ELSEVIER

Coordination Chemistry Reviews
190–192 (1999) 1049–1066

COORDINATION CHEMISTRY REVIEWS

www.elsevier.com/locate/ccr

Protein-bound iron–sulfur centers. Form, function, and assembly

Shumin Bian, J.A. Cowan *

Evans Laboratory of Chemistry, The Ohio State University, 100 West 18th Avenue, Columbus, OH 43210, USA

Contents

* Corresponding author. Tel.: + 1-614-292-2703; fax: + 1-614-292-1685.
E-mail address: cowan.2@osu.edu (J.A. Cowan)

PII: S0010-8545(99)00157-5

Abstract

This review presents a historical perspective of Fe–S cluster biochemistry, ranging from the first identification of the Fe–S core, through the elucidation of their biological diversity of structure and function, to more recent considerations of the pathways of assembly and disassembly. The latter in particular is discussed in the context of biological utility in the regulation of genetic information.

Keywords: Iron–sulfur centers; Fe–S cluster biochemistry; Cluster stability; Assembly/disassembly

1. Introduction

1.1. Historical background

Iron–sulfur proteins are a family of metalloproteins containing iron that is at least partially coordinated by sulfur. They were not recognized as a distinct class from other non-heme iron-containing proteins until the early 1960s, when analytical techniques were first applied to these proteins. In a novel application of EPR spectroscopy to the study of mitochondrial membranes, biochemist H. Beinert at the University of Wisconsin and physicist R. Sands at the University of Michigan made the first discovery of what has now become a hallmark signature of iron–sulfur clusters, 'the $g = 1.94$ signal' [1–3]. While the nature of this signal and the identity of the [2Fe–2S] cluster were being discussed in the following decade, other iron–sulfur clusters were found in a variety of species [4]. In 1962, Mortenson and coworkers [5] reported a low molecular weight iron-containing protein from the bacterium *Clostridium pasteurianum* that was shown to be essential for nitrogen fixation. They named the protein ferredoxin (Fd) because of its iron content and role in redox reactions. In 1965 Lovenberg and Sobel [6] observed a red protein while isolating Fd from *C. pasteurianum*. They found it to be a low molecular weight iron protein (~ 6 kDa) that could substitute for Fd as an electron carrier in several reactions mediated by extracts of *C. pasteurianum*. They named it rubredoxin (Rd). By 1967, about a dozen ferredoxins and other iron–sulfur enzymes had been identified [7].

After extensive studies by a variety of chemical methods and physical techniques (electron paramagnetic resonance (EPR), Mössbauer, extended X-ray absorption fine Structure (EXAFS), X-ray crystallography, Raman spectroscopy, and nuclear magnetic resonance (NMR)), it became evident that these and many other proteins containing iron and sulfur represented a new class of protein, the iron–sulfur proteins. All members of this class have one or more Fe atoms per molecule, and all except the Rds and desulforedoxin (Dx) subclasses have 'acid-labile' or inorganic sulfide also. Methods for the analytical determination of iron [8–10] and sulfide [11–15] are technically demanding and are associated with large errors. However, averaged results from many measurements, together with spectroscopic and/or

structural determinations have established the chemical composition of the prosthetic center in iron–sulfur proteins to be distinct from other nonheme iron proteins. Currently there are over 100 characterized examples of iron–sulfur proteins [16], and the number is rising steadily as novel iron–sulfur cluster centers continue to be discovered, often as a result of the observation of novel spectroscopic signatures in biological systems. Increasingly, DNA sequence information is providing clues to new Fe–S proteins.

1.2. Diverse architecture of Fe–S clusters

As more complicated types of iron–sulfur cluster have been identified, the classification of Fe–S proteins has become increasingly difficult. Currently, iron–sulfur proteins are classified into several main categories according to the composition of the Fe–S center and the ancillary ligands [16]. Table 1 lists some of the common types of Fe–S cluster, the names of characteristic proteins, and typical ranges for their midpoint reduction potentials. Most of the listed references derive from structural determinations of Fe–S clusters. The reduction potential E_m of a particular class of cluster typically varies from species to species.

The most common classes of iron–sulfur clusters are summarized in Table 1 [17]. The rubredoxin-type cluster has only one central iron coordinated to four cysteinyl thiolates. There is no acid-labile sulfide. It is still considered an iron–sulfur cluster because of the coordination by cysteinyl sulfur. A variant of the [2Fe–2S] cluster ferredoxins is the Rieske protein [18], named after Professor J.S. Rieske of The Ohio State University [19]. Rieske proteins contain unique [2Fe–2S] clusters with two histidine nitrogen ligands replacing the terminal cysteines of one iron [20]. They

Table 1
Classification of Fe–S clusters

Type	Protein	E_m range (mV)	Protein ligands	Selected Ref.
$[Fe]^{3+/2+}$	Rubredoxins	+20 to −60	4 Cys	[174]
$[2Fe–2S]^{2+/1+}$	Ferredoxins	−240 to −460	4 Cys	[175]
$[2Fe–2S]^{2+/1+}$	Rieske proteins	+300 to −155	2 Cys, 2 His	[20]
$[3Fe–4S]^{1+/0}$	Ferredoxins, IRP	−50 to −420	3 Cys	[21]
$[4Fe–4S]^{2+/1+}$	Ferredoxins	0 to −645	4 Cys	[176]
$[4Fe–4S]^{3+/2+}$	HiPIP	50 to 450	4 Cys	[177]
H-cluster [4Fe–4S]–[2Fe–2S]	Hydrogenase	[a]	4 Cys, $CO_x/(CN^-)_y$	[30]
P-cluster [8Fe–7S]	Nitrogenase	[a]	6 Cys, 1 Ser	[34]
Fe–Mo cofactor [7Fe–Mo-8S]	Mo Nitrogenase	[a]	1 Cys, 1 His, homocitrate	[178]
Fe–V cofactor[b]	V nitrogenase	[a]	[b]	[36]

[a] These potentials have not been accurately determined but are likely to be negative.
[b] Presumed similar to Mo nitrogenase with V taking the place of Mo.

were first isolated from mitochondrial complex III, but similar proteins are also found in other types of membrane-bound electron-transfer chains. The [3Fe–4S] ferredoxins have been found in several bacterial strains, for example, *Desulfovibrio gigas* [21] and *S. griseolus* [22], while two classes of [4Fe–4S] clusters have been characterized: the low potential ferredoxins with a negative E_m, and the high potential iron proteins (high potential iron–sulfur proteins (HiPIPs)) with a positive E_m [23].

Complexity is introduced by increasing the size of the cluster. The occurrence of [6Fe–6S] clusters has been proposed; especially in proteins derived from *Desulfovibrio* organisms [24]. However, the evidence for such a center has been shown to be flawed in one instance [25], while the determination of a 1.7 Å crystal structure [26] established that the putative [6Fe–6S] protein from *Desulfovibrio vulgaris* (Hildenborough) rather contains two 4Fe clusters separated by 12 Å. One cluster is a conventional [4Fe–4S] center, although the cysteines are arranged in an unusual fashion (Cys 3, 6, 15, 21). The second cluster is a novel 4Fe structure with two μ_2–sulfido bridges, two μ^2-oxo bridges, and a partially occupied, unidentified μ_2 bridging ligand X. Resonance Raman spectroscopy suggests that the bridging ligand X is a solvent-exchangeable oxygen. The H-clusters in Fe hydrogenase of *C. pasteurianum* and *D. gigas* have also been proposed to contain a [6Fe–6S] cluster [27–29], but again recent crystallographic data indicate an unusual configuration of a [4Fe–4S] center bridged to a [2Fe–2S] cluster, the latter showing coordination of CO and CN^- ligands (Table 1) [30].

The P-cluster in the nitrogenase enzymes isolated from several bacterial strains comprise eight iron and seven labile sulfide atoms [31–33]. Further complexity exists for the so-called mixed-type clusters in which Fe–S clusters are complexed with other cofactors in an enzyme. Both the molybdenum–iron protein in nitrogenase and the 'alternative' vanadium–iron nitrogenase have a mixed-metal cluster. In the Mo nitrogenase, the Fe–Mo cofactor has a composition of Fe_7MoS_9 [34,35], while in the V nitrogenase the composition is $Fe_{5-7}VS_{4-6}$ [36]. There is also an iron-only nitrogenase [37], which also contains a P-cluster like the other two types of nitrogenase enzymes. In carbon monoxide dehydrogenase, an unusual Ni–Fe cluster has also been identified [38–40].

The diversity of Fe–S centers is manifest in one other aspect; namely, that iron–sulfur proteins often contain several cofactors of the same or different type. These centers work together in a concerted manner to accomplish a physiologically relevant reaction. The reaction mechanisms for most of these complex systems have yet to be elucidated. The well-known 8Fe ferredoxin in *C. pasteurianum* has two [4Fe–4S] clusters [41]. The *Azotobacter vinelandii* 7Fe ferredoxin has one [3Fe–4S] cluster and one [4Fe–4S] cluster [42]. Most Ni–Fe hydrogenases contain from two to four [4Fe–4S] clusters and a [2Fe–2S] cluster [43–45]. In hydrogen-utilizing bacteria, such as *A. eutrophus*, NiFe hydrogenase requires one more flavin cofactor FMN [46], and in the methanogenic bacterium *M. thermoautotrophicum*, an FAD cofactor [47]. Flavin cofactors are present in many other Fe–S proteins, such as trimethylamine dehydrogenase in the bacterium W3A1, which contains a [4Fe–4S] cluster [48]; and also xanthine oxidase in milk which has 2 × [2Fe–2S] clusters and

a Mo-pterin cofactor [49]. One of the few well-studied complex systems is sulfite reductase from the sulfate-reducing bacteria *D. vulgaris*, and from *Escherichia coli*. Both assimilatory and dissimilatory sulfite reductases, and also some nitrite reductases, contain one [4Fe–4S] cluster bridged with a siroheme [50,51]. The mechanism for the six-electron reduction from sulfite to sulfide has been elucidated [52].

1.3. Diverse functional roles for biological Fe–S clusters

A functional role for iron–sulfur proteins was first recognized in ferredoxins as electron transfer. These proteins are essential to many of the fundamental processes of life: including photosynthesis, respiration, and nitrogen fixation. Now the functions of iron–sulfur proteins have expanded to include the following categories: (1) catalysis (redox or nonredox); (2) stabilization of protein structure; (3) regulation of metabolic pathways and (4) biological sensors of iron, O_2, and O_2^-. There also exists a number of iron–sulfur proteins with unknown functions [16,53].

1.3.1. Ferredoxins

Ferredoxins are the main group of iron–sulfur proteins used for electron transfer. Rubredoxins (Rds) are the simplest, with the Fe-center ligated entirely by cysteinyl sulfurs. One of the few known functions of this protein is as an intermediate electron carrier in ω-hydroxylation of fatty acids, for which a unique rubredoxin with two rubredoxin domains is used [54]. The [2Fe–2S] ferredoxins were identified as photosynthetic electron transfer proteins in chloroplasts of plants, such as spinach, and in cyanobacteria. The chloroplast [2Fe–2S] ferredoxins act as electron donors to enzymes such as nitrite reductase and glutamate synthase [55]. [2Fe–2S] ferredoxins have also been found in kidney, adrenal glands and other animal tissues, acting as electron donor to cytochrome P-450. Like many other eukaryotic proteins, adrenal ferredoxin undergoes phosphorylation which modulates its activity [56,57]. [3Fe–4S] ferredoxins were found in *S. griseolus*, with only three available cysteines for ligation [22]. The [3Fe–4S] ferredoxin II of *D. gigas* was later considered to be the oxidation product of the *D. gigas* [4Fe–4S] ferredoxin I. Ferredoxins that contain 2 × [4Fe–4S] centers have almost two-fold symmetry, and may be the precursors of the [4Fe–4S], the [4Fe–4S][3Fe–4S], and the [3Fe–4S] cluster ferredoxin [58]. They are primarily found in bacteria, though a similar 2 × [4Fe–4S] ferredoxin has been isolated from *Entamoeba histolytica* [59]. Other organisms use the 2 × [4Fe–4S] ferredoxins for a distinct purpose; namely, as low-potential electron carriers in anaerobic metabolism of *C. pasteurianum* [41], for example.

HiPIPs are a class of ferredoxins found in purple photosynthetic bacteria and contain a cubane [4Fe–4S] cluster [60–63]. They have been isolated from many species such as the photosynthetic purple sulfur bacteria *Chromatium vinosum* [64], the photosynthetic purple bacteria *Rhodopseudomonas gelatinosa* [65] and *Rhodoferax fermentans* [66], and denitrifying bacterium *Micrococcus* species [67]. HiPIPs are peculiar among other iron–sulfur proteins as a result of the high redox potential at which the cluster exchanges electrons (from + 50 to + 450 mV) [62]. A much

larger number of ferredoxins show a lower redox potential, ranging from −250 to −650 mV [68]. Although the chemical and biophysical properties of HiPIPs are well defined [69,70], their biological functional role is still uncertain. Their function as electron transfer proteins in photosynthetic reactions has been suggested [66,71], but direct evidence for this as an in vivo activity is still needed.

C. vinosum HiPIP has been the subject of extensive characterization. It is a relatively small soluble protein with a molecular weight of ~9.6 kDa. The amino acid sequence of *C. vinosum* HiPIP is known [72]. The four cysteine residues are all coordinated to the cluster and the three-dimensional structure has been determined by X-ray crystallography [72–74]. An interesting feature of the [4Fe–4S] cluster is that it is surrounded by aromatic residues: Tyr19, Phe48, Phe66, Trp60, Trp76, and Trp 80. The closest point of approach of the cluster to the solvent appears to be two of the inorganic sulfur atoms, which are about 4.5 Å from the surface. Recently, three-dimensional solution structures of both reduced [75,76] and oxidized HiPIP [77] have been determined by NMR. There is no significant deviation between the solid state and solution structures.

Other physiochemical studies on *C. vinosum* HiPIP have provided additional information on the [4Fe–4S] cluster. EPR spectroscopy has been the most versatile method for characterizing iron–sulfur proteins [78,79]. Different types of iron–sulfur clusters, or different oxidation states of the same cluster, usually give rise to distinct EPR signals [80]. The oxidized cluster $[4Fe–4S]^{3+}$ has a ground spin state of $S = 1/2$, and is therefore paramagnetic and EPR active. The EPR spectrum of *C. vinosum* HiPIP gives a characteristic and mostly axial signal with $g_u = 2.11$, and $g_\perp = 2.03$. Minor components at around $g = 2.08$ have been interpreted to arise from possible formation of a HiPIP dimer at high concentrations [23]. Reduced HiPIP has a ground spin state $S = 0$, and is EPR silent. However, residual paramagnetism in the reduced protein has been detected by nuclear magnetic resonance (NMR) spectroscopy [81]. Both the reduced and oxidized protein have been extensively characterized by NMR spectroscopy [69,77,81–84].

1.3.2. Membrane-bound electron carrier proteins

The first group in this category contains membrane-bound electron-carrier Fe–S proteins that interact with quinones in the respiratory chain of mitochondria, chloroplasts and bacteria. These include hydrogenase, formate dehydrogenase, and succinate dehydrogenase [85]; and on the oxidizing side, nitrate reductase [86], and fumarate reductase. All of these proteins have a similar construction, consisting of a membrane anchor subunit or subunits that act as the binding site for quinone, and soluble subunits on the membrane surface that react with the soluble substrates. The iron–sulfur clusters, which act as intermediate electron carriers, are situated between these two components [16]. Rieske proteins with histidine-ligated [2Fe–2S] clusters have relatively high reduction potentials (0–300 mV). They were first isolated from the mitochondrial respiratory chain complex III (ubiquinol: cytochrome c reductase) and later in other types of membrane-bound electron-transfer chains. For example, they are found in aromatic dioxygenases of the bacterium *P. putida* [87,88], the cytochrome b_6f complex of chloroplasts and

cyanobacteria, and in respiratory and photosynthetic bacteria [89,90]. The second group in the membrane-bound category is the photosynthetic electron-transfer chain of plants and cyanobacteria. In photosystem I, the primary electron acceptor complex comprises a large, membrane-bound complex in which electrons from the primary donor chlorophyll are transferred through iron–sulfur clusters to ferredoxin and ultimately NADP [91,92]. One of the three iron–sulfur clusters in this complex, cluster X, is proposed to be a [4Fe–4S] cluster that is bound between the two subunits PS1A1 and PS1A2 (products of the psaA and psaB genes) [93]. The terminal electron acceptors of photosystem I are iron–sulfur clusters A and B. Both of them have a 9-kDa subunit encoded by the psaC gene, which shows a strong homology with the bacterial 2 × [4Fe–4S] ferredoxins [94].

1.3.3. Soluble electron carrier proteins with other cofactors

The iron–sulfur proteins in this category interact with other electron-carrying center such as heme or non-heme iron, molybdenum, nickel, and some organic cofactors, such as flavin, quinone, or thiamin diphosphate. In sulfite and nitrite reductases of plants and bacteria, the [4Fe–4S] cluster is bridged to a specialized chlorin prosthetic center, termed siroheme, by either a cysteinyl sulfur or inorganic sulfide [95,96]. Molybdenum-containing iron–sulfur proteins generally have multiple Fe–S clusters and a molybdopterin cofactor [49,97], with the exception of Mo nitrogenase. These include oxidoreductases such as nitrate and formate reductases [98], and the molybdenum hydroxylases, such as xanthine dehydrogenase and aldehyde oxidase [99]. The iron–sulfur clusters in nickel-containing hydrogenases serve to transfer electrons between the nickel site and donors or acceptors such as ferredoxin, NAD, deazaflavin, quinone, or cytochrome c [100,101]. Flavoproteins such as ferredoxin:NADP reductase are the intermediaries for the iron–sulfur protein to interact with NAD or NADP [102]. In monooxygenases [103] and dioxygenases [104], the iron–sulfur protein donor is either a separate ferredoxin, or is found as part of an iron–sulfur flavoprotein.

1.3.4. Iron–sulfur proteins as redox enzymes

Iron–sulfur proteins having redox catalytic functions include carbon monoxide dehydrogenases (carbon monoxide oxidoreductases), iron hydrogenases, and nitrogenases. Carbon monoxide dehydrogenases catalyze the reversible oxidation of CO to CO_2. In aerobic bacteria a molybdenum-containing iron–sulfur protein with [2Fe–2S] clusters catalyzes the oxidation of CO [105]. All three types of nitrogenase (Mo, V and Fe) have the so-called P-cluster, while the reduction of N_2 to ammonium most likely arises at the Fe–X (X = Mo, V, or Fe) cofactor site [106]. The nitrogenase reductase, or the iron protein, is the second essential component of the nitrogenase system serving as a low-potential, ATP-dependent electron donor to the dinitrogen-reducing protein [107]. It contains one [4Fe–4S] cluster that connects a dimer of two subunits. The Fe hydrogenases, which catalyze the production and consumption of hydrogen gas as in Fe–Ni hydrogenases, also contain a special iron–sulfur cluster, the H-cluster, that has recently been shown to consist of a [4Fe–4S] cluster bridged to a [2Fe–2S] core with an unusual ligand set (Table 1) [30].

1.3.5. Iron–sulfur proteins as nonredox enzymes

There is a large group of iron–sulfur proteins with nonredox functions, some of which catalyze hydration/dehydration of double bonds. The best characterized example, aconitase, belongs to the hydro-lyase family [108]. The inactive enzyme contains a [3Fe–4S] cluster that is converted to a catalytically active [4Fe–4S] cluster [109]. Other enzymes in this family, such as fumarate dehydratase (or fumarase) A and B from *E. coli* [110,111] and dihydroxy-acid dehydratase of *E. coli* [112] also contain a catalytic $[4Fe–4S]^{2+/1+}$ cluster with incomplete cysteine ligation. Spinach dihydroxy-acid dehydratase has a $[2Fe–2S]^{2+/1+}$ cluster with one non-cysteine ligand [113].

Recent studies have demonstrated some novel functions of iron–sulfur clusters. These include the translational regulatory function of the iron regulatory protein (apo cytosolic aconitase), and transcriptional control of the oxygen and/or superoxide sensory proteins SoxR and FNR (fumarate nitrate reduction) [110]. Clusters also serve structural roles in glutamine 5-phosphoribosyl-1-pyrophosphate amidotransferase and in endonuclease III [110].

2. Biological Fe–S cluster assembly and disassembly

2.1. Cluster stability

This topic has been the subject of a recent detailed review [114] and will not be discussed further here.

2.2. Structural versatility of Fe–S clusters

The ubiquity of iron–sulfur clusters in proteins and the diverse functional chemistry that they display rests in part on the interconvertibility of the cluster core. In the synthetic field, a variety of cluster types have been successfully synthesized. These include the mononuclear $[Fe(SR)_4]$, binuclear $[Fe_2S_2(SR)_4]$ and cubane $[Fe_4S_4(SPh)_4]$ clusters [115–118], the hexanuclear cluster $[Fe_6S_9(SR)_2]$ [119], and the cuboidal trinuclear cluster $[Fe_3S_4(SR)_3]$ [120]. In the early 1980s, Hagen and coworkers defined reaction sequences for assembly of $[Fe_4S_4(SR)_4]$ clusters from simple reactants, and demonstrated elaboration of a tetranuclear cluster through mononuclear and binuclear intermediates [116]. Zhou et al. in their synthetic studies have shown that the cuboidal trinuclear cluster $[Fe_3S_4(SR)_3]$ displays special versatility [121,122]. It can be converted into several other cluster types, including a linear form of the trinuclear cluster $[3Fe_4S(SR)_4]$ in the presence of thiol, a cubane cluster when thiol and Fe^{2+} are added, and a mixed-type cubane cluster $[MFe_3S_4(SR)_4]$ when a second metal such as Cu, Mn, or Cd is added.

The versatility of iron–sulfur clusters has also been recognized in biological systems. Fe–S cluster conversion and interconversion have been found in several cases in studies of cluster assembly and disassembly. Christou et al. reported the conversion of *C. pasteurianum* rubredoxin, which has a mononuclear Fe center, into

a four-iron ferredoxin [123]. A molecular variant of *C. pasteurianum* rubredoxin was shown to be able to assemble a binuclear $[2Fe–2S]^{2+}$ cluster [124]. Cluster conversion from [2Fe–2S] to [4Fe–4S] was observed in *E. coli* biotin synthase [125] when the isolated inactive protein was reduced with dithionite anaerobically to form the active protein. The reverse process, [4Fe–4S] cluster to [2Fe–2S] cluster conversion, was observed for the iron protein of *A. vinelandii* nitrogenase [126,127]. More recently, conversion of a [4Fe–4S] cluster to a [2Fe–2S] cluster fragment was observed after exposure of the FNR protein of *E. coli* to dioxygen [128]. Consistent with findings on synthetic cluster complexes, the cuboidal cluster in iron–sulfur proteins demonstrates a remarkable facility in converting to other cluster types [50,51,129,130]. In *D. gigas* ferredoxin II [131], and the enzyme aconitase [132], facile interconversion between the cubane $[4Fe–4S]^{2+}$ and the cuboidal $[3Fe–4S]^{1+}$ structures occurs. Aconitase is inactive in the 3Fe–4S state [133], but addition of a fourth iron center populates a unique substitutionally labile iron subsite where substrate binds and restores catalytic activity. Similarly, in the case of the cytosolic aconitase (the iron regulatory protein 1 (IRP1)), interconversion of the [4Fe–4S] cluster and apo-protein underpins a novel mechanism of control at the translational level [134,135].

2.3. Cluster assembly and disassembly: iron–sulfur proteins as regulatory proteins, biosensors of oxidants and iron

Interest in iron–sulfur proteins has increased in recent years as a result of the finding that they serve as sensors of iron, dioxygen, superoxide ion ($O_2^{\cdot -}$), and possibly nitric oxide [134,135]. This is clearly demonstrated in three proteins: the IRP1 [136,137], SoxR in *E. coli* [138,139] and FNR in *E. coli* [128,140,141].

One of these proteins, SoxR, is a transcriptional activator that senses superoxide and nitric oxide stress in *E. coli*. When post-translationally activated, it stimulates transcription of a second regulatory gene, soxS, by binding to its promoter DNA [142,143]. The product of the soxS gene, SoxS protein, then triggers expression of over ten genes (including the gene for SOD) that are involved in the defense against oxidative damage [144] and antibiotic resistance [145,146]. SoxR contains two [2Fe–2S] clusters, one in each monomer of this dimeric Fe–S protein [147,148]. It has been shown that SoxR Fe–S clusters are essential for promoting open-complex formation by RNA polymerase and triggering expression of the soxS gene, although they are not required for DNA binding [138]. Under reducing conditions, as purified using thiol-containing buffer, the Fe–S cluster undergoes disassembly to form apoprotein with loss of activity. However, full transcriptional activity of SoxR can be restored by in vitro assembly of protein-bound iron–sulfur clusters, either noncatalytically or enzymatically with NifS [149].

IRP1 is another example where cluster assembly and disassembly provide the signals for activation of a regulatory mechanism, in this case responding to the bioavailability of iron and to oxidative stress [135]. The control of intracellular iron levels depends critically on the function of two proteins: the transferrin receptor, which recognizes the iron-loaded transport protein transferrin and ushers it into

cells, and the storage protein ferritin, which provides for reversible and safe storage of excess iron. The concentration of these proteins is regulated at the translational level by IRP. The mRNAs for transferrin receptor and for ferritin contain so-called iron-responsive elements (IREs). These IREs bind with high affinity to IRP, which is found in all higher forms of life from mollusks to insects and vertebrates. The active form of IRP does not have an iron–sulfur cluster. In fact, the active IRP1 is the apo form of the iron–sulfur protein cytosolic aconitase [150–152]. IRP is activated during periods of iron deficiency, and as a result of cytosolic aconitase losing its cluster. IRP is inactivated when the available iron levels for complexation is plentiful, giving rise to the reconstruction of the [4Fe–4S] cluster and activation of aconitase. At low iron levels, IRP binds to the IRE in the 5′ region of ferritin mRNA, resulting in the blockage of protein translation. Active IRP also binds to the IREs in the 3′ region of tranferrin receptor mRNA, resulting in protection of the mRNA from being degraded by nucleases. Thus, the interplay of the [4Fe–4S] cluster assembly and disassembly pathways regulates the levels of transferrin receptor and ferritin in a complementary sense, supporting either the uptake or storage of iron.

3. Mechanisms of Fe–S cluster assembly and disassembly

3.1. Biological examples of Fe–S cluster assembly and disassembly

Both assembly and disassembly of iron–sulfur clusters is fundamental to the function of many biological iron–sulfur proteins. Transcriptional regulation of iron and/or oxygen sensing properties, are found to be closely related to the disassembly and reconstitution of iron–sulfur clusters. Iron–sulfur proteins have been recognized as one of natures modular units [135], and cluster interconversion represents a powerful mechanism for a diverse array of organisms to deal with external stress.

A number of iron–sulfur proteins experience iron–sulfur cluster disassembly under oxidative stress. A group of enzymes are recognized to be involved in the homolytic cleavage of C–C and C–H bonds by radical (5′-deoxyadenosyl) mechanisms that require *S*-adenosylmethionine. These include biotin synthase [125,153], pyruvate formate lyase activating enzyme [18,154], and lysine 2,3-aminomutase [155,156]. A $[4Fe–4S]^{2+/1+}$ cluster that bridges protein subunits in the active enzyme, but can undergo oxidative degradation to form $[2Fe–2S]^{2+}$ clusters is likely to be a common feature of these proteins. It is further suggested that such oxidative cluster conversion to $[2Fe–2S]^{2+}$ centers may play a physiological role by providing a method of regulating enzyme activity in response to oxidative stress, without irreversible cluster degradation [125].

A similar process has been found in the FNR protein of *E. coli*. *E. coli* FNR functions as a transcriptional regulator for a set of genes that provide alternative pathways for energy generation when dioxygen becomes scarce [140]. A [4Fe–4S] cluster contained in FNR mediates the sensitivity of this transcription factor to oxygen, thus, limiting FNR activity to anaerobic conditions. Active FNR with an

integrated [4Fe–4S] cluster tends to dimerize and shows specific DNA binding activity [141]. Upon exposure to dioxygen the [4Fe–4S] cluster disassembles to form a [2Fe–2S] cluster in about 60% yield, with accompanying loss of the DNA-binding ability. It has been further demonstrated that DNA-binding and the absorption spectrum characteristic of a $[4Fe–4S]^{2+}$ cluster could be largely restored from the $[2Fe–2S]^{2+}$ form after addition of Cys, Fe, DTT, and the NifS protein. This finding led to the suggestion that the form of FNR containing the $[2Fe–2S]^{2+}$ cluster may be an in vivo intermediate that is more rapidly converted to the active form than the apoprotein [128].

An interesting example of cluster disassembly is IRP1. Cellular iron homeostasis is modulated and maintained through changes in the synthesis of proteins involved in the uptake (transferrin receptor), storage (ferritin), and utilization (erythroid 5-aminolevulinate synthase or eALAS and other Fe proteins) of this essential mineral. It has been established that synthesis of these proteins is post-transcriptionally regulated through the action of cytosolic IRP1 [136,150–152,157,158]. IRP1 is the apo form of cytoplasmic aconitase. When iron is abundant, aconitase has an intact [4Fe–4S] cluster and catalytic activity. When the iron level is low, the [4Fe–4S] cluster disassembles, which is accompanied by structural changes in the protein [134]. The apo form of IRP1 then binds to IREs that are stem-loop structures present in the 5′ untranslated region (UTR) of ferritin, and eALAS mRNAs, and in the 3′ UTR of transferrin mRNA. Formation of the IRP–IRE complex results in the repression of translation of ferritin as well as the simultaneous stabilization of transferrin receptor mRNA and consequent increase in iron loaded transferrin intake. Thus, disassembly (and assembly) of the iron–sulfur cluster is the regulatory step [137].

Many other iron sulfur proteins exhibit oxidative disassembly of their [4Fe–4S] cluster, for example, *Desulfovibrio africanus* ferredoxin III [159], mammalian aconitase [160,161], and the hydro-lyase class in *E. coli* (dihydroxy-acid dehydratase, fumarase A, fumarase B). Unlike superoxide inactivation in aconitase and the 8Fe ferredoxin III, which leads to formation of [3Fe–4S] clusters, oxidative degradation appears to lead to a complete breakdown of the Fe–S clusters in the hydro-lyase class [53,112,160,161].

While there is mounting evidence that Fe–S cluster disassembly plays an important role in the function of Fe–S proteins, the mechanism and pathways of Fe–S cluster disassembly, and the correlation between protein conformational change and cluster disassembly are areas requiring further exploration.

3.2. Reaction pathways for Fe–S cluster assembly

3.2.1. In vivo

As stated previously, the correct assembly of iron–sulfur clusters is a requirement for the normal biological function of many iron–sulfur proteins. In turn, these proteins display a remarkable diversity of functional chemistry: including electron-transfer [55,135], catalysis [53,162,163] stabilization of protein structure [110], and sensors of redox state [138,139]. The mechanism of assembly of the Fe–S prosthetic center is also of functional relevance in iron regulatory proteins [136].

Assembly of 4Fe–4S clusters in vivo involves multiple protein components working together in ways that are not fully understood. The NifS protein has been identified as a sulfur transferase, involved in Fe–S cluster formation and the biological function of nitrogenase systems. Other gene products such as NifU have also been implicated in cluster assembly [164–166]. Surprisingly, it has been discovered that NifS- and NifU-like proteins exist throughout a broad realm of living systems, from *E. coli*, *A. vinelandii*, to yeast and mammals [161,167]. Beside the NifSU system, a gene cluster known as iscSUA-hscBA-fdx has been identified in *A. vinelandii* [166] which produces multiple enzymes that apparently function in iron–sulfur cluster assembly (either in formation or repair). IscS has the same L-cysteine desulfurization activity as NifS, and iscU is a homologue of nifU, whose product NifU is involved in iron mobilization for nitrogenase-specific iron–sulfur cluster formation. Similar to the nif system, a cysE-like sequence (which produces *O*-acetylserine synthase; the catalyst for cysteine biosynthesis) is found directly upstream of the isc (= iron sulfur cluster) gene, and the hscA-like gene (produces heat-shock chaperonins) is located downstream of the iscSUA gene. It has been proposed that this complex system is involved in Fe–S cluster assembly in vivo for proteins other than the nif system.

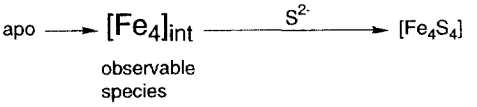
In vitro iron–sulfur protein reconstitution with sulfur transferases has been carried out in the presence of externally added iron and reducing agents such as DTT [168,169], or β-mercaptoethanol [170]. Rhodanese and NifS are the primary choice for providing sulfide [149,165,171]. A regulatory function has been proposed for rhodanese in the aerobic energy metabolism of liver cells and of *E. coli* by interaction with Fe–S clusters of the electron transport chain [172].

Although studies of in vivo iron–sulfur cluster assembly have been advanced by the rapidly developing tools of molecular biology, the molecular level understanding of the reaction pathways, and structural characterization of reaction intermediates in cluster assembly has been severely limited by the complexity of the system. Crude extracts have typically been used since it is apparently difficult to study iron–sulfur cluster assembly in vitro with all of the necessary biological components in place [53,160,161]. Several key proteins and enzymes may yet have eluded detection and characterization, especially with regard to iron transport enzymes and proteins. Even in vitro enzymatic reconstitution studies have proven difficult to follow; especially with regard to identification of cluster intermediates by biophysical methods. These facts have provided motivation for studies of iron–sulfur cluster assembly in vitro using the well characterized *C. vinosum* HiPIP as a protein model.

3.2.2. In vitro

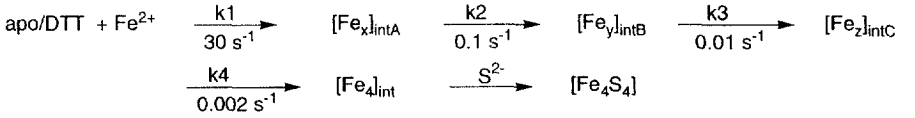
Cluster assembly in low molecular weight cluster proteins is an interesting phenomenon inasmuch as cluster assembly is intimately related to protein folding. In fact the early stages of an iron–sulfur cluster assembly pathway has been evaluated for native *C. vinosum* HiPIP during measurements to monitor backbone folding by use of ^{1}H–^{15}N heteronuclear single quantum coherence (HSQC) and matrix assisted laser desorption ionization time of flight (MALDI-TOF) mass spectrometry experiments [173]. These studies suggested that in the absence of

inorganic sulfide, the $[Fe_4S_4]$ cluster might initially assemble through a key structural intermediate that already possessed to a large extent the secondary and tertiary structural elements associated with the native protein (Scheme 1). Subsequent addition of inorganic sulfide generated the holocluster. Such a pathway might be relevant to biosynthetic cluster assembly.



Scheme 1.

This reaction pathway has also been examined kinetically using intrinsic protein fluorescence [173]. It has been shown that both native and apo protein, and other reaction intermediates show distinct emission characteristics that allow the kinetic profile for HiPIP cluster assembly to be evaluated by time-resolved fluorescence methods. Binding of iron to apo-HiPIP (probably to one or more cysteine residues) resulted in an initial rapid quenching of Trp fluorescence, and further reduction in the fluorescence intensity most likely reflected the binding of additional iron centers, and intramolecular quenching mechanisms arising from structural changes as protein residues begin to form a more compact structure. A subsequent increase in fluorescence intensity is attributed to the exclusion of water molecules as the cluster intermediate develops and the protein folds around it, thereby establishing the tertiary structure. The kinetics of intermediate formation measured by these fluorescence experiments (Scheme 2), is consistent with the folding pathway to intermediate **I** previously characterized by NMR and mass spectrometric measurements [173]. Such studies have allowed identification of several kinetic intermediates, and provide welcome insight on this important biological problem.



Scheme 2.

4. Overview

In summary, this review has attempted to detail some of the historical background to Fe–S proteins, to describe the breadth of the field and the multifunctional uses of the Fe–S cluster prosthetic center, and finally to highlight some exciting new perspectives in the area of biological assembly/disassembly reactions.

Acknowledgements

Supported by the National Science Foundation (CHE-9706904) and by a grant from the Petroleum Research Fund, administered by the American Chemical Society. J.A.C. is a Camille Dreyfus Teacher-Scholar (1994–99).

References

[1] H. Beinert, R.H. Sands, Biochem. Biophys. Res. Commun. 3 (1960) 41.
[2] H. Beinert, Iron-Sulfur Proteins, in: W. Lovenberg (Ed.), Academic Press, NY, 1 (1973), 1–36.
[3] R.H. Sands, H. Beinert, Biochem. Biophys. Res. Commun. 3 (1960) 47.
[4] L.H. Jensen, Pap. Int. Symp. Front. Iron–Sulfur Protein Res., Japan Sci. Press, Tokyo (1987), pp. 3–21.
[5] L.E. Mortenson, R.C. Valentine, J.E. Carnahan, Biochem. Biophys. Res. Commun. 7 (1962) 448.
[6] W. Lovenberg, B.E. Sobel, Proc. Natl. Acad. Sci. USA 54 (1965) 193.
[7] R. Malkin, J.C. Rabinowitz, Annu. Rev. Biochem. 36 (1967) 113.
[8] L.L. Stookey, Anal. Chem. 42 (7) (1970) 779.
[9] W.W. Fish, Methods Enzymol. 158 (1988) 357.
[10] H. Beinert, Methods in Enzymol. L IV (1978) 435.
[11] L.M. Siegel, Anal. Biochem. 11 (1965) 126.
[12] J.-S. Chen, H.L.E. Mortenson, Anal. Biochem. 79 (1977) 157.
[13] H. Sakurai, L. Stephen, P.A. San, Anal. Biochem. 119 (1982) 372.
[14] J. Beinert, Anal. Biochem. 131 (1983) 373.
[15] J.-M. Moulis, M. Jacques, Bichemistry 21 (1982) 4762.
[16] R. Cammack, Adv. Inorg. Chem. 38 (1992) 281.
[17] H. Beinert, FASEB 4 (1990) 2484.
[18] J. Knappe, G. Sawers, FEMS Microbiol. Rev. 75 (1990) 303.
[19] J.S. Rieske, R.E. Hansen, W.S. Zaugg, J. Biol. Chem. 239 (1964) 3017.
[20] R.J. Gurbriel, C.J. Batie, M. Sivaraja, A.E. True, J.A. Fee, B.M. Hoffman, D.P. Ballou, Biochemistry 28 (1989) 4861.
[21] C.R. Kissinger, L.C. Sieker, E.T. Adman, L.H. Jensen, J. Mol. Biol. 219 (1991) 693.
[22] D.P. O'Keefe, K.J. Gibson, M.H. Emptage, R. Lenstra, J.A. Romeesser, P.J. Litle, C.A. Omer, Biochemistry 30 (1991) 447.
[23] W.R. Dunham, W.R. Hagen, J.A. Fee, R.H. Sands, J.B. Dunbar, C. Humblet, Biochim. Biophys. Acta 1079 (1991) 253.
[24] W.R. Hagen, A.J. Pierik, C. Veeger, J. Chem. Soc. Faraday Trans. 85 (1989) 4083.
[25] B.M. Wolfe, S.M. Lui, J.A. Cowan, Eur. J. Biochem. 223 (1994) 79–89.
[26] F. Arendsen, J. Hadden, G. Card, A.S. Mcalpine, S. Bailey, V. Zaitsev, E.H.M. Duke, P.F. Lindley, M. Krockel, A.X. Trautwein, M.C. Feiters, J.M. Charnock, C.D. Garner, S.J. Marritt, A.J. Thomson, I.M. Kooter, M.K. Johnson, W.A.M. Van Den Berg, W.M.A.M. Van Dongen, W.R. Hagen, J. Biol. Inorg. Chem. 3 (1998) 81.
[27] M.W.W. Adams, E. Eccleston, J.B. Howard, Proc. Natl. Acad. Sci. U.S.A. 86 (1989) 4932.
[28] M.W.W. Adams, Biochim. Biophys. Acta 1020 (1990) 115.
[29] C. Van Dijk, S.G. Mayhew, H.J. Grande, C. Veeger, Eur. J. Biochem. 102 (1979) 317.
[30] J.W. Peters, W.N. Lanziloth, B.J. Lemon, B.J. Seefeldt, Nature 282 (1998) 1853–1858.
[31] J.T. Bolin, A.E. Ronco, L.E. Mortenson, T.V. Morgan, M. Williamson, N.H. Xuong, Proceedings on the Eighth International Congress, Nitrogen Fixation: Achievements and Objectives, Chapman and Hall, NY (1990), 117–124.
[32] B.K. Burgess, Chem. Rev. 90 (1990) 1377.
[33] B.R. Crane, M.L. Siegel, E.D. Getzoff, Science 270 (1995) 59.

[34] J.W. Peters, M.H.B. Stowell, S.M. Soltis, M.G. Finnegan, M.K. Johnson, D.C. Rees, Biochemistry 36 (1997) 1181.
[35] M.K. Chan, J. Kim, D.C. Rees, Science 260 (1993) 792.
[36] R.R. Eady, R.L. Robson, T.H. Richardson, R.W. Miller, M. Howkins, Biochemistry 244 (1987) 197.
[37] J.R. Chisnell, R. Premakumar, P.E. Bishop, J. Bacteriol. 170 (1988) 27.
[38] D.R. Abbanat, J.G. Ferry, J. Bacteriol. 172 (1990) 7145.
[39] S.W. Ragsdale, M. Kumar, Chem. Rev. 96 (1996) 2515–2539.
[40] S.W. Ragsdale, J.R. Bauer, C.M. Gorst, S.R. Harder, W.P. Lu, D.L. Roberts, J.A. Runquist, I. Schiau, FEMS Microbiol. Rev. 87 (1990) 397.
[41] M.G. Graves, G.T. Mullenbach, J.C. Robinowitz, Proc. Natl. Acad. Sci. USA 82 (1985) 6153.
[42] M.K. Johnson, D.E. Bennett, J.A. Fee, W.V. Sweeney, Biochim. Biophys. Acta 911 (1987) 81.
[43] R. Cammack, D.S. Patil, E.C. Hatchikian, V.M. Fernandez, Biochim. Biophys. Acta 912 (1987) 98.
[44] J.N. Reeve, G.S. Beckler, D.S. Cram, P.T. Hamilton, J.W. Brown, J.A. Krzycki, A.F. Kolodziej, L. Alex, W.H. Orme-Johnson, C.T. Walsh, Proc. Natl. Acad. Sci. USA 86 (1989) 3031.
[45] B.H. Huynh, D.S. Patil, I. Moura, M. Teixeira, J.J.G. Moura, D.V. DerVartanian, M.H. Czechowiski, B.C. Prickril, H.D. Peck, J. LeGall, J. Biol. Chem. 262 (1987) 795.
[46] K. Schneider, R. Cammack, H.G. Schlegel, Eur. J. Biochem. 142 (1984) 75.
[47] L.A. Alex, J.N. Reeve, W.H. OrmeJohnson, C.T. Walsh, Biochemistry 29 (1990) 7237.
[48] H.D. Bellamy, L.W. Lim, F. Mathews, W.R. Dunham, J. Biol. Chem. 264 (1989) 11887.
[49] R.C.Q. Bray, Rev. Biophys. 21 (1988) 299.
[50] I. Moura, J.J.G. Moura, E. Münck, V. PaPaefthymiou, J. LeGall, J. Am. Chem. Soc. 108 (1986) 349.
[51] I. Moura, A.R. Lino, J.J.G. Moura, A.V. Xavier, G. Fauque, H.D. Peck, J. LeGall, Biochem. Biophys. Res. Commun. 141 (1986) 1032.
[52] S.M. Lui, J.A. Cowan, J. Am. Chem. Soc. 116 (1994) 25.
[53] D.H. Flint, R.M. Allen, Chem. Rev. 96 (1996) 2315.
[54] E.T. Lode, M.J. Coon, J. Biol. Chem. 246 (1971) 791.
[55] D.B. Knaff, M. Hirasawa, Biochim. Biophys. Acta 93 (1991) 1056.
[56] N. Monnier, G. Defaye, E.M. Chambez, Eur. J. Biochem. 169 (1987) 147.
[57] R. Nemani, J.G. Ghazarian, B. Moorthy, N. Wongsurawat, R. Strong, H.J. Armbrecht, J. Biol. Chem. 264 (1989) 15361.
[58] K. Fukuyama, Y. Nakahara, T. Tsukihara, Y. Katsube, T. Hase, H. Matsubara, J. Mol. Biol. 199 (1988) 183.
[59] R.E. Reeves, J.D. Guthrie, P. Lobelle-Rich, Exp. Parasitol. 49 (1980) 83.
[60] T.E. Meyer, Methods Enzymol. 243 (1994) 435.
[61] J. Meyer, J. Fujinaga, J. Gaillard, M. Lutz, Biochemistry 33 (1994) 13642.
[62] T.E. Meyer, C.T. Przysiecki, J.A. Watkins, A. Bhattacharyya, R.P. Simondsen, M.A. Cusanovich, G. Tollin, Proc. Natl. Acad. Sci. USA 80 (1983) 6740.
[63] R.G. Bartsch, Methods Enzymol. 53 (1978) 329.
[64] R.G. Bartsch, Methods Enzymol. 23 (P + A) (1971) 644.
[65] H. DeKlerk, M.D. Kamen, Biochim. Biophys. Acta 112 (1966) 175.
[66] A. Hochkoeppler, S. Ciurli, G. Venturoli, D. Zannoni, FEBS Lett. 357 (1995) 70.
[67] K. Hori, J. Biochem. 50 (1961) 481.
[68] F.A. Armstrong, S.J. George, A.J. Thomson, M.G. Yates, FEBS Lett. 234 (1988) 107.
[69] I. Bertini, S. Cuirli, C. Luchinat, Struct. Bonding 83 (1995) 1.
[70] H.A. Heering, Y.B.M. Bulsink, W.R. Hagen, T.E. Meyer, Eur. J. Biochem. 232 (1995) 811.
[71] S.J. Kennel, R.G. Bartsch, M.D. Kamen, Biophys. J. 12 (1972) 882.
[72] K. Dus, S. Tedro, R.G. Bartsch, J. Biol. Chem. 248 (1973) 7318.
[73] C.W. Carter, J. Kraut, S.T. Freer, N.H. Yuong. R.A. Alden, R.G. Bartsch, J. Biol. Chem. 249 (1974a) 4212.
[74] C.W. Carter, J. Kraut, S.T. Freer, R.A. Alden, J. Biol. Chem. 249 (1974b) 6339.

[75] L. Banci, I. Bertini, L.D. Eltis, I. Felli, D.H.W. Kastrau, C. Luchinat, M. Piccioli, R. Pieratteli, M. Smith, Eur. J. Biochem. 225 (1994) 715.
[76] L. Banci, I. Bertini, A. Dikiy, D.H.W. Kastrau, C. Luchinat, P. Sompornpisut, Biochemistry 34 (1995) 206.
[77] I. Bertini, L.D. Eltis, I.C. Felli, D.H.W. Kastrau, C. Luchinat, M. Piccioli, Eur. J. 1 (1996) 598.
[78] W.R. Hagen, Adv. Inorg. Chem. 38 (1992) 165.
[79] R. Cammack, C.E. Cooper, Methods Enzymol. 227 (1993) 353.
[80] J.R. Pilbrow, G.R. Hanson, Methods Enzymol. 227 (1993) 330.
[81] I. Bertini, F. Briganti, C. Luchinat, A. Scozzafava, M. Sola, J. Am. Chem. Soc. 113 (1991) 1237.
[82] J. Gaillard, J.P. Albrand, J.M. Moulis, D.E. Wemmer, Biochemistry 31 (1992) 5632.
[83] I. Bertini, G. Gori-Savellini, C. Luchinat, J. Biol. Inorg. Chem. 2 (1997) 114.
[84] J.A. Cowan, M. Sola, Biochemistry 29 (1990) 5633.
[85] T. Ohnishi, Curr. Top. Bioenerg. (1987).
[86] M.K. Johnson, D.E. Bennett, J.E. Morningstar, M.W.W. Adams, L.E. Mortonson, J. Biol. Chem. 260 (1985) 5456.
[87] D. Kuila, J.A. Fee, J.R. Schoonover, W.H. Woodruff, C.J. Batie, D.P. Ballou, J. Am. Chem. Soc. 109 (1987) 1559.
[88] G.J. Zylstra, D.T. Gibson, J. Biol. Chem. 264 (1989) 14940.
[89] E. Davidson, F. Daldal, J. Mol. Biol. 195 (1987) 13.
[90] B.L. Trumpower, Microbiol. Rev. 54 (1990) 101.
[91] B. Lagoutte, P. Mathis, Photochem. Photobiol. 49 (1989) 833.
[92] M.C.W. Evans, G. Bredencamp, Physiol. Plant. 79 (1990) 415.
[93] J.H. Golbeck, D.A. Bryant, Curr. Top. Bioenerg. 16 (1991) 83.
[94] H. Oh-oka, S. Itoh, K. Saeki, Y. Takahashi, H. Matsubara, Plant Cell. Physiol. 32 (1991) 11.
[95] D.E. McRee, D.C. Richardson, J.S. Richardson, L.M. Siegel, J. Biol. Chem. 261 (1986) 10277.
[96] S. Ida, B. Mikami, Biochim. Biophys. 871 (1986) 167.
[97] K.V. Rajagopalan, Biochem. Soc. Trans. 13 (1985) 401.
[98] M.W.W. Adams, L.E. Mortenson, Molybd. Enzym. (1985) 519.
[99] J.C. Wootton, R.E. Nicolson, J.M.E. Cock, D.E. Walters, J.F. Burke, W.A. Doyle, R.C. Bray, Biochim. Biophys. Acta 1057 (1991) 157.
[100] G. Voordouw, Adv. Inorg. Chem. 38 (1992) 397.
[101] R. Cammack, V.M. Fernandez, K. Schneider, in: J.R. Lancaster Jr. (Ed.), The Bioinorganic Chemistry of Nickel, VCH, Vienna (1988), pp. 167–190.
[102] R. Cammack, Chem. Scr. 21 (1983) 87.
[103] A. Berg, J.-A. Gustafsson, M. Ingelman-Sundberg, K. Carlström, J. Biol. Chem. 251 (1976) 2831.
[104] C.J. Batie, D.P. Ballou, C.J. Correll (1991).
[105] O. Meyer, S. Jacobitz, B. Kruger, Microbiol. Rev. 39 (1986) 161.
[106] R.N. Pau, Trends Biochem. Sci. 14 (1989) 183.
[107] A.S. Moffat, Science 250 (1990) 1513.
[108] M.C. Kennedy, C.D. Stout, Adv. Inorg. Chem. 38 (1992).
[109] H. Beinert, M.C. Kennedy, Eur. J. Biochem. 186 (1989) 5.
[110] D.H. Flint, M.H. Emptage, J.R. Guest, Biochemistry 31 (1992) 10331.
[111] Y. Ueda, N. Yumoto, M. Tokushige, K. Fukui, H. Ohya-Nishiguchi, J. Biochem. 109 (1991) 728.
[112] D.H. Flint, J.F. Tuminello, M.H. Emptage, J. Biol. Chem. 268 (1993) 22369.
[113] D.H. Flint, M.H. Emptage, J. Biol. Chem. 263 (1988) 3558.
[114] J.A. Cowan, S.M. Lui, Adv. Inorg. Chem. 45 (1998) 313.
[115] J.M. Berg, R.H. Holm, Metal ions in Biology, in: T.G. Spiro (Ed.), Wiley, NY (1982), pp. 1–66.
[116] K.S. Hagen, J.G. Reynolds, R.H. Holm, J. Am. Chem. Soc. 103 (1981) 4054.
[117] R.H. Holm, Acc. Chem. Res. 16 (1977) 2565.
[118] D.M. Kurtz Jr., W.C. Stevens, J. Am. Chem. Soc. 106 (5) (1984) 1523.
[119] G. Christou, R.H. Holm, M. Sabat, J.A. Ibers, J. Am. Chem. Soc. 103 (20) (1981) 6269.
[120] J. Zhou, Z. Hu, E. Münck, R.H. Holm, J. Am. Chem. Soc. 118 (1996) 1966.
[121] J. Zhou, M.J. Scott, Z. Hu, G. Peng, E. Münck, R.H. Holm, J. Am. Chem. Soc. 114 (1992) 10843.
[122] H.X. Zhou, J. Biol. Inorg. Chem. 2 (1997) 109.

[123] G. Christou, B. Ridge, H.N.J.C.S. Rydon, Chem. Commun. (1979) 20.
[124] J. Meyer, J. Gagnon, J. Gaillard, M. Lutz, C. Achim, E. Münck, Y. Pétillot, C. Colangelo, R.A. Scott, Biochemistry 36 (1997) 13374.
[125] E.C. Duin, M.E. Lafferty, B.R. Crouse, R.M. Allen, I. Sanyal, D.H. Flint, M.K. Johnson, Biochemistry 36 (1997) 11811.
[126] G.L. Anderson, J.B. Howard, Biochemistry 23 (10) (1984) 2118.
[127] W. Fu, T.V. Morgan, L.E. Mortenson, M.K. Johnson, FEBS Lett. 284 (2) (1991) 165.
[128] N. Khoroshilova, C. Popescu, E. Münck, H. Beinert, P.J. Kiley, Proc. Natl. Acad. Sci. USA 94 (1997) 6087.
[129] M.G. Finnegan, R.C. Conover, J.-B. Park, Z.H. Zhou, M.W.W. Adams, M.K. Johnson, Inorg. Chem. 34 (1995) 5358.
[130] J.N. Butt, F.A. Armstrong, J. Breton, S.J. George, A.J. Thomson, E.C. Hatchikian, J. Am. Chem. Soc. 113 (1991) 6663.
[131] J.J.G. Moura, I. Moura, T.A. Kent, J.D. Lipscomb, B.H. Huynh, J. LeGall, A.V. Xavier, E. Münck, J. Biol. Chem. 257 (1982) 6259.
[132] T.A. Kent, J.L. Dreyer, M.C. Kennedy, B.H. Huynh, M.H. Emptage, H. Beinert, E. Münck, Proc. Natl. Acad. Sci. USA 79 (1982) 1096.
[133] T.A. Kent, M.H. Emptage, H. Merkle, M.C. Kennedy, H. Beinert, E. Münck, J. Biol. Chem. 260 (1985) 6871.
[134] T.A. Rouault, R.D. Klausner, J. Biol. Inorg. Chem. 1 (1996) 494.
[135] H. Beinert, R.H. Holm, E. Münck, Science 277 (1997) 653.
[136] W. Hentze, L.C. Kuhn, Proc. Natl. Acad. Sci. USA 93 (1996) 8175.
[137] H. Beinert, M.C. Kennedy, C.D. Stout, Chem. Rev. 96 (1996) 2335.
[138] E. Hidalgo, J.M. Bollinger Jr., T.M. Bradley, C.T. Walsh, B. Demple, J. Biol. Chem. 270 (1995) 20908.
[139] P. Gaudu, B. Weiss, Proc. Natl. Acad. Sci. USA 93 (1996) 10094.
[140] N. Khoroshilova, H. Beinert, P. Kiley, J. Proc. Natl. Acad. Sci. USA 92 (1995) 2499.
[141] B.A. Lazazzera, H. Beinert, N. Khoroshilova, M.C. Kennedy, P.J. Kiley, J. Biol. Chem. 271 (1996) 2762.
[142] T. Nunoshiba, E. Hidalgo, C. Amabile-Cuevas, B. Demple, J. Bacteriol. 174 (1992) 6054.
[143] J. Wu, B. Weiss, J. Bacteriol. 174 (1992) 3915.
[144] Z. Li, B. Demple, J. Biol. Chem. 269 (1994) 18371.
[145] J.H. Chou, J.T. Greenberg, B. Demple, J. Bacteriol. 175 (1993) 1026.
[146] D. Ma, M. Alberti, C. Lynch, H. Nikaido, J.E. Hearst, Mol. Microbiol. 19 (1996) 101.
[147] E. Hidalgo, B. Demple, EMBO J. 13 (1994) 138.
[148] J. Wu, W.R. Dunham, B. Weiss, J. Biol. Chem. 270 (1995) 10323.
[149] E. Hidalgo, B. Demple, J. Biol. Chem. 271 (1996) 7269.
[150] T.A. Rouault, D.J. Haile, W.E. Downey, C.C. Philpott, C. Tang, F. Samaniego, J. Chin, I. Paul, D. Orloff, J.B. Harford, R.D. Klauser, Biometals 5 (1992) 131.
[151] D.J. Haile, T.A. Rouault, C.K. Tang, J. Chin, J.B. Harford, R.D. Klausner, Proc. Natl. Acad. Sci. USA 89 (1992) 7536.
[152] D.J. Haile, T.A. Rouault, J.B. Harford, M.C. Kennedy, G.A. Blondin, H. Beinert, R.D. Klausner, Proc. Natl. Acad. Sci. USA 89 (1992) 11735.
[153] E. Jestin, F. Moreau, D. Florentin, A. Marquet, Bioorg. Med. Chem. 4 (1996) 1065.
[154] J.B. Broderick, R.E. Duderstadt, D.C. Fernandez, K. Wojtuszewski, T.F. Henshaw, M.K. Johnson, J. Am. Chem. Soc. 119 (1997) 77396.
[155] R.M. Petrovich, F.J. Ruzicka, G.H. Reed, P.A. Frey, Biochemistry 31 (1992) 10774.
[156] P.A. Frey, G.H. Reed, Adv. Enzymol. Relat. Areas Mol. Biol. 66 (1993) 1.
[157] H. Hirling, B.R. Henderson, L.C. Kühn, EMBO J. 13 (1994) 453.
[158] K.L. Schalinske, S.A. Anderson, P.T. Tuazon, O.S. Chen, M.C. Kennedy, R.S. Eisenstein, Biochemistry 36 (1997) 3950.
[159] J.L.H. Busch, J.L. Breton, B.M. Bartlett, F.A. Armstrong, R. James, A.J. Thomson, Biochem. J. 323 (1997) 95.
[160] D.H. Flint, J.F. Tuminello, T. Miller, J. Biol. Chem. 271 (1996) 16053.

[161] D.H. Flint, J. Biol. Chem. 271 (1996) 16068.
[162] M.H. Emptage, T.A. Kent, M.C. Kennedy, H. Beinert, E. Münck, Proc. Natl. Acad. Sci. USA 80 (1988) 4674.
[163] R.H. Holm, P. Kennepohl, E.I. Solomon, Chem. Rev. 96 (1996) 2239.
[164] L. Zheng, R.H. White, V.L. Cash, R.F. Jack, D.R. Dean, Proc. Natl. Acad. Sci. USA 90 (1993) 2754.
[165] L. Zheng, D.R. Dean, J. Biol. Chem. 269 (1994) 18723.
[166] L. Zheng, V.L. Cash, D.H. Flint, D.R. Dean, J. Biol. Chem. 273 (1998) 13264.
[167] D.M. Hwang, A. Dempsey, K.T. Tan, C.C. Liew, J. Mol. Evol. 43 (1996) 536.
[168] H. Cheng, B. Xia, G.H. Reed, J.L. Markley, Biochemistry 33 (1994) 3155.
[169] J.L.H. Busch, J.L.J. Breton, B.M. Bartlett, R. James, E.C. Hatchikian, A.J. Thomson, Biochem. J. 314 (1996) 63.
[170] M. Yamaguchi, H. Fujisawa, J. Biol. Chem. 256 (1981) 6783.
[171] S. Pagani, F. Bonomi, P. Cerletti, Eur. J. Biochem. 142 (1984) 361.
[172] V. Marguerite, Adv. Inorg. Biochem. 11 (1996) 135.
[173] K. Natarajan, J.A. Cowan, J. Am. Chem. Soc. 119 (1997) 4082.
[174] R.E. Stenkamp, L.C. Sieker, L.H. Jensen, Proteins: Struct. Funct. Genet. 8 (1990) 352.
[175] W.R. Rypniewski, D.R. Breiter, M.M. Benning, G. Wesenberg, B.H. Oh, J.L. Markley, I. Rayment, H.M. Holden, Biochemistry 30 (1991) 4126.
[176] K. Fukuyama, H. Matsubara, T. Tsukihara, Y. Katsube, J. Mol. Biol. 210 (1989) 383.
[177] S.T. Freer, R.A. Alden, C.W.J. Carter, J. Kraut, J. Biol. Chem. 250 (1975) 46.
[178] J. Kim, D. Woo, D.C. Rees, Biochemistry 32 (1993) 7104.