Structural and function changes in photosynthetic bacterial reaction center proteins induced by incorporating different metal ions

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Introduction

Photoinduced electron transfer reactions in a series of pigments imbedded in photosynthetic bacterial reaction center (RC) proteins are the fundamental processes in photosynthesis. The third step of the electron transfer occurs between the two ubiquinones, Q_A and Q_B (Figure 1). There is an Fe(II) binding site situated between Q_A and Q_B and in the middle of the putative path of the electron transfer. Recent studies show evidence of large nuclear rearrangements in this region upon electron transfer [1]. Although the x-ray structures for several bacterial reaction center proteins are available, the accuracy of the structure around the Fe(II) site is limited due to the global fitting of the entire protein in data analyses. X-ray absorption fine structure (XAFS), on the other hand, can be used to obtain more accurate local structures around the metal ions. Because of the strategic location of the metal ions, the metal ion binding site structure from an XAFS study could be a good probe for the protein matrix in the surrounding area.

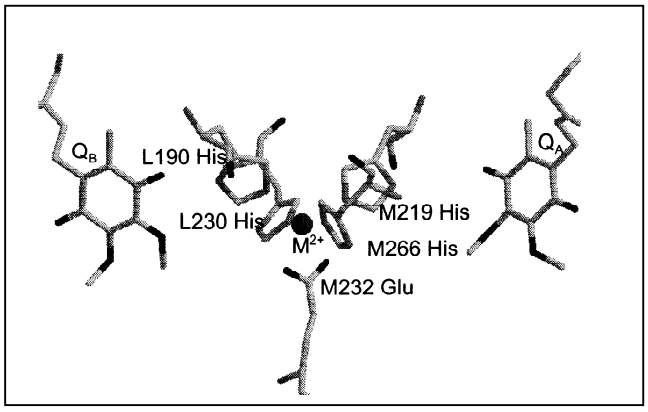

Figure 1: Protein surrounding structure of the metal-ion-binding site in the reaction center.

The goal of our study is to seek a structure/function relationship in the RC proteins by determining the changes in function and structure of the RC proteins with different metal ions replacing the native Fe(II). The study will provide information on the roles of the metal in the electron transfer and the possibility of manipulating the electron transfer rates by varying the structures.

Methods and Materials

Preparation of the reaction center (RC) proteins with Zn(II), Fe(II), and Mn(II) has been described in detail elsewhere [2]. The lipid-imbedded proteins were then 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 [3] with samples at 22ºC.

XAFS spectra were collected at beamline 12-BM with a Si(111) crystal in the monochromator. A Pt-coated focusing mirror reduced the beam size and removed 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 Mn(II) concentration around 2×10^{-4} M. A shaping time of 0.5 μs was used. A Z-1 filter was placed in front of the detector for reducing elastic scattering from the sample.

Conventional XAFS data analysis programs and FEFF 7.0 were used in data analysis. Oxalate dihydrate salts of Fe(II), Zn(II), and Mn(II) were used as reference compounds. The structural parameters were extracted from the reference compounds as well as from the FEFF 7.0 calculations, based on their x-ray diffraction data. The fittings used one-shell models (including six N atoms) and two-shell models (including, four N atoms and two O atoms).

Results

Figure 2 shows the Fourier transform (FT) XAFS of K-edge for Mn(II)-, Fe(II)-, and Zn(II)-bound RC proteins. The most obvious changes are the sequential shifts of the peaks corresponding to the nearest neighbor where Mn(II) has the longest nearest neighbor distance, followed by Fe(II) and Zn(II). This indicates that the protein structure around this region could be metal ion dependent. Figure 3 depicts the fitting results for the nearest neighbors in the three RCs. Structural parameters based on FEFF 7.0 calculations and the x-ray structures of the model compounds are listed in Table 1 (the average distances are used).

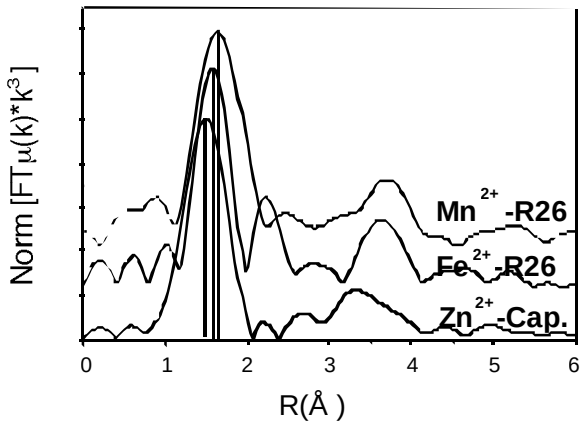

Figure 2: FT-XAFS spectra of three RC proteins with different metal ions (not corrected for the phases).

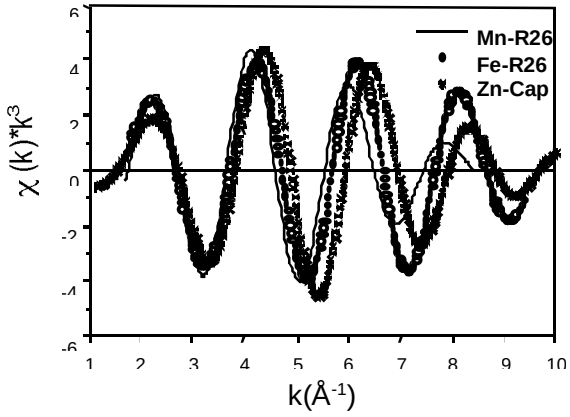

Figure 3: Fitting results for the nearest neighbors in Mn(II)-, Fe(II)-, and Zn(II)-bound RCs.

Table 1: Structural parameters using average distances based on FEFF 7.0 calculations and the x-ray structures of the model compounds.

	N	R(Å)	σ^2(Å^2)
Zn^{2+}-Cap	6.0 ± 0.5	1.96 ± 0.02	0.0083
Fe^{2+}-R26	6.0 ± 0.5	2.08(0.02)	0.0051
Mn^{2+}-R26	6.0 ± 0.5	2.20(0.02)	0.0142

These results provide evidence for metal-ion dependence of the nearest neighbor distances, which is parallel to the variation of the ionic radii for the metal (shown in Figure 4). Metal ion substitution also affects the kinetics for the electron transfer from Q_A to Q_B, showing a trend that the RC with the shorter metal-to-ligand distance gives the fastest electron transfer rate. Figure 5 shows the kinetics traces in our preliminary measurements for Zn(II)-, Fe(II)-, and Mn(II)-bound RCs by optical transient absorption spectroscopy.

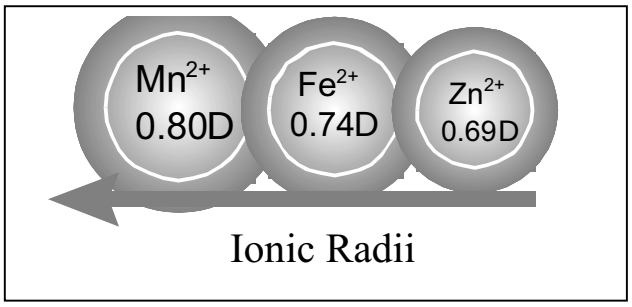

Figure 4: Ionic radii of the metals.

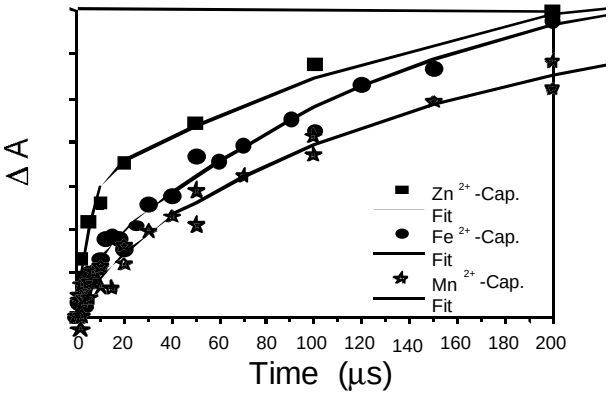

Figure 5: Optical transient absorption traces for the three metal-bound RC proteins. Zn(II) shows the fastest change in ΔA corresponding to the rate of the electron transfer from Q_A to Q_B.

Discussion

The observations from XAFS and transient optical spectra on the metal-ion dependent structures and the kinetics for the electron transfer from Q_A to Q_B suggest that 1) the RC protein in the region near the metal ion binding site is flexible and capable of adapting different metal ions with various ionic radii and 2) the structural changes induced by incorporating metal ions are related to changes on the kinetics of the electron transfer from Q_A to Q_B. The Zn(II), with a smaller ionic radius, could pull the ligands tighter, making a more compact protein surrounding with a faster electron transfer rate, whereas Mn(II), with a larger ionic radius, causes looser packed protein surroundings, giving a slower rate for the electron transfer. This behavior is in contrast with the metal ions in bacterial chlorophylls where the metal-ion-radii dependence is significantly reduced in a much more rigid surrounding protein matrix [4]. This study reveals a direct correlation between the protein structure and the function of the electron transfer.

Acknowledgements

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