# Human Cytosolic Iron Regulatory Protein 1 Contains a Linear Iron−Sulfur Cluster

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The chemistry of iron−sulfur clusters is among the most intensively studied areas of bioinorganic chemistry, and synthetic chemists have had considerable success in reproducing many of the iron−sulfur clusters found in natural systems. This synthetic work has provided a wealth of information on iron−sulfur cluster chemistry and electronic structure, together with unequivocal confirmation of the biological structures.[1] To date, however, no category of biological cluster has been synthesized chemically before being structurally characterized in a biological system. The only potential exception to this is the linear [3Fe−4S] cluster of mitochondrial aconitase formed when the enzyme is inactivated by high pH or concentrated urea,[2] although whether this cluster occurs in vivo remains unclear. We report herein a spectroscopic characterization of recombinant human iron regulatory protein 1 (IRP1) and show that it contains a similar linear [3Fe−4S] cluster under physiological conditions. This is possibly the first instance of a native biological iron−sulfur cluster previously unknown in biology that is already well-known from synthetic chemistry.

IRP1 is a ∼100 KDa protein that is found in all higher forms of life from mollusks to insects and vertebrates.[3] In mammals, IRP1 regulates cellular iron uptake and storage by controlling the synthesis of both the transferrin receptor and ferritin in an opposing sense at the translational level.[4] IRP1 binds to stem−loop structures in the mRNAs only when it does not contain iron.[3,5] With ferritin it binds at the 5′ end, and blocks translation, but with the transferrin receptor it binds at the 3′ end and stabilizes the mRNA from degredation. It has considerable sequence homology with mitochondrial aconitase and can also have aconitase activity.[3,5] Indeed, it has recently been discovered that the so-called cytosolic aconitase is in fact IRP1.

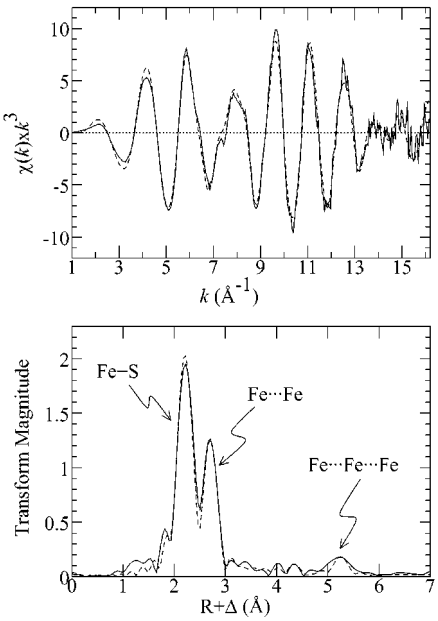


**Figure 1.** Fe K-edge EXAFS (top) and Fourier transform (bottom) of human IRP1. The solid lines show experimental data, and the broken lines, the results of full multiple scattering analysis: 4 Fe−S at 2.250(1) Å with $\sigma^2$ = 0.0035(2) and $1^1/_3$ Fe···Fe at 2.700(1) Å, with $\sigma^2$ = 0.0029(1). $\sigma^2$ are the mean-square deviation in interatomic distances, and the values in parentheses are the estimated standard deviations obtained from the diagonal elements of the covariance matrix.

The Fe K-edge extended X-ray absorption fine structure (EXAFS)[6] spectrum of recombinant human IRP1 containing the iron−sulfur cluster[7] and corresponding Fourier transform are shown in Figure 1, along with the results of EXAFS curve-fitting analysis. The Fourier transform is dominated by intense Fe−S interactions, giving rise to the strong peak at 2.25 Å, with a smaller feature at about 2.7 Å, attributable to Fe···Fe interactions. Using

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(4) (a) Klausner, R. D.; Rouault, T. A.; Harford, J. B. *Cell* **1993**, *72*, 19−28. (b) Rouault, T. A.; Klausner, R. D. *Trends Biochem. Sci.* **1996**, *21*, 174−177.
(5) Beinert, H.; Kennedy, M. C.; Stout, C. D. *Chem. Rev.* **1996**, *96*, 2335−2373.

(6) X-ray absorption spectra were recorded on beamline 7-3 at the Stanford Synchrotron Radiation Laboratory (SSRL) using a Si(220) double crystal monochromator, an upstream vertical aperture of 1 mm, and no specular optics. Data were collected as the Fe Kα excitation spectrum using an array of 13 Ge detectors. Incident intensity was monitored using a $N_2$-filled ionization chamber. The sample was maintained at 10 K in a liquid He flow cryostat during data collection. Twenty-one 35-minute scans were averaged, and the spectrum of an iron metal foil was simultaneously measured for energy calibration (by reference to the lowest energy K-edge inflection point, assumed to be 7111.3 eV). The EXAFSPAK programs [http://ssrl.slac.stanford.edu/exafspak.html] were used to analyze the data; EXAFS curve-fitting employed ab initio phase and amplitude functions generated with the program FEFF v8.2 (Rehr, J. J.; Mustre de Leon, J.; Zabinsky, S. I.; Albers, R. C. *J. Am. Chem. Soc.* **1991**, *113*, 5135−5140).

(7) N-terminal His-tagged human IRP1 (a kind gift from Dr. M. Hentze, EMBL, Heidelberg, Germany) transfected *Escherichia coli* [Stratagene Epicurian Coli BL21] were grown in PBS-buffered Luria broth (pH 7.2, 100 μg/mL ampicillin) at 30 °C for 20 h (without IPTG). Following cell lysis, IRP1 was purified by immobilized metal affinity and anion-exchange chromatography (Gailer, J.; Yu, Y.; George, G. N.; Pickering, I. J.; Prince, R. C.; Kohlhepp, P.; Zhang, D.; Walker, F. A.; Winzerling, J. J. unpublished) and stored overnight at 4 °C. Reconstitution of the iron−sulfur cluster followed Gegout et al. (Gegout, V.; Schlegl, J.; Schläger, B.; Hentze, M. W.; Reinboldt, J.; Ehresmann, B.; Ehresmann, C.; Romby, P. *J. Biol. Chem.* **1999**, *274*, 15052−15058.), except that the initial washing buffer did not contain glycerol and $Fe(NH_4)_2(SO_4)_2$ was used instead of $FeSO_4$. The reconstitution mixture was incubated at 37 °C for 10 min (at no time did the reaction mixture pH rise above 7.6) and then concentrated at 4 °C in a 25 000 MW Collodion nitrocellulose membrane against 30 mM TRICINE (all procedures were performed under an atmosphere of helium and used degassed buffers at pH 7.5). The protein was then subjected to size-exclusion chromatography (Superdex 200 HR 10/30; Amersham Pharmacia Biotech., Uppsala, Sweden) using 20 mM TRICINE and 1.5 mM $MgCl_2$. Colored protein fractions were further concentrated (Centricon YM-30, 4 °C) and either transferred to an EPR tube or mixed with glycerol (6:4, v/v), transferred to a Lucite sample holder, and frozen in liquid nitrogen for later spectroscopy.

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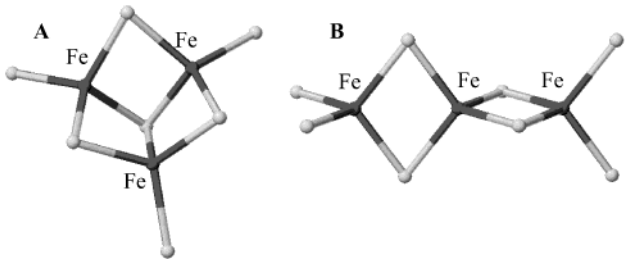


**Figure 2.** Structures of [3Fe−4S] clusters. (A) The thiocubane fragment found in ferredoxins and inactive mitochondrial aconitase. (B) The linear iron−sulfur cluster found in denatured aconitase and in reconstituted human IRP1. The nonlabeled atoms are sulfurs, and the external sulfurs are derived from cysteine residues in the proteins.

simple single scattering EXAFS theory, a best fit was obtained with 4 Fe−S at 2.25 Å and $1^1/_3$ Fe···Fe at 2.69 Å. The experimental Fourier transform (Figure 1) indicates that there are additional weak long-distance interactions giving rise to the small transform peak at 5.4 Å, plus more subtle features at shorter distances. Observation of long-distance (i.e. >5 Å) interactions in EXAFS of biological systems is not common, and usually only occurs when multiple scattering interactions are enhanced by a linear arrangement of atoms.[8] This, together with the fact that the 5.4 Å peak occurs at twice the distance of the major Fe···Fe interaction, suggests a linear Fe···Fe···Fe arrangement in the cluster. We therefore modeled the EXAFS using a full multiple scattering treatment assuming the linear $(RS)_2FeS_2FeS_2Fe(SR)_2$ core (Figure 2B) constructed using the bond lengths from the single scattering EXAFS analysis, and assuming a cluster with $D_2$ symmetry. An excellent fit to the data was achieved, reproducing all of the low-intensity features in the transform (Figure 1), and including six three-leg and three four-leg multiple scattering paths.[9] Thus, the EXAFS data indicate the presence of the linear iron−sulfur cluster shown in Figure 2B. Comparison of the near-edge portion of the spectrum of IRP1 with those of oxidized and reduced rubredoxin[10] suggested an all-ferric cluster (not illustrated).

The structural parameters, as determined by EXAFS, are very similar to the low-molecular weight model compound $[(PhS)_2FeS_2FeS_2Fe(SPh)_2]^{3-}$ characterized by Hagen et al.[11] The average Fe−S and Fe···Fe interatomic distances are 2.26 and 2.71 Å in the model, and 2.25 and 2.70 Å in human IRP1. The Fe···Fe···Fe distance is 5.42 Å in the model and 5.40 Å in the protein.

(8) See, e.g.: Laplaza, C. E.; Johnson, M. A.; Peters, J.; Odom, A. L.; Kim, E.; Cummins, C. C.; George, G. N.; Pickering, I. J. *J. Am. Chem. Soc.* **1996**, *118*, 8623−8638.

(9) Single scattering analyses assuming different scatterer types (e.g., carbon) did not adequately reproduce the long distance transform peaks, and required unreasonably large numbers of backscatterers. Other candidates for possible long-range interactions (e.g., phosphorus, sulfur) can be excluded, as they also required an unreasonably large number of scatterers and gave EXAFS that were close to 180° out of phase with the EXAFS of the 5.4 Å feature. On the other hand, the full multiple scattering linear three-iron model not only reproduced the 5.4 Å feature but also several smaller peaks between 3 and 4.5 Å.

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(11) (a) Hagen, K. S.; Holm, R. H. *J. Am. Chem. Soc.* **1982**, *104*, 5496−5497. (b) Hagen, K. S.; Watson, A. D.; Holm, R. H. *J. Am. Chem. Soc.* **1983**, *105*, 3905−3913.

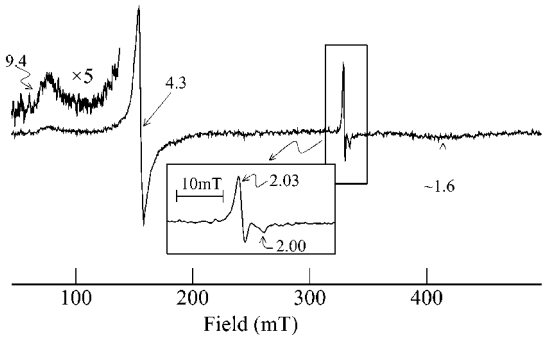


**Figure 3.** EPR spectrum of human IRP1. The vertically expanded region (left) shows the $g \approx 9$ ground-state feature of the $S = {}^5/_2$ system, and the inset shows the axial $S = {}^1/_2$ signal.

The electron paramagnetic resonance (EPR) spectrum[12] of human IRP1 showed a small peak at $g \approx 9.4$, an isotropic feature at 4.3, a possible broad feature near $g \approx 1.6$, and a sharper axial signal near $g \approx 2.03$ (Figure 3). The $g \approx 9.4$ and 4.3 features are characteristic of an $S = {}^5/_2$ system in a rhombic environment, arising from transitions within the ground-state and first excited-state Kramers doublets, respectively. The characteristic asymmetric shape of the $g \approx 9.4$ feature indicates the presence of considerable $D$-strain.[13] The signal at $g \approx 2.03$ originates from an $S = {}^1/_2$ system, is relatively isotropic, and arises from a small quantity[14] of an unknown species, possibly a thiocubane fragment [3Fe−4S] cluster. The $S = {}^5/_2$ signal is very similar to that previously reported for the linear [3Fe−4S] cluster of mitochondrial aconitase, and to the model compound of Hagen et al.,[11] both of which have large positive zero-field splittings and are almost fully rhombic (e.g., aconitase has $D \approx 1.5$ cm$^{-1}$ and $\lambda \approx 0.31$).[15]

In summary, we have purified recombinant human IRP1, and reconstituted the iron−sulfur cluster using an established method[7] that yielded thiocubane-type clusters in other proteins. We have shown that reconstituted IRP1 contains a linear species, which is likely to be $Cys_2[FeS_2FeS_2Fe]Cys_2$ (Figure 2B). It remains to be seen whether this linear cluster actually occurs in vivo.

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(12) EPR spectra were obtained on a Bruker 300 E instrument at 9.344 GHz, with 0.2 mW applied power, 0.4 mT modulation amplitude, and a sample temperature of about 4.2 K.

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(14) While the peak intensities of the EPR signals at $g = 4.3$ and $g = 2.03$ are similar, the integrated intensity of the broader $S = {}^5/_2$ signals will be far greater.

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