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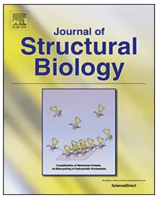

Review

Structure and electrochemistry of proteins harboring iron-sulfur clusters of different nuclearities. Part II. [4Fe-4S] and [3Fe-4S] iron-sulfur proteins

Piero Zanello

Dipartimento di Biotecnologie, Chimica e Farmacia dell'Università di Siena, Via A. De Gasperi 2, 53100 Siena, Italy

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ABSTRACT

In the context of the plethora of proteins harboring iron-sulfur clusters we have already reviewed structure/electrochemistry of metalloproteins expressing single types of iron-sulfur clusters (namely: $\{Fe(Cys)_4\}$, $\{[Fe_2S_2](Cys)_4\}$, $\{[Fe_2S_2](Cys)_3(X)\}$ (X = Asp, Arg, His), $\{[Fe_2S_2](Cys)_2(His)_2\}$, $\{[Fe_3S_4](Cys)_3\}$, $\{[Fe_4S_4](Cys)_4\}$ and $\{[Fe_4S_4](S^{\gamma}_{Cys})_3(\text{nonthiolate ligand})\}$ cores) and their synthetic analogs. More recently we are focussing on structure/electrochemistry of metalloproteins harboring iron-sulfur centres of different nuclearities. Having started such a subject with proteins harboring [4Fe-4S] and [2Fe-2S] clusters, we now depict the state of art of proteins containing [4Fe-4S] and [3Fe-4S] clusters.

1. Introduction

Having a detailed picture of the hundreds of proteins that host iron-sulfur clusters and their different biological functions is not a trivial task, also because an overall rationalization of such a field has not yet appeared. In the attempt to cover such a subject at least from the structural-to-electrochemical viewpoint, in recent years we have carefully selected those proteins that host single iron-sulfur clusters, also giving evidence to those that have not yet been studied from the electrochemical viewpoint. Thus we have reviewed structure/electrochemistry of proteins harboring the following iron-sulfur centres: $\{Fe(Cys)_4\}$ (Zanello, 2013), $\{[Fe_2S_2](Cys)_4\}$ (Zanello, 2014), $\{[Fe_2S_2](Cys)_3(X)\}$ (X = Asp, Arg, His) and $\{[Fe_2S_2](Cys)_2(His)_2\}$ (Zanello, 2016), $\{[Fe_3S_4](Cys)_3\}$ (Fabrizi de Biani and Zanello, 2017), $\{[Fe_4S_4](Cys)_4\}$ (Zanello, 2017a) and $\{[Fe_4S_4](S^{\gamma}_{Cys})_3(\text{nonthiolate ligand})\}$ (Zanello and Corsini, 2017), also looking at their respective synthetic analogs. After such a sequence of surveys, we planned to extend such a systematic approach to proteins harboring iron-sulfur clusters of different nuclearities. In this perspective we started with proteins containing [4Fe-4S] and [2Fe-2S] clusters (Zanello, 2017b). We are now looking at structures and electrochemistry of proteins harboring [4Fe-4S] and [3Fe-4S] clusters. As in previous papers, we will examine exclusively those proteins the molecular structure of which has been ascertained by X-ray crystallography, NMR spectroscopy or cryo-electron microscopy, giving also evidence to those structurally characterized proteins which still lack electrochemical investigation.

2. 7-Fe ferredoxins

Ferredoxins are enzymes essentially deputed to biological electron-transfer processes in different metabolic pathways.

[3Fe-4S] ferredoxins perform their biological functions through a reversible one-electron process that can be formally represented as:

$$\{[Fe_3^{III}S_4](S^{\gamma}_{Cys})_3\}^{2-} \underset{-e}{\overset{+e}{\rightleftarrows}} \{[Fe_2^{III}Fe^{II}S_4](S^{\gamma}_{Cys})_3\}^{3-}$$

or:

$$[Fe_3^{III}S_4]^{+} \underset{-e}{\overset{+e}{\rightleftarrows}} [Fe_2^{III}Fe^{II}S_4]^{0}$$

Analogously, [4Fe-4S] ferredoxins can exploit a sequence of reversible one-electron processes formally represented as[1]:

$$\{[Fe_3^{III}Fe^{II}S_4](S^{\gamma}_{Cys})_4\}^{-} \underset{-e}{\overset{+e}{\rightleftarrows}} \{[Fe_2^{III}Fe_2^{II}S_4](S^{\gamma}_{Cys})_4\}^{2-} \underset{-e}{\overset{+e}{\rightleftarrows}} \{[Fe^{III}Fe_3^{II}S_4](S^{\gamma}_{Cys})_4\}^{3-}$$

or:

$$\underset{(\text{HiPIP})^{1}}{[Fe_3^{III}Fe^{II}S_4]^{3+}} \underset{-e}{\overset{+e}{\rightleftarrows}} [Fe_2^{III}Fe_2^{II}S_4]^{2+} \underset{-e}{\overset{+e}{\rightleftarrows}} [Fe^{III}Fe_3^{II}S_4]^{+}$$

Since the subject of 7-Fe ferredoxins has been accurately reviewed more than ten years ago (Stout, 2006) making use of literature data until 2000, a more recent revisitation is hopefully useful.

The most structurally and electrochemically investigated 7-Fe ferredoxin is that expressed by *Azotobacter vinelandii* [an aerobic soil-dwelling bacterium with a wide variety of metabolic capabilities which

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E-mail address: piero.zanello@unisi.it.

[1] Structure and electrochemistry of HiPIP (i.e. High Potential Iron-sulfur Protein) has been recently reviewed (Zanello, 2017a).

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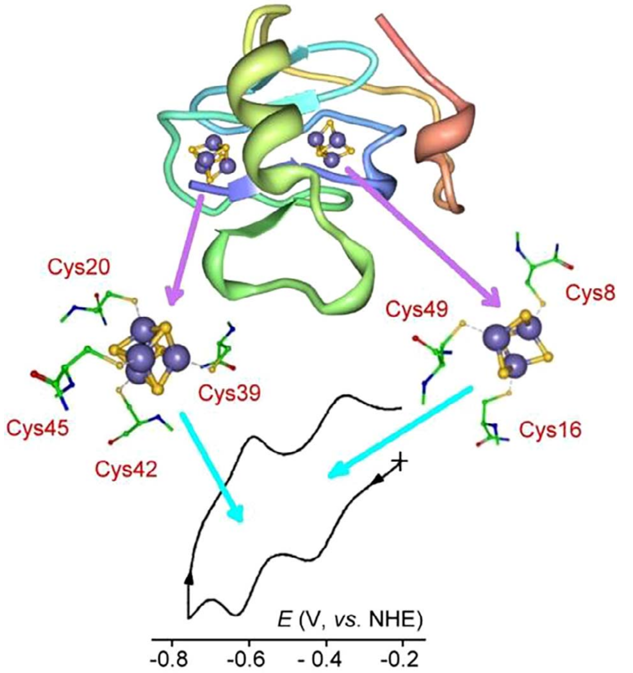

Fig. 1. Crystal structure of *Av*FdI (isolated at pH 6.5) and its cyclic voltammetric behavior (recorded at an edge-oriented pyrolytic graphite electrode at pH 7.0 in the presence of neomicyn promoter; scan rate 0.05 Vs^{-1}). The distance between the two iron-sulfur clusters is about 9 Å. Adapted from (Merritt et al., 1993 (PDB 1FER)) and (Chen et al., 2002a,b), respectively.

include the ability to fix atmospheric nitrogen by converting it to ammonia (Kennedy et al., 2005; Setubal et al., 2009)], commonly known as ferredoxin I (hereafter *Av*FdI). X-ray structures of the monomeric form of the enzyme crystallized at pH from 6.0 to 8.5 have been solved (Stout, 1993; Merritt et al., 1993; Stout et al., 1998; Sridhar et al., 1998; Jung et al., 2000; Schipke et al., 1999).

Fig. 1 illustrates the molecular structure of *Av*FdI (that substantialy reflects that of all 7-Fe ferredoxins) as well as its redox activity.

The enzyme displays two separate reductions with features of chemical reversibility that are attributed to the sequence $[Fe_3S_4]^{+/0}$ and $[Fe_4S_4]^{2+/+}$, respectively. Such a behavior agrees with the fact that only the $[3Fe\text{-}4S]^+$ cluster is reduced by dithionite.[2]

The redox potentials of the $[Fe_3S_4]^{+/0}$ and $[Fe_4S_4]^{2+/+}$ processes of *Av*FdI are compiled in Table 1 together with those of a few mutants.

We point out that a number of crystallographically characterized mutants still lack electrochemical investigation {i.e. [Phe2Tyr, (PDB 1FRH)], Asp23Asn [(PDB 1FRI)], His35Asp [(PDB 1FRK)], Glu38Ser [(PDB 1FRL)] and [(PDB 1FRM)] (Shen et al., 1994)}.

It is noted that the structural differences between the *oxidized* {[Stout, 1993 (PDBs: 5Fd1, 1FDA)], [Merritt et al., 1993 (PDB 1FER)], [Stout et al., 1998 (PDB 6Fd1)], [Schipke et al., 1999 (PDB 7Fd1)]} and *reduced* {[Stout, 1993 (PDB 1FDB)], [Schipke et al., 1999 (PDBs: 6FDR, 7FDR)]} forms of *Av*FdI are really minimal.

From the electrochemical viewpoint, attention has been paid to the fact that the $[Fe_3S_4]^{+/0}$ process shows an almost linear shift of redox potential towards more negative values of about 50 mV *per* pH unit in the pH range from 5 to 8 (Shen et al., 1994), whereas no substantial dependence from pH is exhibited by the $[Fe_4S_4]^{2+/+}$ process [about 15 mV *per* pH unit (Iismaa et al., 1991)]. Such a dependence suggests that the $[Fe_3S_4]^{+/0}$ electron-transfer is coupled to cluster protonation (Chen et al., 2000; Armstrong, 2002) schematically represented as:

$$[Fe_3S_4]^+ \underset{-e}{\overset{+e}{\rightleftarrows}} [Fe_3S_4]^0 \underset{-H^+}{\overset{+H^+}{\rightleftarrows}} [Fe_3S_4]^0{-}H^+$$

Concerned with the redox potentials of the $[Fe_4S_4]^{2+/+}$ and $[Fe_3S_4]^{+/0}$ transitions of the different mutants, under the same pH values they are generally substantially similar to those of the native protein. A significant difference holds by replacing the Cys20 of the $[4Fe\text{-}4S]^{2+}$ cluster for Ala. In fact, at pH 7.8 the $[4Fe\text{-}4S]^{2+/+}$ process is −0.648 V for the native protein and −0.75 V for the Cys20Ala mutant. In contrast in the case of Cys20Ser, the redox potential of the $[4Fe\text{-}4S]^{2+/+}$ process is substantially similar to that of the native protein. Such different effects can be explained by taking into account that both cysteine and serine are hydrophilic residues thus favoring the electron exchange, whereas the hydrophobic alanine disfavors the electron exchange. Also in the case of Cys42Asp no significant difference in the $[4Fe\text{-}4S]^{2+/+}$ redox potential occurs with respect to the native protein in that the asparagine residue is hydrophilic.

Let us now pass to the 7-Fe ferredoxin expressed by *Bacillus schlegelii* [formerly *Hydrogenobacillus schlegelii*, a thermophilic facultatively anaerobic and chemolithotrophic bacterium able to oxidize hydrogen, first isolated from the surface layer of the sediment of a small eutrophic lake near Neuchatel, Switzerland, that grows optimally at 55 °C (Kämpfer et al., 2013)]) (hereafter *Bs*Fd). Its structure (determined in solution by NMR spectroscopy) is available (Aono et al., 1998). The redox potentials of the pertinent $[3Fe\text{-}4S]^{+/0}$ and $[4Fe\text{-}4S]^{2+/+}$ processes are reported in Table 2 also in comparison with those of *Av*FdI and *Aa*Fd (*Acidianus ambivalens* 7-Fe ferredoxin, which will be below discussed) under the same experimental conditions.

As shown, both the iron-sulfur clusters of *Bs*Fd reduce easier by about 100 mV with respect to those of *Av*FdI. Since the amino acid residues surrounding the two iron-sulfur clusters of *Bs*Fd and *Av*FdI possess substantially the same physicochemical properties (charge, polarity, hydrophobicity) one cannot invoke different coulombic effects or water penetrability, so that it is possible that the relatively high stability of the oxidized states of the two iron-sulfur clusters of *Av*FdI might arise from the presence of 31 backbone hydrogen bonds in *Av*FdI with respect to 29 in *Bs*Fd (Aono et al., 1998). Given that the pH dependence of the redox potentials of the $[3Fe\text{-}4S]^{+/0}$ passage of *Bs*Fd and *Aa*Fd is quite similar to that of *Av*FdI (Bentrop et al., 2000; Battistuzzi et al., 2000), it is possible that the easier $[3Fe\text{-}4S]^{+/0}$ reduction of *Bs*Fd with respect to *Av*FdI might depend not so much on pH dependence as on electrostatic effects in that in *Bs*Fd the $[3Fe\text{-}4S]^+$ cluster forms a preferential hydrogen bond with the positively charged (polar and hydrophilic) Lys12, whereas in *Av*FdI it forms a preferential hydrogen bond with the non-charged (polar and hydrophilic) Thr14.

A further structurally resolved 7-Fe ferredoxin is expressed by *Thermus thermophilus* [also known as *Thermus aquaticus*, a thermophilic bacterium that has a maximum temperature for growth of 79 °C (Brock and Freeze, 1969; Oshima and Inahori, 1974)] (hereafter *Tt*Fd), whose crystal structure is available (Macedo-Ribeiro et al., 2001).

A direct comparison of the redox potentials of the $[3Fe\text{-}4S]^{+/0}$ and $[4Fe\text{-}4S]^{2+/+}$ processes of *Tt*Fd with *Av*FdI and *Bs*Fd under the same experimental conditions is not possible. In fact at moment the only pertinent investigation on *Tt*Fd has been carried out by potentiometric titration (monitored by EPR spectroscopy) at pH 9.0, that assignes potential values of −0.25 V and −0.53 V to the sequential $[3Fe\text{-}4S]^{+/0}$ and $[4Fe\text{-}4S]^{2+/+}$ reductions, respectively (V, vs. NHE) (Ohnishi et al., 1980; Hille et al., 1983).

Finally we look at the 7-Fe ferredoxin expressed by *Acidianus ambivalens* [formerly *Desulfurolobus ambivalens*, a thermoacidophilic archaebacterium that grows optimally at 80 °C and pH between 1 and 3.5 (Zillig et al., 1985; Fuchs et al., 1996)] in the Zn^{2+} containing form (hereafter *Da*Fd),[3] whose crystal structure is available (Frazão et al.,

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[2] Taking into account that the reduction potential of dithionite is conditioned either by pH and dithionite itself concentration, a value of about −0.5 V (vs. NHE) is commonly estimated in the pH range from 7 to 9 (Mayhew, 1978).

[3] *Acidianus ambivalens* also expresses a spectroscopically and electrochemically characterized non-Zn containing 7-Fe ferredoxin (Janssen et al., 2001).