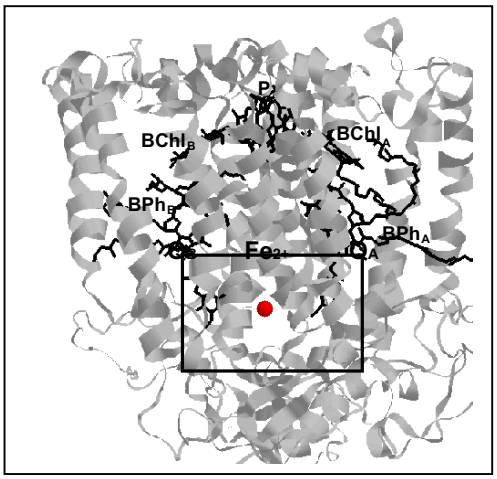

Figure 1: *Rhodobacter sphaeroides* RC structure (Stowell *et al*. 1997).

global fitting in data analyses of the protein x-ray diffraction. Thus, some subtle structural variations around the Fe sites reflecting the protein structural changes could be overlooked. XAFS, on the other hand, can be used to obtain more accurate local structures around the metal ions. Because of its strategic location between the two ubiquinones, the Fe^{2+} binding site structure may be used to probe local structural changes of the protein matrix due to environmental change, which may affect the kinetics of electron transfer from Q_A to Q_B. Previous studies on electron transfer from Q_A to Q_B in the RCs from *Rhodobacter sphaeroides* R-26, *R. sphaeroides* PUC 705BA, and *R. capsulatus* in different media strongly suggested a correlation between the structures and the kinetics of the electron transfer in this region [4].

Methods and Materials

RC proteins from a light-grown, LH II-depleted strain of *R. sphaeroides* PUC 705-BA were prepared according to methods described previously [4]. The RC proteins before and after a DEAE Sephacel column in the purification protocol, respectively, were collected. The lipid-imbedded proteins then were concentrated by ultracentrifugation, and the paste of the protein/lipid was collected and spread onto a Mylar film substrate for XAFS experiments.

Spectroscopic measurements were made with a single-beam, pump-probe, diode array instrument using procedures described previously [5] with samples at 22%C.
XAFS spectra were collected at beamline 12-BM with a Si(111) crystal in the monochromator. A Pt-coated focusing mirror reduces the beam size and removes x-ray photons of higher harmonics. The actual beam size at the sample was about 0.4 mm(v) x 1 mm(h). A nine-element Ge solid-state detector (Canberra) was used to collect x-ray fluorescence signals from the RC paste sample with Fe(II) concentration around 2×10^{-4} M. A shaping time of 0.5 µs was used. A maganese filter was placed in front of the detector for reducing elastic scattering from the sample. In the case of the Fe(II)-containing RC protein, this method increased the relative ratio between the signal and the elastic scattering. An ion chamber was placed before the sample for I_0 reference and the second and third ion chamber detectors were after the sample. An iron foil was inserted between the two detectors as a reference for the energy of the x-ray photons.

Conventional XAFS data analysis programs and FEFF 7.0 were used in data analysis. Iron oxalate dihydrate (FeC_2O_4ß$2H_2O$), iron picolylamine dichloride ([Fe(α-picolylamine)$_3$]Cl_2•EtOH) and Fe(II) bispyridine-phthalocyanine ($FePcPy_2$) were used as reference compounds for O and N backscatterings. The XAFS spectra of these compounds were calculated using FEFF 7.0 based on their atomic coordinates from x-ray diffraction studies. Then the individual shells of neighbors were isolated via Fourier back transform to obtain the phase and amplitude parameters for a particular neighbor. Parameters from the calculation were used to fit the experimental data.

Results

1. Kinetics of Electron Transfer from Q_A and Q_B
The kinetics of electron transfer from Q_A and Q_B were monitored through the electrochromic effect of bacterial pheophytin H_A and H_B. There is a three- to four-fold increase in the average rate constant for the electron transfer from chromatophores to precolumn environment, and another factor of two increase after the final column. These observations indicate that prolonged purification changes the protein environment and has a direct influence on the kinetics of the electron transfer reactions in the RC. However, the structural origin for the electron transfer rate change is unclear.

2. Structures around Fe(II) Binding Site
According to the crystal structures of RC for *R. sphaeroides* [1], the Fe(II) atom between Q_A and Q_B is chelated by four N atoms from four His residue and two O atoms from a carboxylate group of a Glu residue. Thus, the Fe(II) atom is situated in a distorted octahedral site. Figure 2 shows x-ray absorption near-edge spectra (XANES) of the PUC RC proteins at the Fe K-edge of RC proteins before and after the DEAE Sephacel column.

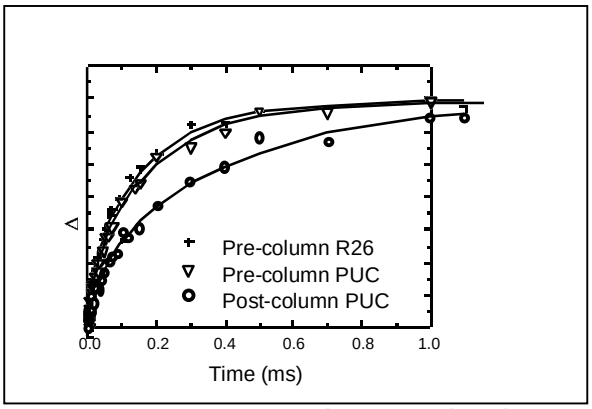

Figure 2: Kinetic traces monitored at 774 nm based on electrochromic effects that follow the electron transfer from Q_A to Q_B for PUC and R-26 *sphaeroides* RCs in different environments.

The shapes of the XANES at the transition edge are different and they can not be overlapped by normalization at the white light peak. The relative intensities of the white light peak at 16 eV above the edge to that of a shoulder around 20 eV is smaller for the RC before than after the column. Normalization at the white light peak at 16 eV results in a higher intensity of the pre-edge feature at about 2 eV for the RC before the column corresponding to 1s -3d transition, an indication of more distorted geometry for Fe(II) from that of octahedral coordination.

Figure 3 displays Fourier back transformed XAFS spectra for the first shell and the fits where an oscillation frequency difference is clearly visible. A one-shell fit (assuming all six

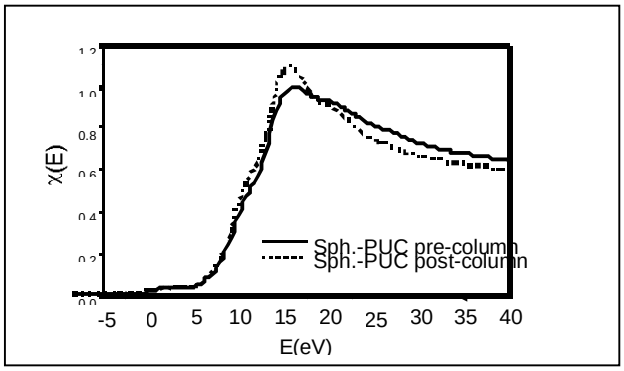

Figure 3: Fe K-edge XANES spectra of RC before and after the column.

nearest neighbors are O atoms) for the average nearest neighbor distance gives 2.06 Å for the Fe(II) site before the column and 2.11 Å after the column, a 0.05 Å increase (Figure 4). In addition, the oscillation in the spectrum before the column appears to have more than one oscillation frequency which causes the amplitude of the oscillation to decreases with the wave vector k first then increases again after k = 9 $Å^{-1}$. Such a diversity on the average nearest neighbor distances can be demonstrated by a larger Debye-Weller factor for the RC before the column (0.0129 $Å^2$) than that after the column
(0.0097 $Å^2$). The differences between each distance for the RC before and after the column are 0.06 Å, similar to the difference in the one-distance fitting. This result suggests that the differences in the first shell neighbor distances between the two RC samples are distributed to all the ligand-to-Fe(II) bonds other than particular bonds. This is an indication of protein matrix expansion or loosening near the Fe site after the column.

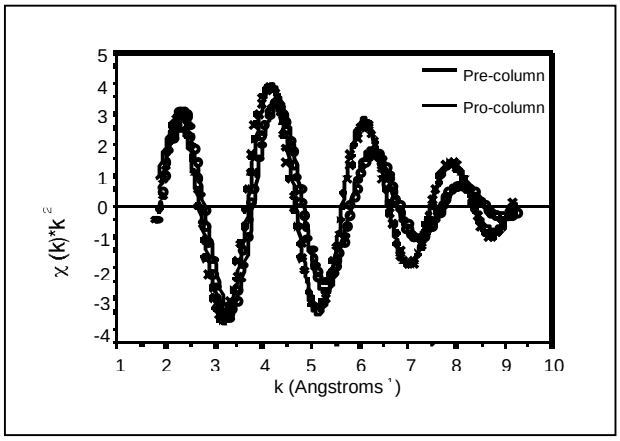

Figure 4: The EXAFS due to the nearest neighbors of Fe(II) and the fits for PUC *sphaeroides* RC before and after the column.

Discussion

The strategic location of the Fe atom and the H-bond channel of Q_A, His(M219), His(L190), and Q_B suggests that

the structural changes around the Fe site are very likely the result of the structural changes of the protein matrix in this region. If a 0.05 Å distance increase is observed for the Fe to the nearest-neighbor distance, which averages about 2 Å, a 0.5 Å increase in the Q_A to Q_B distance of about 20 Å is likely to occur if the protein matrix expansion due to the extensive purification is linear. Although this assumption could be oversimplified, it is likely to happen in protein molecules with different conformational substates. The RC protein after the final column still retains its function, but its structure has been altered, which is observed through our XAFS results. We observed elongation of the Fe-to-ligand distance due to prolonged purification of the protein, which is likely an indication of protein matrix loosening. This structural change is directly related to slowing down of the electron transfer rates from Q_A to Q_B after the protein undergoes the same purification procedure.

Acknowledgments

This work is conducted at the Advanced Photon Source (Argonne National Laboratory, Argonne, IL) supported by the U.S. Department of Energy, Office of Sciences, Basic Energy Science and Division of Chemical Sciences, under contract W-31-109-Eng-38. We thank personnel from BESSRC-CAT for their assistance. Special thanks to Dr. Guy Jennings for his invaluable help in multi-element x-ray detector and data acquisition.

References

[1] M.H.B. Stowell, *et. al*., *Science* **276**, 812–816 (1997).
[2] P. Eisenberger, M.Y. Okamura, and G. Feher, *Biophys. J.* **37**, 523 (1982).
[3] G. Bunker *et. al*., *Biophys. J.* **37**, 539 (1982).
[4] D.M. Tiede *et. al*., *Photosynthesis Res.* **55**, 267 (1998).
[5] D.M. Tiede *et. al*., *Biochemistry* **35**, 10763–10775 (1996).