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# EXAFS Structural Study of $F_X$, the Low-Potential Fe-S Center in Photosystem I†

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ABSTRACT: We present iron extended X-ray absorption fine structure (EXAFS) spectra of a photosystem I core preparation containing $F_X$, the very low potential iron–sulfur cluster in photosystem I. The preparation lacks $F_A$ and $F_B$. The amplitude of Fe–Fe backscattering in the EXAFS spectrum indicates that $F_X$ may be a [4Fe-4S] cluster and is not a [2Fe-2S] cluster or clusters.

There are contradictory data in the literature concerning the structure of an Fe-S cluster called $F_X$,[1] which serves as an electron acceptor in photosystem I of green plants and cyanobacteria. In its reduced state, $F_X$ is characterized by an EPR spectrum similar to those of [2Fe-2S] and [4Fe-4S] ferredoxins but with a somewhat more anisotropic $g$ tensor. This cluster operates at approximately −720 mV, in contrast to typical ferredoxins, which have reduction potentials of −250 to −450 mV. The properties of $F_X$ have been reviewed (Evans, 1982). The fact that its reduction potential is so low makes it an interesting target for structural studies. In the present work we are concerned with identifying whether $F_X$ is of cluster type [2Fe-2S], [4Fe-4S], or something other than these. Previous structural analyses by spectroscopic methods have been consistent with [4Fe-4S] (Evans et al., 1981) or [2Fe-2S] (Golbeck et al., 1987; McDermott et al., 1988; Bertrand et al., 1988) or a distorted [4Fe-4S] cluster (McDermott et al., 1988). Examination of the polypeptide sequences of psaA and psaB (Fish et al., 1985), which are the putative binding sites of $F_X$ (Golbeck et al., 1988), reveals none of the characteristic sequence elements of [2Fe-2S] or [4Fe-4S] ferredoxins [reviewed in Stout (1982)]. A consideration of the stoichiometries of Fe, acid-labile sulfide, and cysteines per complex would argue against the presence of [2Fe-2S] clusters on the psaA and psaB polypeptides (Bruce & Malkin, 1988; Golbeck et

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[1] Abbreviations: EPR, electron paramagnetic resonance; EXAFS, extended X-ray absorption fine structure; $F_A$, $F_B$, and $F_X$, ferredoxins in photosystem I, alternatively referred to as centers A, B, and X; kDa, kilodaltons; PS I, photosystem I; psaA and psaB, reaction center polypeptides in PS I, also referred to as PSI-A1 and PSI-A2; P700, primary electron donor in PS I; Tiron, 4,5-dihydroxy-1,3-benzenedisulfonic acid; Tris, tris(hydroxymethyl)aminomethane.

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al., 1988; Scheller et al., 1989).

A difficulty associated with the previous spectroscopic work is that the preparations of PS I have contained additional Fe-S clusters ($F_A$ and $F_B$) that interfere with the spectral signatures of $F_X$. Recently, a preparation of PS I with intact $F_X$ clusters but lacking $F_A$ and $F_B$ has been developed (Golbeck et al., 1988). In the present work we report the analysis for X-ray absorption data that favor a [4Fe-4S] structure for $F_X$.

## Experimental Methods

The PS I core preparation containing P700 and $F_X$ was prepared from *Synechococcus* 6301 as described previously (Golbeck et al., 1988). To remove adventitious iron resulting from destruction of $F_A$ and $F_B$, the preparation was dialyzed for 12 h in 50 mM Tris, pH 8.3, and 5 mM Tiron. Samples were then pelleted from 50 mM Tris buffer, pH 7.0, approximately 30% glycerol was added to the pellet, and the resulting mixture was homogenized. The samples were packed into Lucite sample holders and dark adapted for 5 min before freezing. The resulting concentration of iron was 1–2 mM. Chemical analyses of the samples for iron, P700, and acid-labile sulfide were done as described (McDermott et al., 1988; McDermott, 1987).

X-ray absorption data were collected as X-ray fluorescence yield measurements (Jaklevic et al., 1977) by using a lithium-drifted silicon detector similar to that described previously (Goulding et al., 1983) at Stanford Synchrotron Radiation Laboratory on beamline IV-2 during dedicated operation. During beam exposure the sample temperature was maintained between 160 and 200 K. X-ray experimental methods and data analysis have been described (McDermott et al., 1988; McDermott, 1987).

## Results and Discussion

*Characterization of the PS I Core Preparation.* The PS I core preparation has been previously described (Golbeck et al., 1988; Parrett et al., 1989). It contains the high molecular weight (82 000, 83 000) proteins, called psaA and psaB, which bind P700, $A_0$, $A_1$, and $F_X$. It is lacking in the low molecular weight proteins (<22 000) normally associated with PS I complexes with $F_X$ intact. In particular, the 8.9-kDa protein, recently assigned as the $F_A$ and $F_B$ binding site (Oh-oka et al., 1987), cannot be seen on Coomassie- or silver-stained gels. The PS I core preparation contains 5 ± 1 Fe and 4 ± 1 acid-labile sulfide atoms per P700. The EPR signals usually associated with $F_A$ or $F_B$ were not detectable either upon reduction with dithionite at pH 10 or following low-temperature illumination. However, following treatment with ascorbate and illumination at 77 K, the samples exhibit an EPR signal with line shape, $g$ values, and magnitude characteristic of $F_X$. Optical detection at room temperature of the back-reaction of $P700^+$ in this preparation indicates a monophasic 1.2-ms transient accounting for over 90% of the P700 in the sample. This rate has previously been associated with back-reaction from $F_X^-$ in the absence of $F_A$ and $F_B$ (Warden & Golbeck, 1986). The backreaction kinetics indicate that >95% of $F_A$ and $F_B$ has been removed. Thus, the EPR, chemical analysis, and electron-transfer kinetics confirm the presence of an intact $F_X$ cluster in the PS I core preparation and the absence of $F_A$ and $F_B$.

*X-ray K Edge Spectra of the $F_X$ Preparation.* The Fe X-ray K edge spectrum of the $F_X$-containing core preparation (Figure 1a) exhibits a pre-edge transition near 7112 eV, with an intensity characteristic of four-coordinate iron complexes (Roe et al., 1986). The general shape of the edge is quite similar to that of a [4Fe-4S] inorganic complex (Figure 1b) and that

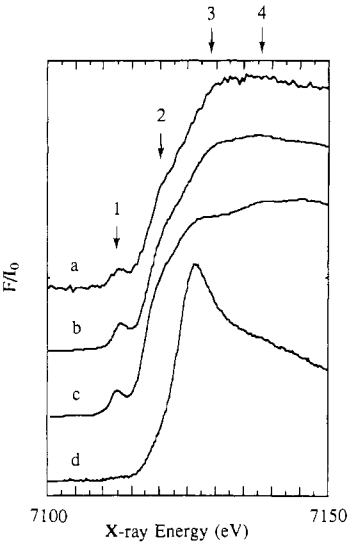


FIGURE 1: Iron X-ray K edge spectra of the PS I core preparation containing $F_X$ (a), $(Et_4N)_2Fe_4S_4(S\text{-benzyl})_4$ (b), PS I complex from *Synechococcus* containing $F_A$, $F_B$, and $F_X$ (c), and a heat-denatured sample of PS I (d). Spectra b and c are taken from McDermott et al. (1988), and all edge spectra were analyzed as described therein. Note the similarity between the spectrum of $F_X$ and that of a [4Fe-4S] model complex, particularly the presence of a strong 1s–3d transition near 7112 eV and lack of a strong 1s–4p transition near 7127 eV; note also the distinctly different spectrum of the denatured ferredoxin, which is typical of centrosymmetric complexes, exhibiting a strong 1s–4p transition near 7127 eV and lack of a 1s–3d transition.

of the intact PS I complex (Figure 1c). In contrast, spectra of a heat-denatured PS I (Figure 1d) or oxidatively denatured ferredoxin sample (data not shown) are distinctly different. The spectra of denatured ferredoxins resemble those of six-coordinate Fe complexes; such centrosymmetric complexes exhibit a pronounced 1s–4p transition and lack a 1s–3d transition since 3d–4p orbital mixing is not symmetry allowed. The X-ray K edge spectrum confirms the presence of an intact [Fe-S] complex in the PS I core preparation and indicates that this [Fe-S] complex accounts for the majority (greater than 80%) of the iron present in the complex.

The transitions underlying the broad edge spectra are better revealed by examining the second-derivative spectra, in which minima correspond to positions of the absorption spectral components. Second-derivative spectra of the same four samples are displayed in Figure 2. The spectra were smoothed by fitting 2-eV sections of the absorption spectrum to second-order polynomials, and an analytic second derivative was then computed. For the spectrum of the PS I core preparation a 4-eV window was used, because of the higher noise level in the data. In the second derivatives of the spectra of the PS I core preparation (a), of the [4Fe-4S] inorganic complex (b), and of whole PS I (c) four transitions can be seen at approximately 7112, 7120, 7129, and 7138 eV. It is likely that the first transition is predominantly 1s–3d (Roe et al. 1986), the second 1s–4p, since it is the first strongly allowed transition; the other two have not been assigned. For the spectrum of denatured PS I one predominant transition is seen that is presumably a 1s–4p transition.

*EXAFS of the $F_X$ Preparation.* A Fourier amplitude Fe-EXAFS spectrum of the $F_X$-containing PS I core preparation is shown in Figure 3. As shown in Figure 4, the k-space spectrum may be adequately simulated by assuming the presence of four S neighbors at 2.27 Å and of three Fe neighbors at 2.7 Å, as would be typical of a [4Fe-4S] cluster, but cannot be simulated by assuming the presence of four S neighbors and one Fe neighbor, as would occur in a [2Fe-2S]

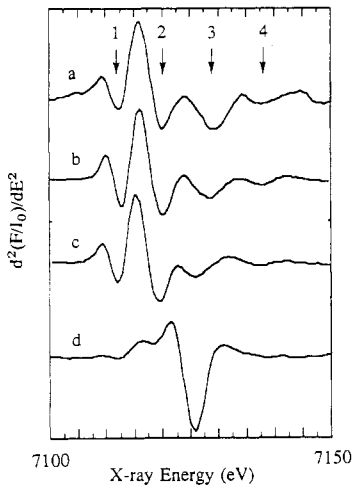


FIGURE 2: Second derivatives of iron X-ray K edge spectra of the PS I core preparation containing $F_X$ (a), $(Et_4N)_2Fe_4S_4(S\text{-benzyl})_4$ (b), PS I complex from *Synechococcus* containing $F_A$, $F_B$, and $F_X$ (c), and a heat-denatured sample of PS I (d). Second derivatives were generated by fitting regions (2-eV sections for spectra b–d and 4-ev sections for spectrum a) to second-order polynomials and computing the second derivative analytically. For spectra a–c, which are all Fe-S complexes or mixtures of Fe-S complexes, four predominant transitions appear as minima in the second derivative and are indicated with arrows. We note the similarity of these three spectra with respect to position and intensity of these features. The spectrum of the denatured PS I sample is distinctly different, showing mainly only one strong transition.

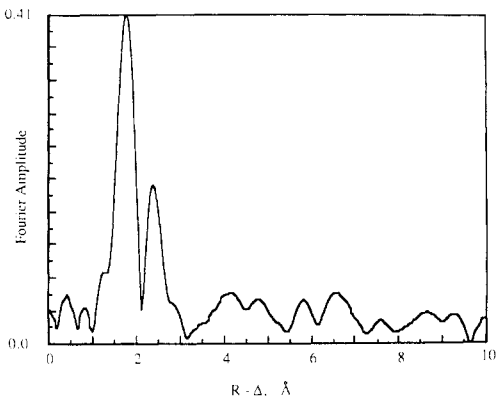


FIGURE 3: Fourier amplitude spectrum of the Fe EXAFS of the $F_X$-containing PS I core preparation. Following normalization and background removal, data from $k$ = 3.5 to $k$ = 11.6 Å$^{-1}$, weighted by $k^3$, were included. Features appear shifted to a lower apparent distance by an amount Δ, which results from the $k$ dependence of the photoelectron phase shift. The feature at an apparent distance of 1.76 Å results from sulfur backscattering, and the feature that appears at 2.3 Å results from Fe backscattering.

cluster. The difference between the simulation that mimics a [2Fe-2S] cluster and the simulation that mimics a [4Fe-4S] cluster is primarily the amplitude of Fe–Fe backscattering, resulting from the larger number of Fe neighbors in the [4Fe-4S] clusters. The data from the PS I core preparation can be simulated with Fe backscattering amplitudes corresponding to a range from two to three Fe neighbors. The result that in $F_X$ each Fe has approximately three Fe neighbors is essentially unchanged by including up to one O atom at 2.0–2.3 Å to compensate for the fact that the sample may contain 10–20% adventitious and probably hexacoordinate Fe.

Previously we reported that a PS I complex containing $F_A$, $F_B$, and $F_X$ was characterized by a smaller Fe–Fe backscattering amplitude as compared with [4Fe-4S] clusters. From these data we were unable to discern whether PS I contains a mixture of clusters with various Fe–Fe bond lengths that give

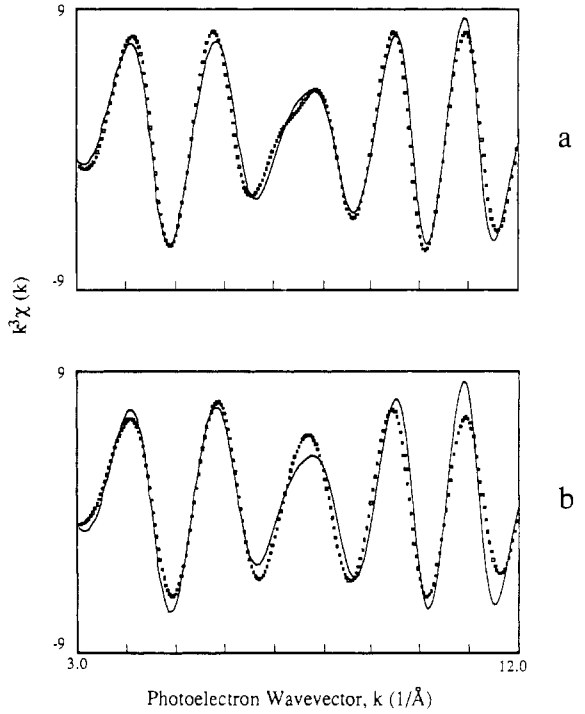


FIGURE 4: Simulation of the $k^3$-weighted Fe EXAFS data from the $F_X$-containing PS I preparation [(solid line) experiment; (dotted line) simulation]. After background removal and weighting by $k^3$, data from $k$ = 3 to $k$ = 12 Å$^{-1}$ were Fourier filtered with window limits at $R'$ = 0.5 and $R'$ = 3.3 Å. The simulation shown was performed by the method of Teo and Lee (1979) using two shells. The parameters for simulation a are typical for a [4Fe-4S] center and employ four S atoms at 2.27 Å with a Debye–Waller disorder parameter of 0.075 Å and three Fe neighbors at 2.7 Å with a disorder parameter of 0.1 Å. The parameters for simulation b are typical for a [2Fe-2S] center and employ four S atoms at 2.26 Å with a Debye–Waller disorder parameter of 0.08 Å and one Fe neighbor at 2.7 Å with a disorder parameter of 0.07 Å. Approximate error in the number of scatterers is 20% and in $R$ is 0.03 Å. The threshold energy for photoelectron release was allowed to vary by 15 eV relative to the initial guess of 7130 eV. Within the range of physically reasonable values, spectrum b represents the best simulation consistent with a [2Fe-2S] cluster, and we conclude that such a structure is unlikely for $F_X$. While a [4Fe-4S] cluster is consistent with these data, as shown in spectrum a, there may be other cluster types that are also consistent with the data.

rise to damped Fe–Fe backscattering or whether PS I contained some [2Fe-2S] clusters, which would contribute a small amplitude to the Fe–Fe backscattering. In the present study, the isolation of $F_X$ from the other clusters allows us to clearly determine that $F_X$ is not a [2Fe-2S] cluster. It is probably a [4Fe-4S] cluster or some similar type of cluster with two to three Fe neighbors for each Fe. The reduced amplitude of Fe–Fe backscattering in PS I presumably reflects a difference in Fe–Fe bond lengths for $F_A$ and $F_B$ as compared with $F_X$. We note that the EXAFS simulations of the whole PS I preparation including $F_A$, $F_B$, and $F_X$ suggest an average Fe–Fe bond length of 2.76–2.78 Å (McDermott et al., 1988), while in the PS I core complex containing only $F_X$ the average Fe–Fe bond length is 2.69–2.70 Å.

Besides a [4Fe-4S] cluster, one may propose other models for $F_X$ that would be consistent with these EXAFS results. For example, the [3Fe-4S] clusters should show an EXAFS spectrum similar to that measured for $F_X$. However, the EPR spectra and magnetic properties of these centers are very different from those of $F_X$ (Beinert & Thomson, 1983). Another model consistent with the EXAFS spectrum would be a [4Fe-4S] cluster with an additional S ligand on one Fe, such as the cluster synthesized by Kanatzidis et al. (1983).

This cluster exhibits a lower midpoint potential than other [4Fe-4S] clusters due to the addition of an extra electron-donating ligand, which makes it an interesting model for $F_X$. Alternatively, because of the relatively small number of cysteines in psaA and psaB, one might propose that $F_X$ has O or N ligands in place of some of the S ligands. Such a mixed ligation has been reported for the [2Fe-2S] Rieske proteins, which have at least one N ligand (Fee et al., 1984). As a result of the substitutiuon of a relatively electron poor ligand for a S (Johnson et al., 1983), the Rieske centers exhibit a more positive midpoint potential. To date, no [4Fe-4S] clusters with O or N ligands have been reported in ferredoxins. Such a model for $F_X$ cannot be proven or excluded on the basis of our EXAFS results, especially as there may be a small amount of contaminating iron in the preparation that is coordinated to oxygen. However, this suggestion seems unlikely in light of the very low midpoint potential of $F_X$.

The assignment of $F_X$ as a tetranuclear structure rather than as a pair of binuclear clusters is more consistent with the number of available cysteines in the PS I core complex. The psaA and psaB polypeptides have fewer than four cysteine residues each (Fish et al., 1985), which implies that the $F_X$ cluster must be ligated by cysteines from at least two polypeptides. Recent evidence indicates that there is only one copy each of the psaA and psaB polypeptides per PS I complex (Bruce & Malkin, 1988; Scheller et al., 1989). One copy of each of these two polypeptides would contain enough cysteine residues to ligate one [4Fe-4S] cluster, but not two [2Fe-2S] clusters. Thus, a model for $F_X$ consistent with all of the data would be that $F_X$ is a single tetranuclear Fe-S cluster, with ligands from both of the reaction center polypeptides.

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