Three-Iron Clusters in Iron-Sulfur Proteins

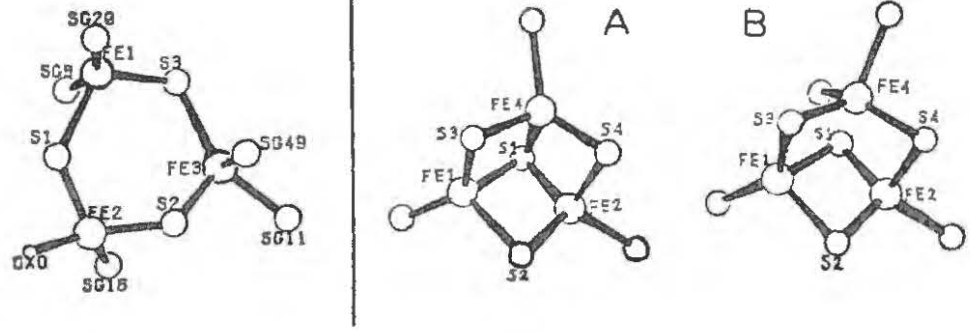
Sten A. Wallin Literature Seminar March 27, 1984

The existence of [3Fe-xS] clusters [1] was first postulated in late 1979/early 1980 based on a combined EPR and Mössbauer study of the *Azotobacter vinelandii* ferredoxin I (*A.v.* Fd) [2] and an x-ray crystallographic study of *A.v.* Fd [3]. Shortly thereafter, this rather convincing evidence for a novel Fe/S cluster type was corroborated by the results of EPR and Mössbauer studies on *Desulfovibrio gigas* Fd II (*D. gigas* Fd II) [4] and beef heart mitochondrial aconitase [5]. At the present time, at least a dozen "native" proteins (*i.e.*, obtained upon ordinary aerobic purification) have been shown to contain 3Fe clusters with an additional dozen displaying some of the characteristic features of this cluster type.

Mössbauer spectroscopy provides convincing evidence for the presence of 3Fe clusters [6]. Other spectroscopic probes that exhibit signals characteristic of [3Fe-xS] sites are low temperature magnetic circular dichroism [7], low temperature resonance raman [8] and the linear field effect in EPR [9].

The subject of 3Fe cluster containing proteins has had more than its share of controversy. By late 1981/1982, it had been demonstrated that, in a number of cases, 3Fe clusters are definitely man-made artifacts. For example, oxidation of certain native $[4Fe\text{-}4S]^{2+,+}$ ferredoxins with $Fe(CN)_6^{3-}$ yields not the expected $[4Fe\text{-}4S]^{3+}$ oxidized HiPIP state, but rather, a [3Fe-xS] species [10,11]. In other systems, 3Fe clusters can be converted, under appropriate conditions, into [4Fe-4S] clusters with or without the addition of Fe^{2+} or $S^{=}$ [5,12,13,14].

Another area of some controversy concerns the structure(s) of 3Fe clusters. Stout's 2.5 Å resolution x-ray crystallographic analysis of *A.v.* Fd (a 7Fe protein) showed, in addition to a conventional [4Fe-4S] site, a planar, twist-boat six-membered ring [3Fe-3S] structure with $d_{Fe\text{-}Fe}$ = 4.1 Å [3,15]. In contrast, Fe EXAFS studies on *D. gigas* Fd II [16] and aconitase [12] clearly indicate a much more closed cluster structure with $d_{Fe\text{-}Fe}$ = 2.7 Å.



[3Fe-3S] [3Fe-4S]

$d_{Fe\text{-}Fe}$ = 4.1Å $d_{Fe\text{-}Fe}$ = 2.7Å

Earlier studies on the activation of inactive "native" aconitase suggested a [3Fe-4S] cluster for aconitase [5,12,13]. This has been confirmed by a recent high-precision determination of the $Fe/S^{=}$ stoichiometry showing that "native" aconitase contains 2.9 ± 0.2 Fe/molecule and 3.9 ± 0.2 $S^{=}$/molecule [17]. Very recently, it has been shown that the Fe EXAFS of [4Fe-4S] depleted *A.v.* Fd (*i.e.*, containing only the 3Fe cluster) is virtually identical to that of "native" aconitase [18].

On the basis of the above, as well as on the strong similarity of the RR spectra for all studied [3Fe-xS] proteins and the results of normal mode calculations for various 3Fe cluster geometries [19], it has been suggested that all 3Fe clusters are of a "closed" (*i.e.*, d_{Fe-Fe} = 2.7 Å) [3Fe-4S] form.

References

1. For a review current through Jan. 1983: Beinert, H.; Thomson, A. J., "Three-Iron Clusters in Iron-Sulfur Proteins", Arch. Biochem. Biophys. 1983, 222(2), 333-361.

2. Emptage, M. H.; Kent, T. A.; Huynh, B. H.; Rawlings, J.; Orme-Johnson, W. H.; Münck, E., "On the Nature of the Iron-Sulfur Centers in a Ferredoxin from *Azotobacter vinlandii*, J. Biol. Chem. 1980, 255, 1793-1796.

3. Stout, C. D.; Ghosh, D.; Patthabi, R.; Robbins, A. H., "Iron-Sulfur Clusters in *Azotobacter* Ferredoxin at 2.5 Å Resolution", J. Biol. Chem. 1980, 255, 1797-1800.

4. Huynh, B. H.; Moura, J. J. G.; Moura, I.; Kent, T. A.; LeGall, J.; Xavier, A. V.; Münck, E., "Evidence for a Three-Iron Center in a Ferredoxin from *Desulfovibrio gigas*", J. Biol. Chem. 1980, 255, 3242-3244.

5. Kent, T. A.; Dreyer, J.-L.; Kennedy, M. C.; Huynh, B. H.; Emptage, M. H.; Münck, E., "Mössbauer Studies of beef heart aconitase: Evidence for facile interconversion of iron-sulfur clusters", Proc. Nat. Acad. Sci. USA 1982, 79, 1096-1100.

6. Münck, E., "Mössbauer studies of [3Fe-3S] Clusters and Sulfite Reductase", In "Iron-Sulfur Proteins", T. G. Spiro, Ed.; John Wiley & Sons: New York, 1982; pp 148-175.

7. a) Thomson, A. J.; Robinson, A. E.; Johnson, M. K.; Moura, J. J. G.; Moura, I.; Xavier, A. V.; LeGall, J., "The Three-Iron Cluster in a Ferredoxin from *Desulfovibrio gigas* A Low-Temperature Magnetic Circular Dichroism Study", Biochim. Biophys. Acta 1981, 670, 93-100.
   b) Johnson, M. K.; Robinson, A. E.; Thomson, A. J., "Low-Temperature Magnetic Circular Dichroism Studies of Iron-Sulfur Proteins", In "Iron-Sulfur Proteins", T. G. Spiro, Ed.; John Wiley & Sons: New York, 1982; pp 368-406.

8. Spiro, T. G.; Hare, J.; Yachandra, V.; Gewirth, A.; Johnson, M. K.; Remsen, E., "Resonance Raman Spectra of Iron-Sulfur Proteins and Analogs", In "Iron-Sulfur Proteins", T. G. Spiro, Ed.; John Wiley & Sons: New York, 1982; pp 408-423.

9. Peisach, J.; Beinert, H.; Emptage, M. H.; Mims, W. B.; Fee, J. A.; Orme-Johnson, W. H.; Rendina, A. R.; Orme-Johnson, N. R., "Characterization of 3-Iron Ferredoxins by Means of the Linear Electric Field Effect in EPR", J. Biol. Chem. 1983, 258, 13014-13016.

10. Thomson, A. J.; Robinson, A. E.; Johnson, M. K.; Cammack, R.; Rao, K. K.; Hill, D. O., "Low-Temperature Magnetic Circular Dichroism Evidence for the Conversion of Four-Iron-Sulfur Clusters in a Ferredoxin from *Clostridium pasteurianum* into Three-Iron-Sulfur Clusters", Biochim. Biophys. Acta 1981, 637, 423-432.

11. Johnson, M. K.; Spiro, T. G.; Mortenson, L. E., "Resonance Raman and Electron Paramagnetic Resonance Studies on Oxidized and Ferricyanide-treated *Clostridium pasteurianum* Ferredoxin", J. Biol. Chem. 1982, 257, 2447-2452.

12. Beinert, H.; Emptage, M. H.; Dreyer, J.-L.; Scott, R. A.; Hahn, J. E.; Hodgson, K. O.; Thomson, A. J., "Iron-sulfur stoichiometry and structure of iron-sulfur clusters in three-iron proteins: Evidence for [3Fe-4S] clusters", Proc. Nat. Acad. Sci. USA 1983, 80, 393-396.

13. a) Kennedy, M. C.; Emptage, M. H.; Dreyer, J.-L.; Beinert, H., "The role of Iron in the Activation-Inactivation of Aconitase", J. Biol. Chem. 1983, 253, 11098-11105.
b) Emptage, M. H.; Dreyer, J.-L.; Kennedy, M. C.; Beinert, H., "Optical and EPR Characterization of Different Species of Active and Inactive Aconitase", J. Biol. Chem. 1983, 258, 11106-11111.

14. a) Kent, T. A.; Moura, I.; Moura, J. J. G.; Lipscomb, J. D.; Huynh, B. H.; LeGall, J.; Xavier, A. V.; Münck, E., "Conversion of [3Fe-3S] into [4Fe-4S] Clusters in a *Desulfovibrio gigas* Ferredoxin and Isotopic Labelling of Iron-Sulfur Subsites", FEBS Lett. 1982, 138, 55-58.
b) Moura, J. J. G.; Moura, I.; Kent, T. A.; Lipscomb, J. D.; Huynh, B. H.; LeGall, J.; Xavier, A. V.; Münck, E., "Interconversion of [3Fe-3S] and [4Fe-4S] Clusters", J. Biol. Chem. 1982, 257, 6259-6267.

15. Stout, C. D., "Iron-Sulfur Protein Crystallography", In "Iron-Sulfur Proteins", T. G. Spiro, Ed.; John Wiley & Sons: New York, 1982; pp 98-146.

16. Antonio, M. R.; Averill, B. A.; Moura, I.; Moura, J. J. G.; Orme-Johnson, W. H.; Teo, B.-K.; Xavier, A. V., "Core Dimensions in the 3Fe Cluster of *Desulfovibrio gigas* Ferredoxin II by Extended X-ray Absorption Fine Structure Spectroscopy", J. Biol. Chem. 1982, 257, 6646-6649.

17. Rydén, L.; Öfverstedt, L.-G.; Beinert, H.; Emptage, M. H.; Kennedy, M. C., "Molecular Weight of Beef Heart Aconitase and Stoichiometry of the Components of Its Iron-Sulfur Cluster", J. Biol. Chem. 1984, 259 (in press).

18. a) Stephens, P. J., personal communication.
b) Scott, R. A., personal communication.

19. a) Johnson, M. K.; Czernuszewicz, R. S.; Spiro, T. G.; Fee, J. A.; Sweeny, W. V., "Resonance Raman Spectroscopic Evidence for a common [3Fe-4S] Structure among Proteins Containing Three-Iron Centers", J. Am. Chem. Soc. 1983, 105, 6671-6678.
b) Johnson, M. K.; Czernuszewicz, R. S.; Spiro, T. G.; Ramsay, R. R.; Singer, T. P., "Resonance Raman Studies of Beef Heart Aconitase and a Bacterial Hydrogenase", J. Biol. Chem. 1983, 258, 12771-12774.