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IRON–SULFUR CLUSTERS IN ENZYMES: THEMES AND VARIATIONS

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I. Introduction

It would be interesting to know how many different types of iron–sulfur proteins there are. From the inventory of about a dozen ferredoxins and enzymes in 1967 (*1*), the number is now well over 100, and rising steadily. This has come about as more enzymes containing iron and acid-labile sulfide have been isolated, as more types of iron–sulfur clusters have been found, and as more spectroscopic signals have been observed in biological systems. The large amount of gene sequence information, now emerging, has also revealed many ferredoxin-like sequences in open reading frames.

An even more interesting question is regarding how many functions iron–sulfur clusters may have in nature. They have long been known to function as secondary electron carriers, within or between redox enzyme systems. Now iron–sulfur clusters, long suspected, have been found that have catalytic functions of their own. Different properties of the iron–sulfur clusters are exploited by these systems, including electron transfer, coordination chemistry, and the ability to undergo cluster interconversions.

This review considers the diversity of iron–sulfur proteins. Differences are exhibited at several levels: (1) in the different types of iron–sulfur clusters, which have been identified by structure determination and by spectroscopy; (2) in the proteins that bind these clusters (the same type of cluster may be coordinated to protein domains of different sequences and structure); (3) in the other prosthetic groups that are present; and (4) in the different catalytic activities of the clusters. If we classify the iron–sulfur proteins according to these criteria (Table I), it becomes apparent that there are a number of different distinct structural themes. Within these themes there are variations, in the number and types of iron–sulfur clusters and other prosthetic groups, and in the catalytic functions performed. These relationships have implications for the evolution of the iron–sulfur proteins, which have been discussed elsewhere (*2–6*).

II. Methods of Identifying Iron–Sulfur Clusters in Proteins

From studies of the ferredoxins and other small iron–sulfur proteins it is known that there is a variety of iron–sulfur cluster types. These clusters are usually, but not invariably, coordinated to cysteine residues of the protein in conserved arrangements (e.g., Figs. 1 and 2). The types of clusters that have been identified, and some of their properties, are summarized in Table II.

A. CHEMICAL COMPOSITION

The most obvious method of detecting iron–sulfur clusters is by the presence of nonheme iron in the isolated protein. The iron–sulfur proteins are distinguished from other nonheme iron proteins in that the

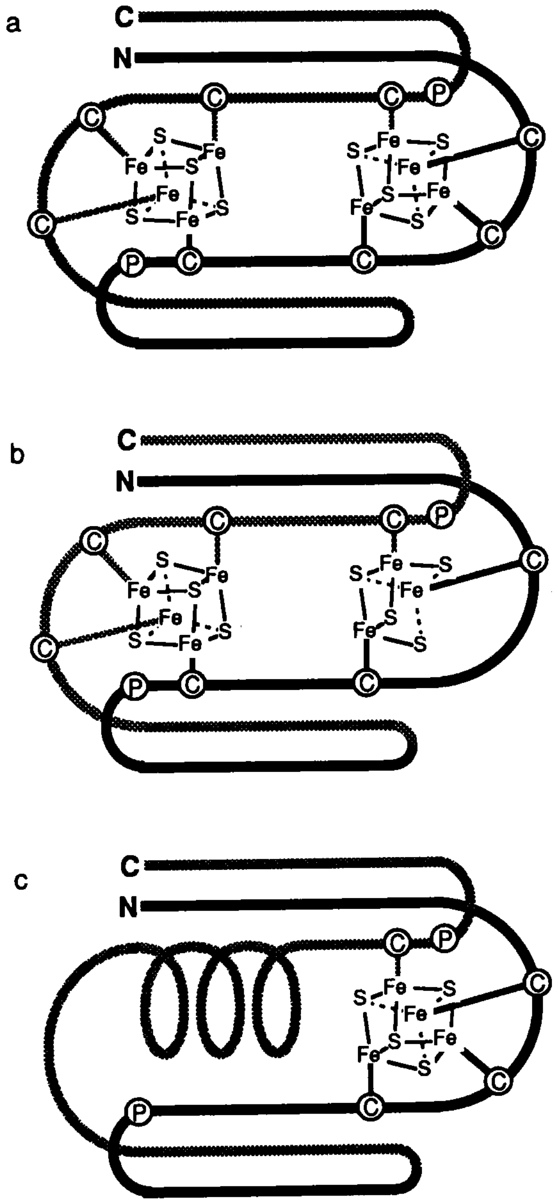

FIG. 1. (a) Outline of folding pattern of the 2[4Fe–4S] ferredoxins, showing the relevant cysteine residues (C) and prolines (P) (*260*). To emphasize the approximate twofold symmetry of the protein, the two halves are differently shaded. (b) The formation of a [3Fe–4S] cluster by omission of one of the cysteines (*54*, *55*). (c) The fold for a [4Fe–4S] ferredoxin (*52*).

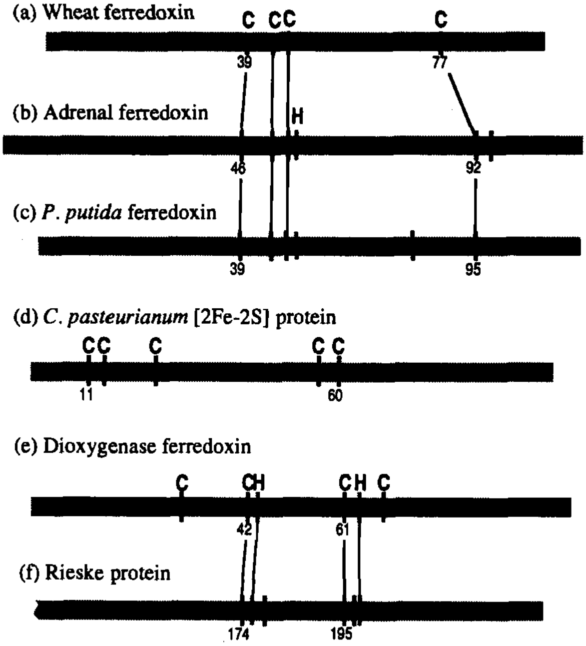

FIG. 2. Cysteine spacings for different types of [2Fe–2S] ferredoxins. (a) Wheat ferredoxin (*266*); (b) adrenal *P*-450-reducing ferredoxin (*267*); (c) *P. putida P*-450-reducing ferredoxin (*268*); (d) *C. pasteurianum* [2Fe–2S] ferredoxin (*45*); (e) *P. putida* strain ML2 benzene dioxygenase ferredoxin (*104*); (f) *Neurospora crassa* Rieske protein (*269*).

iron is coordinated to cysteine sulfurs from the protein, and, in all but the rubredoxins, by the presence of acid-labile sulfide. It must be said that the analytical values for iron and sulfide are rarely reliable on their own. There are cumulative uncertainties in the analyses for iron, sulfide, and protein, in the determination of the molecular mass, and in the purity and integrity of enzyme preparations. In most cases the number of iron and sulfide atoms per mole, originally estimated, has had to be revised as a result of spectroscopic or structural determinations. An honorable exception is the thorough study of aconitase and other proteins by Beinert and co-workers, where the values of 3Fe + 4S, which were contrary to all structural and spectroscopic precedents, proved to be correct (*7, 8*).

B. PROTEIN STRUCTURES

The existence of the [2Fe–2S], [3Fe–4S], and [4Fe–4S] clusters in proteins has been established by high-resolution X-ray crystallography

TABLE I

EXAMPLES OF IRON–SULFUR PROTEINS[a]

Protein	Typical source	Function	Subunit M_r ($\times 10^{-3}$)	Fe–S clusters	Other groups	Ref.
Simple iron–sulfur proteins						
[2Fe–2S] ferredoxin, plant type	Chloroplasts, cyanobacteria	Photosynthetic reduction of NADP, nitrite, thioredoxin, etc.	10.5	[2Fe–2S]	—	*36*
[3Fe–4S] ferredoxin	Bacteria, e.g., *D. gigas*, *S. griseolus*	Electron transfer	6–12	[3Fe–4S]	—	*57, 58*
[4Fe–4S] ferredoxin	Bacteria, e.g. *Bacillus*, *Desulfovibrio* spp.	Electron transfer	6–8	[4Fe–4S]	—	*52*
High-potential iron–sulfur protein (HiPIP)	Photosynthetic bacteria, e.g., *C. vinosum*	Not known	10	$[4Fe–4S]^{2+/3+}$	—	*171*
7Fe ferredoxin	Bacteria, e.g., *A. vinelandii*	Not known	6–10	[4Fe–4S] + [3Fe–4S]	—	*172*
8Fe ferredoxin	Anaerobic bacteria, e.g., *C. pasteurianum*	Electron transfer	6–10	2[4Fe–4S]	—	*173*
Rubredoxin	Anaerobic bacteria, e.g., *Desulfovibrio, Clostridium*	Electron transfer	6	[Rd]	—	*174*
Desulforedoxin	*D. vulgaris*	Not known	2(7.9)	2[Rd]	—	*175*
Membrane-bound electron transfer proteins						
NADH ubiquinone oxidoreductase (complex I)	Mitochondria, aerobic bacteria	$NADH + UQ \leftrightarrow NAD^+ + UQH_2$	>30 subunits, depending on species	*n*[2Fe–2S] + *m*[4Fe–4S]	FMN	*69*
Succinate dehydrogenase (complex II)	Mitochondria, aerobic bacteria	Succinate + UQ → fumarate + UQH_2	70 + 27 + 13 + 15	[2Fe–2S] + [3Fe–4S] + [4Fe–4S]	FAD + cyt *b*	*176*
Complex III–UQH_2 : cyt *c* reductase	Mitochondria, aerobic bacteria	$UQH_2 + \text{cyt } c_{ox} \rightarrow UQ + \text{cyt } c_{red}$	22 (Rieske protein)	$[2Fe–2S]_R$	2 cyt *b* + cyt c_1	*177*
Cytochrome b_6f complex	Chloroplasts	$PQH_2 + PC_{ox} \rightarrow PQ + PC_{red}$	20 (Rieske) + 33 + 23 + 17	$[2Fe–2S]_R$	2 cyt *b* + cyt *f*	*178*
ETF–UQ reductase	Mitochondria	$ETF \cdot H_2 + UQ \rightarrow ETF + UQH_2$	≈70	[4Fe–4S]	FAD	*179, 180*
Formate–hydrogen lyase	*E. coli*	$H \cdot COO^- + H^+ \rightarrow H_2 + CO_2$	110 + 32 + 20	*n*[4Fe–4S]	Mo-pterin, Se, Ni	*181, 182*
[NiFe] hydrogenase—(respiratory)	Bacteria, e.g., *E. coli*, *C. vinosum; A. vinelandii*	$H_2 + MK \rightarrow MKH_2$	60 + 30 (+30)	2[4Fe–4S] + [3Fe–4S]	Nickel center (cyt *b*)	*183*

(Continued)

TABLE I (*Continued*)

Protein	Typical source	Function	Subunit M_r ($\times 10^{-3}$)	Fe–S clusters	Other groups	Ref.
Glycerol phosphate dehydrogenase (anaerobic)	*E. coli*	Glycerol phosphate + MK $\rightarrow$ glyceraldehyde phosphate + MKH_2	62 + 43 + 44	2[4Fe–4S] + [2Fe–2S] (predicted)	FAD,FMN?	*64*
Fumarate reductase	—	Fumarate + $MKH_2 \rightarrow$ succinate + MK	70 + 30 + 12 + 12	[2Fe–2S] + [3Fe–4S] + [4Fe–4S]	FAD	(*14, 184, 185*)
Nitrate reductase (respiratory)	Anaerobic bacteria, e.g., *E. coli*	$NO_3^- + MKH_2 \rightarrow NO_2^- + MK$		3[4Fe–4S] + [3Fe–4S]	Mo-pterin	*186*
DMSO/TMAO reductase (respiratory)	Anaerobic bacteria, e.g., *E. coli*	DMSO + $MKH_2 \rightarrow$ DMS + MK + H_2O	87 + 23 + 31	4[4Fe–4S]	Mo-pterin	*19*
Methylenetetrahydrofolate reductase	*C. thermoaceticum*	$CH_2{=}FH_4 + D_{red} \rightarrow CH_3 \cdot FH_4 + D_{ox}$	8(36)	2[4Fe–4S]	FAD, Zn	*187*
Photosystem I	Chloroplasts, cyanobacteria	Photosynthetic electron transfer from plastocyanin to ferredoxin	2(84), 22, 19, 9	3[4Fe–4S]	Chlorophyll, phaeophytin, vitamin K_1	*77*
Soluble iron–sulfur enzymes						
NAD(P)H-glutamate synthase	Plants, *E. coli*	Glutamine + 2-oxoglutarate + NAD(P)H $\rightarrow$ 2 glutamate + NAD(P)$^+$	166 + 52	[4Fe–4S]	FAD, FMN	*188, 189*
Ferredoxin-glutamate synthase	Plants	Glutamine + 2-oxoglutarate + $2Fd_{ox} \rightarrow$ 2 glutamate + $2Fd_{red}$	140–160	[4Fe–4S] + ?[2Fe–2S]	FAD, FMN	*190*
Trimethylamine dehydrogenase	Bacterium W3A1	$(CH_3)_3NH^+ + [ETF]_{ox} \rightarrow (CH_3)_2NH_2^+ + [ETF]_{red} + [C_1]$	2(83)	[4Fe–4S]	FMN, ADP	*191*
2-Enoate reductase	*C. kluyveri*	Reduction of 2-enoates $R_2R_3{=}R_1COO^- + NADH \rightarrow R_2R_3CH{-}CH \cdot R_1 \cdot COO^-$	6(78)	4Fe + 4S	FAD	*192*
Adenylylsulfate reductase	Sulfate-reducing bacteria, e.g., *D. gigas*	$AMP \cdot SO_4 + 2e^- \leftrightarrow HSO_3^- + AMP$	4(70 + 23)	2[4Fe–4S] clusters per dimer	FAD	*193*
Dihydroorotate dehydrogenase	*Clostridium oroticum*	Dihydroorotate + $NAD^+ \leftrightarrow$ orotate + NADH	115	2[2Fe–2S]	FAD, FMN	*194*
Pyruvate:NADP$^+$ oxidoreductase	*Euglena gracilis*	Pyruvate + CoA + $NADP^+ \leftrightarrow$ acetyl-CoA + CO_2 + NADPH	2(166)	4Fe, 4S	FAD	*195*
Pyruvate:ferredoxin oxidoreductase	Cyanobacteria, clostridia, protozoa	Pyruvate + CoA + $Fd_{ox<} \leftrightarrow$ acetyl-CoA + CO_2 + Fd_{red}	123	2[4Fe–4S]	Thiamin diphosphate	*196*

Pyruvate : ferredoxin oxidoreductase	*Halobacterium halobium*	Pyruvate + CoA + $Fd_{ox<}$ ↔ acetyl-CoA + CO_2 + Fd_{red}	2(86 + 42)	[4Fe–4S]	Thiamin diphosphate	*91*
Pyruvate : flavodoxin oxidoreductase	Bacteria, e.g., *K. pneumoniae*	Pyruvate + CoA + $2Fld_{ox<}$ ↔ acetyl-CoA + CO_2 + $2Fld_{red}$	2(120)	2[4Fe–4S] per subunit	Thiamin diphosphate	*89, 197*
Oxoglutarate : ferredoxin oxidoreductase	*Halobacterium halobium*	Oxoglutarate + CoA + $Fd_{ox<}$ ↔ succinyl-CoA + CO_2 + Fd_{red}	88 + 36; total 248	[4Fe–4S]	Thiamin diphosphate	*91*
[NiFe] hydrogenase (cytochrome *c* reducing)	*Desulfovibrio* spp.	H_2 + cyt c_{ox} ↔ $2H^+$ + cyt c_{red}	≈60 + ≈30	2[4Fe–4S] + [3Fe–4S]	Ni	*29, 198*
[NiFe] hydrogenase (NAD reducing)	Hydrogen bacteria, e.g., *A. eutrophus, Nocardia opaca*	$H_2 + NAD^+ \rightarrow H^+ + NADH$	63 + 56 + 30 + 26	3–4[4Fe–4S] + [2Fe–2S]	Ni, FMN	*199*
[NiFe] hydrogenase (MV reducing)	Methanogenic bacteria, e.g., *M. thermoautotrophicum*	$H_2 + A_{ox} \leftrightarrow 2H^+ + A_{red}$	53 + 33 + 16	3–4[4Fe–4S] + [2Fe–2S]	Ni	*165*
[NiFe] hydrogenase (F_{420} reducing)	Methanogenic bacteria, e.g., *M. thermoautotrophicum*	$H_2 + F_{420} \rightarrow F_{420} \cdot H_2$	45 + 31 + 26 + 18	3–4[4Fe–4S] + [2Fe–2S]	Ni, FAD	*200*
[NiFeSe] hydrogenase	Sulfate-reducing and methanogenic bacteria	H_2 + cyt c_{ox} ↔ $2H^+$ + cyt c_{red}	≈60 + ≈30; total 90	2[4Fe–4S]	Ni, selenocysteine	*201*
[NiFeSe] hydrogenase (F_{420} reducing)	Methanogenic bacteria, e.g., *Methanococcus voltae*	$H_2 + F_{420} \rightarrow F_{420} \cdot H_2$	55 + 45 + 37 + 27	3–4[4Fe–4S] + [2Fe–2S]	Ni, FAD	*202*
Corrinoid/iron–sulfur protein	Acetogenic and methanogenic bacteria	Transfer of methyl from $CH_3 \cdot FH_4$ or $CH_3 \cdot H_4MPT$ to acetyl-CoA synthase	88	[4Fe–4S]	Cobalamin	*203*
Hydroxylases and dioxygenases						
Ferredoxin (*P*-450 reducing)	Mitochondria, bacteria, e.g., *P. putida*	Electron transfer from flavoprotein reductase to cytochrome *P*-450	11.5	[2Fe–2S]	—	*204, 205*
Methane monooxygenase reductase	*Methylococcus capsulatus*	Reduction of methane monooxygenase (NADH)	38.5	[2Fe–2S]	FAD	*206, 207*
Rubredoxin	*P. oleovorans*	Electron transfer to ω-hydroxylase	—	2[Rd]	—	*34*
4-Methoxybenzoate monooxygenase (putidamonooxin)	*P. putida*	O-demethylation of 4-methoxybenzoate	3(41)	$[2Fe–2S]_R$	Fe	*101*
4-Methoxybenzoate monooxygenase reductase	*P. putida*	—	42	[2Fe–2S]	FMN	*208*
Benzene dioxygenase	*P. putida*	Benzene + NADH + O_2 → *cis*-benzene glycol + NAD^+	2(54.5 + 23.5)	$[2Fe–2S]_R$	Fe	*63, 103*
Benzene dioxygenase ferredoxin	*P. putida*	—	11.9	[2Fe–2S]	—	*63, 104*
Toluene dioxygenase	*P. putida*	Dihydroxylation of toluene	2(529 + 21)	$[2Fe–2S]_R$	Fe	*105*
Toluene dioxygenase ferredoxin	*P. putida*	—	12	[2Fe–2S]	—	*105*
Naphthalene dioxygenase	*Pseudomonas* sp. strain NCIB-9816	Dihydroxylation of naphthalene	2(55 + 20)	$[2Fe–2S]_R$	Fe	*208a, 271*

(Continued)

TABLE I (*Continued*)

Protein	Typical source	Function	Subunit M_r ($\times 10^{-3}$)	Fe–S clusters	Other groups	Ref.
Naphthalene dioxygenase ferredoxin	*Pseudomonas* sp. strain NCIB-9816	—	15	[2Fe–2S]	—	*209*
Naphthalene dioxygenase reductase	*Pseudomonas* sp. strain NCIB-9816	—	36	[2Fe–2S]	FAD	*210*
Phthalate dioxygenase	*P. cepacia*	Dihydroxylation of phthalate	4(48)	$[2Fe-2S]_R$	Fe	*211*
Phthalate dioxygenase reductase	*P. cepacia*	—	34	[2Fe–2S]	FMN	*98*
Pyrazon dioxygenase	—	Dihydroxylation of pyrazon	180	$[2Fe-2S]_R$	Fe	*212*
Pyrazon dioxygenase ferredoxin	—	—	12	[2Fe–2S]	—	*212*
Benzoate dioxygenase	*P. arvilla*	Dihydroxylation of benzoate	3(50 + 20)	$[2Fe-2S]_R$	Fe	*100, 102*
Benzoate dioxygenase reductase	*P. arvilla*	—	37.5	[2Fe–2S]	FAD	*100, 213*
4-Chlorophenylacetate 3,4-Dioxygenase	—	Dihydroxylation of 4-chlorophenylacetate	3(46)	[2Fe–2S]	Fe	*214*
Enzymes with the molybdopterin cofactor						
Xanthine dehydrogenase	Liver, bacteria	Xanthine + $NAD^+ \leftrightarrow$ urate + NADH	2(146)	2[2Fe–2S]	Mo-pterin, FAD	*215, 216*
Xanthine oxidase	Milk	Xanthine + $O_2 \rightarrow$ urate + $H_2O_2 + O_2^-$	2(140)	2[2Fe–2S]	Mo-pterin, FAD	*107*
Aldehyde oxidase	Liver	Aldehyde + $O_2 \rightarrow$ carboxylate + H_2O_2	2(140)	2[2Fe–2S]	Mo-pterin, FAD	*217*
Aldehyde dehydrogenase	*D. gigas, P. testosteroni*	$R \cdot CHO + D_{ox} \rightarrow R \cdot COO^- + D_{red}$	2(92)	2[2Fe–2S]	Mo-pterin, FAD	*218, 219*
Aldehyde: ferredoxin oxidoreductase (tungsten containing)	*Pyrococcus furiosus*	$R \cdot CHO + Fd_{ox} \leftrightarrow R \cdot COO^- + Fd_{red}$	85	≈7Fe, 5S	W	*220*
Purine hydroxylase II	*Anacystis nidulans*	Ring hydroxylation of purines	2(153)	2[2Fe–2S]	Mo, FAD	*221*
Carbon monoxide oxidoreductase	Carboxydovoric aerobic bacteria	$CO + A_{ox} \rightarrow CO_2 + A_{red}$	230	2[2Fe–2S]	Mo-pterin, FAD	*222*
Furoyl-coenzyme-A dehydrogenase	*P. putida* Fu1	2-[furoyl-CoA + $A_{ox} \rightarrow$ 5-hydroxy-2-furoyl-CoA + A_{red}	2(55 + 25)	Fe, S	Mo, cyt *b*	*223*
Nicotinate dehydrogenase	*B. niacini*	Hydroxylation of nicotinate	2(85 + 34 + 20)	8.3Fe, 1.5S	2FAD, 2Mo	*224*

6-Hydroxynicotinate dehydrogenase	*B. niacini*	Hydroxylation of 6-hydroxynicotinate	85 + 34 + 20	Fe + S	FAD, Mo	*224*
Arsenite oxidase	*A. faecalis*	Arsenite + $O_2 \rightarrow$ arsenate + H_2O_2	85	2[2Fe–2S]	Mo-pterin	*225*
Ferredoxin-nitrate reductase	Cyanobacteria, e.g., *Plectonema boryanum*	$NO_3^- + Fd_{red} + 2H^+ \rightarrow NO_2^- + Fd_{ox} + H_2O$	83	2[2Fe–2S]	Mo-pterin	*226*
Formate dehydrogenase (F_{420} reducing)	Methanogens, e.g., *M. formicicum*	$H \cdot COO^- + H^+ + F_{420} \rightarrow CO_2 + F_{420} \cdot H_2$	76 + 34	24Fe, 28S as [4Fe–4S] clusters	Mo-pterin	*227*
Formate dehydrogenase (NAD reducing)	Methanotrophs, e.g., *Methylosinus trichosporium*	$H \cdot COO^- + NAD^+ \rightarrow CO_2 + NADH$	2(98 + 56 + 19 + 11.5)	40Fe, 40S (≈5 Fe–S clusters)	2 flavins/dimer	*228*
Formate dehydrogenase (NAD reducing)	*P. oxalaticus*	$H \cdot COO^- + NAD^+ \rightarrow CO_2 + NADH$	320	17–20Fe, 17–20S	2FMN	*229*
Formate dehydrogenase (ferredoxin reducing)	*C. pasteurianum*	$H \cdot COO^- + Fd_{ox} \leftrightarrow CO_2 + Fd_{red}$	76 + 34	3[4Fe–4S]	Mo-pterin	*230, 231*
Formate dehydrogenase (tungsten containing)	*C. thermoaceticum*	$CO_2 + Fd_{red} \rightarrow H \cdot COO^- + Fd_{ox}$	$\alpha_2\beta_2$; total 340	[4Fe–4S] + [2Fe–2S]	W-pterin, Se	*117*
Enzymes containing sirohaem						
Ferredoxin : sulfite reductase	Plants, bacteria	$SO_3^{2-} + 6Fd_{red} \rightarrow S^{2-} + 6Fd_{ox}$	69	[4Fe–4S]	Siroheme	*127*
Sulfite reductase (assimilatory)	*E. coli*	$HSO_3^- + 3\ NADPH + 3H^+ \rightarrow HS^- + 3\ NADP^+ + 3H_2O$	8(59 + 4 × 55)	[4Fe–4S]	Siroheme, FAD, FMN	*88*
Sulfite reductase (dissimilatory)	Sulfate-reducing bacteria, e.g., *D. vulgaris*	$HSO_3^- + 6D_{red} + 6H^+ \rightarrow HS^- + 6D_{ox} + 3H_2O$	$\alpha_2\beta_2$; total 200	2[4Fe–4S]	Siroheme	*232*
Sulfite reductase (assimilatory)	Sulfate-reducing bacteria, e.g., *D. vulgaris*	$HSO_3^- + 6D_{red} + 6H^+ \rightarrow HS^- + 6D_{ox}\ 3H_2O$	27	[4Fe–4S]	Siroheme (low spin)	*233*
Ferredoxin : nitrite reductase	Plants	$NO_2^- + 6Fd_{red} + 8H^+ \rightarrow NH_4^+ + 6Fd_{ox} + 2H_2O$	67	[4Fe–4S]	Siroheme	*126*
NADH : nitrite reductase	*E. coli*	$NO_2^- + 3NADH + 5H^+ \rightarrow NH_4^+ + 3NAD^+ + 2H_2O$	92	[2Fe–2S]	Siroheme, FAD	*129*
Proteins with catalytic Fe–S or mixed-metal clusters						
[Fe] hydrogenase	Anaerobic bacteria, e.g., *C. pasteurianum*, *D. gigas*	$2H^+ + 2e^- \leftrightarrow H_2$	≈60	H cluster + 2–4[4Fe–4S]	—	*234*
Carbon monoxide dehydrogenase	Photosynthetic bacteria, e.g., *Rhodospirillum rubrum*	$CO + H_2O \rightarrow CO_2 + 2e^- + 2H^+$	61.8	≥2[4Fe–4S] + $NiFe_{3-4}S_{\geq 4}$ + ?[6Fe–6S]	Zn	*144*
Carbon monoxide dehydrogenase (acetyl-CoA synthase)	Acetogenic and methanogenic bacteria, e.g., *C. thermoaceticum*, *Methanothrix soehngenii*	CH_3-[CP] + CO + CoA ↔ CH_3 CO-CoA + [CP]	Acetogens: 3(71 + 78) Methanogens: 90 + 21	≥2[4Fe–4S] + $NiFe_{3-4}S_{\geq 4}$ + ?[6Fe–6S]		*138,139 235, 236*

(Continued)

TABLE I *(Continued)*

Protein	Typical source	Function	Subunit M_r ($\times 10^{-3}$)	Fe–S clusters	Other groups	Ref.
Mo nitrogenase	Nitrogen-fixing bacteria, e.g., *Rhizobium, Azotobacter, Klebsiella*	$N_2 + 8e^- + 10H^+ \rightarrow 2NH_4^+ + H_2$	2(50 + 60)	P clusters, Fe–Mo cofactor		*131, 237*
Vanadium nitrogenase	Nitrogen-fixing bacteria, e.g., *Rhizobium, Azotobacter*	$N_2 + 8e^- + 10H^+ \rightarrow 2NH_4^+ + H_2$	2(50 + 55 + 13)	VFe protein: P clusters + Fe–V cofactor		*238*
Iron nitrogenase	Nitrogen-fixing bacteria, e.g. *Azotobacter, Rhodobacter*	$N_2 + 8e^- + 10H^+ \rightarrow 2NH_4^+ + H_2$	2(58 + 51 + 15)	P clusters + Fe–Fe cofactor		*239*
Nitrogenase Fe protein (nitrogenase reductase)	Nitrogen-fixing bacteria, e.g. *Rhizobium, Azotobacter, Klebsiella*	$[\text{MoFe-protein}] + D_{red} + ATP \rightarrow [\text{MoFe-protein}]_{red} + D_{ox} + ADP + P_i$	2(68)	[4Fe–4S] per two subunits		*237*
Enzymes with nonredox Fe–S clusters						
Glutamine PRPP amidotransferase	Humans, *B. subtilis*	Glutamine + PRPP → 5-phosphoribosylamine + glutamine + PP_i	4(50)	[4Fe–4S]		*148*
Aconitase (aconitate hydratase)	Mitochondria, cytoplasm, bacteria	Citrate ↔ isocitrate	81	[4Fe–4S]		*147*
Maleate hydratase	Rabbit kidney	D-malate ↔ maleate + H_2O	68	[4Fe–4S]		*240*
Lactyl-CoA dehydratase	*C. propionicum*	Lactyl-CoA ↔ acrylyl-CoA + H_2O	*n*(48 + 41)	[3Fe–4S], [4Fe–4S]		*241*
2-Hydroxyglutaryl-CoA dehydratase	*Acetaminococcus fermentans*	2-Hydroxyglutaryl-CoA ↔ glutanoyl-CoA + H_2O	2(55 + 42)	[4Fe–4S]		*242*
Dihydroxyacid dehydratase	Plants	$(CH_3)_2 \cdot C(OH){-}CH(OH) \cdot COO^- \leftrightarrow (CH_3)_2 \cdot CH{-}CO \cdot COO^- + H_2O$	≈2(60)	[2Fe–2S]		*243*
Dihydroxyacid dehydratase	*E. coli*	$(CH_3)_2 \cdot C(OH){-}CH(OH) \cdot COO^- \leftrightarrow (CH_3)_2 \cdot CH{-}CO \cdot COO^- + H_2O$	—	[4Fe–4S]		*244*
L(+)-Tartrate dehydratase	*P. putida*	L-tartrate → oxaloacetate + H_2O	2(23 + 27)	[4Fe–4S]	—	*245*
L-Serine dehydratase	*Peptostreptococcus asaccharolyticus*	Serine → pyruvate + NH_4^+	30 + 25; total 200	[4Fe–4S]	—	*246*
6-Phosphogluconate dehydratase	*Zymomonas mobilis*	—	126	6–8Fe, S/mole	—	*247*
Nitrile hydratase	*Brevibacterium* sp.	$R \cdot CN + H_2O \rightarrow R \cdot CO \cdot NH_2$	2(23 + 23.5)	2Fe	—	*158*
Fumarases A and B	*E. coli*	Fumarate + H_2O ↔ L-malate	60	[4Fe–4S]	—	*149*

DNA endonuclease III	*E. coli*	Apurinic and apyridimic endonuclease	24	[4Fe–4S]	—	*157*
Regulatory proteins						
Iron-responsive element binding protein	Humans	Regulation of translation of ferritin, etc., mRNA	89	[3Fe–4S]?	—	*161*
FNR protein	*E. coli*	Aerobic/anaerobic switch	30	1.1 Fe in isolated protein	—	*163*
Ferredoxin : thioredoxin reductase	Chloroplasts, cyanobacteria	Thioredoxin + $Fd_{red} \rightarrow$ thioredoxin$_{red}$ + Fd_{ox}	13 + (7–16)	[4Fe–4S] cluster	Disulfide bridge	*273*
Miscellaneous iron–sulfur proteins of unknown function						
[6Fe–6S] protein	*D. vulgaris*	Not known	52	[6Fe–6S] cluster?	—	*248*
Rubrerythrin	*D. vulgaris*	Phosphatase?		Fe	$[2Fe]_H$	*168*
Desulfoferrodoxin	*Desulfovibrio* spp.	Not known		[Rd]	Fe	*169*
Polyferredoxin	Methanogens, e.g., *M. thermoautotrophicum*	?-electron transfer to hydrogenase	48	12[4Fe–4S] predicted	—	*165*
Molybdenum iron–sulfur protein	*D. africanus*	Unknown	10(11.5)	20Fe, 20S	5–6Mo	*249*

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[a] Unless otherwise stated, [4Fe–4S] clusters are $[4Fe–4S]^{2+/1+}$ type. Composition of iron–sulfur clusters and other groups is per minimum catalytic unit, e.g., where a protein is an $(\alpha_2\beta)_3$, the composition would be expressed per $\alpha_2\beta$ unit. Abbreviations: A_{ox}, A_{red}, oxidized and reduced electron acceptor; cyt, cytochrome; D_{red}, D_{ox}, reduced and oxidized electron donor; [Rd], rubredoxin-type Fe center; $[2Fe–2S]_R$, Rieske iron–sulfur cluster; $[2Fe]_H$, hemerythrin-like binuclear iron cluster; UQ, ubiquinone; UQH_2, ubiquinol.

TABLE II

TYPES OF IRON–SULFUR CLUSTERS

Type	Source	Typical E_m range (mV)
$[Fe]^{3+/2+}$	Rubredoxins	+20 to −60
$[2Fe–2S]^{2+/1+}$	Ferredoxins	−240 to −460
$[Fe_2S_2(RS)_2N_2]^{0/-1}$	Rieske proteins	+300 to −155
$[3Fe–4S]^{1+/0}$	Ferredoxins	−50 to −420
$[4Fe–4S]^{2+/1+}$	Bacterial ferredoxins	0 to −645
$[4Fe–4S]^{3+/2+}$	HiPIP	50 to 450
[6Fe–6S]	*D. vulgaris* protein	—
H-cluster	Hydrogenase	—
P-clusters	Nitrogenase protein 1	—
Fe–Mo cofactor	Mo Nitrogenase protein 1	—
Fe–V cofactor	V nitrogenase protein 1	—
Fe–Fe cofactor	Alternative nitrogenase protein 1	—
Fe–Ni cluster	CO dehydrogenase	—

of simple iron–sulfur proteins. Iron–sulfur proteins, for which the crystal structure has been determined crystallographically, are listed in Table III. These structures are extremely valuable for the information they provide about the environment and disposition of the clusters. It is also possible to investigate the binding sites for substrates and for other proteins, and the residues that influence the properties of the clusters. For future studies, two-dimensional nuclear magnetic resonance (NMR) is a promising technique for the determination of the structures of small proteins (up to 20 kDa) (*9*). In the region of the iron–sulfur clusters, contact-shifted resonances have been assigned, giving information about the paramagnetic states of the individual iron atoms and about their ligands (*10a, 11*).

C. AMINO ACID SEQUENCES

The sequences for genes encoding many iron–sulfur proteins from different species are emerging rapidly. From an analysis of sequences, consensus sequences may be derived for each type of iron–sulfur cluster. The most prominent features of the cluster-binding "motifs" are the cysteines that bind the clusters, with characteristic spacings in between (see Ref. *5*).

TABLE III

IRON–SULFUR PROTEINS OF WHICH THE STRUCTURE HAS BEEN DETERMINED

Protein	Source	Resolution (Å)[a]	Ref.
Rubredoxin	*Clostridium pasteurianum*	1.2	*250*
Rubredoxin	*Desulfovibrio vulgaris*	1.5	*174*
Rubredoxin	*Desulfovibrio desulfuricans*	1.5	*251*
Rubredoxin	*Desulfovibrio desulfuricans* strain 27774	1.5	*252*
Rubredoxin	*Desulfovibrio gigas*	1.4	*253*
[2Fe–2S] ferredoxin	*Anabaena* 7120	2.5	*254*
[2Fe–2S] ferredoxin	*Spirulina platensis*	2.5	*255*
[2Fe–2S] ferredoxin I	*Aphanothece sacrum*	2.2	*256*
[2Fe–2S] ferredoxin I	*Halobacterium* (Dead Sea)	2.5	*257*
[3Fe–4S] ferredoxin II	*Desulfovibrio gigas*	1.7	*57*
[4Fe–4S] ferredoxin	*Bacillus thermoproteolyticus*	2.3	*52*
[4Fe–4S] HiPIP	*Chromatium vinosum*	2.0	*258*
[4Fe–4S][3Fe–4S] ferredoxin I	*Azotobacter vinelandii*	1.9	*259*
2[4Fe–4S] ferredoxin	*Peptostreptococcus asaccharolyticus*[b]	2	*260*
2[4Fe–4S] ferredoxin	*Clostridium acidi-urici*	5	*261*
Trimethylamine dehydrogenase	Bacterium W3A1	2.4	*262*
Sulfite reductase hemoprotein subunit	*Escherichia coli*	3	*124*
Phthalate dioxygenase reductase	*P. cepacia*		*98*
Aconitase	Beef heart:	2.1	*162*
	[3Fe–4S] form	2.5	
	[4Fe–4S] form		
Nitrogenase Fe–Mo protein	*Clostridium pasteurianum*	3.5	*136*
Nitrogenase Fe protein	*Clostridium pasteurianum*	3.0	*137*

[a] Resolution given is that of the electron density map, or of the refined structure if available.

[b] Formerly *Peptococcus aerogenes,* formerly *Micrococcus aerogenes.*

An example of a motif is the consensus sequence for the 2[4Fe–4S] ferredoxins, which contain two groups of cysteines of the following form:

(-Cys-X-X-Cys-X-X-Cys-X-X-X-Cys-Pro-)$_2$

As illustrated in Fig. 1a, each cluster is coordinated by three cysteine sulfurs from one group and one from the other. Thus, in the [4Fe–4S] ferredoxins, the cluster is bound by three closely spaced cysteines and one distant cysteine (Fig. 1c) (*12*). If the second cysteine of the group is missing, the result is usually a [3Fe–4S] cluster (Fig. 1b). Such arrangements have been found in a number of complex iron–sulfur proteins.

Not all sequences that bind iron–sulfur clusters will conform to these motifs. There may be many ways to fold a polypeptide around a particular type of cluster. In other words, the same type of cluster may arise in different proteins by convergent evolution. For example, there are at least four different arrangements of cysteines in the [2Fe–2S] proteins (Fig. 2). Thus sequence information can help to identify different families of iron–sulfur proteins.

By examination of the sequence of an iron–sulfur protein it is sometimes possible to identify the iron–sulfur-binding motifs. This will determine the types of iron–sulfur clusters present, and their location. An example is the iron–sulfur protein subunit of fumarate reductase/succinate dehydrogenase, in which homologies to the 2Fe and 7Fe ferredoxins were observed (*3, 13*) before the presence of [2Fe–2S], [3Fe–4S], and [4Fe–4S] clusters was confirmed spectroscopically (*14*). Some examples of ferredoxin-like sequences in complex iron–sulfur proteins, which almost certainly bind equivalent clusters, are listed in Table IV.

It is possible for an iron–sulfur cluster to be coordinated by cysteine ligands from different protein domains or even different subunits. Examples are the iron protein of nitrogenase, and cluster X in photosystem I (see below). With such an arrangement it is difficult to identify the binding site by examination of an individual sequence, but in these cases the specific cysteines were found by comparison of sequences from diverse species and by site-directed mutagenesis.

With the accumulation of genomic libraries from different species, it is possible to search for new types of iron–sulfur proteins. Some interesting potential proteins of unknown function have already been found, such as the ferredoxin-like proteins associated with nitrogenase genes (*15, 16*), and an operon resembling a membrane-bound iron–sulfur protein in *Escherichia coli* (*17*). However, it should be remembered that such searches cannot be exhaustive, and some iron–sulfur proteins will be missed. None of the features of a motif is invariant. For example, in the [4Fe–4S] cluster motif in Fig. 1, cases are known where the spacings of the cysteines are different, where the terminal proline is absent, and even where one of the cysteines is replaced by another amino acid.

D. SITE-DIRECTED MUTAGENESIS EXPERIMENTS

The predictions about iron–sulfur cluster composition may be tested by site-directed mutagenesis experiments, wherein cysteines or other

TABLE IV

SEQUENCES IN ENZYMES THAT ARE HOMOLOGOUS TO FERREDOXIN SEQUENCES

Protein	Source	Homologous to	Ref.
Pyruvate : flavodoxin reductase	*Klebsiella pneumoniae*	2[4Fe–4S] ferredoxins	*197*
[Fe] hydrogenase	*Desulfovibrio vulgaris*	2[4Fe–4S] ferredoxins	*263*
F_{420}-reducing hydrogenase	*Methanobacterium thermoautotropicum*	2[4Fe–4S] ferredoxins	*200*
Acetyl-CoA synthase	*Methanothrix soehngenii*	2[4Fe–4S] ferredoxins	*236*
Formate dehydrogenase	*Methanobacterium formicicum*	2[4Fe–4S] ferredoxins	*231*
DMSO reductase DmsB	*Escherichia coli*	4[4Fe–4S]	*264*
Nitrate reductase NarH	*Escherichia coli*	3[4Fe–4S], [3Fe–4S]	*20*
NAD-reducing [Ni] hydrogenase	*Alcaligines eutrophus*	[2Fe–2S], [4Fe–4S]	*265*
Succinate dehydrogenase	Beef heart	[2Fe–2S], [3Fe–4S], [4Fe–4S]	[*265a*]
Fumarate reductase	*Escherichia coli*	[2Fe–2S], [3Fe–4S], [4Fe–4S]	*184*
Xanthine dehydrogenase	*Drosophila melanogaster*	Plant-type [2Fe–2S]	*112*
Xanthine dehydrogenase	Rat liver	Plant-type [2Fe–2S]	*114*
Benzene dioxygenase	*Pseudomonas putida*	Rieske proteins	*99*
Benzoate dioxygenase	*Acinetobacter calcoaceticus*	Rieske proteins	*100*
Benzoate dioxygenase reductase	*Acinetobacter calcoaceticus*	Plant-type [2Fe–2S]	*100*
Methane monooxygenase reductase	*Methylococcus capsulatus*	Plant-type [2Fe–2S]	*94*

putative ligands to the cluster may be substituted by other amino acids. In some cases this causes the loss of the cluster or failure of the protein to assemble; in other cases the protein retains its structure and function. Generally one would conclude that if the cluster is retained unchanged, the cysteine is not a ligand. However, the results of such experiments may be unpredictable and any conclusions require corroboration by other methods.

An instructive example is provided by the experiments (*18*) on the [4Fe–4S][3Fe–4S] ferredoxin I from *Azotobacter vinelandii.* A mutant protein, in which Cys 20 was substituted by alanine, retained both iron–sulfur clusters with properties apparently little changed. From this it might have been concluded that Cys 20 was not a ligand to a cluster. However X-ray crystallography demonstrated that Cys 20 is a ligand in the native protein, but that a structural rearrangement took place in the mutant, in which the missing cysteine was replaced by the free Cys 24.

In proteins that contain multiple iron–sulfur clusters, such as the membrane-bound electron transfer proteins, the functions of individual clusters may be studied by their removal or modification. For example, both the dissimilatory nitrate reductase and the dimethyl sulfoxide (DMSO) reductase of *E. coli* have similar structures, with an intermediate iron–sulfur protein containing four iron–sulfur clusters. In DMSO reductase the subunit contains four [4Fe–4S] clusters (*19*), whereas in nitrate reductase it contains three [4Fe–4S] clusters and one [3Fe–4S] cluster (*20*). The difference has been attributed to the replacement of a cysteine in the sequence of DMSO reductase, by a tryptophan in nitrate reductase. A DMSO reductase gene was constructed, in which the corresponding Cys 102 was substituted by Trp. Electron paramagnetic resonance (EPR) spectroscopy showed that the [4Fe–4S] cluster was converted to the predicted [3Fe–4S] cluster. In this case the capability to transfer electrons from quinols was lost (*21*).

The iron–sulfur protein subunits of *E. coli* fumarate reductase and succinate dehydrogenase, which each contain [2Fe–2S], [3Fe–4S], and [4Fe–4S] clusters, provide an ideal test system for the investigation of iron–sulfur cluster assembly and function. Cysteines 57, 62, 65, and 77 (numbering for fumarate reductase) were predicted to bind the [2Fe–2S] cluster, by analogy with the plant-type ferredoxins. These are invariant in all the fumarate reductases and succinate dehydrogenases, except that Cys 65 is replaced by aspartate in the succinate dehydrogenase of *E. coli* (*13*). Replacement of each of these cysteines by serines in fumarate reductase, replacing —SH groups by —OH, still permitted assembly of the [2Fe–2S] cluster, but with significantly modified redox potentials and EPR spectroscopic properties (*22*). An interesting conclusion from this is that the [2Fe–2S] cluster can accommodate at least one alternative nonsulfur ligand. However, substitution of cysteines by histidines, which are believed to be the ligands in the Rieske proteins, prevented assembly of the protein (*23*). The [3Fe–4S] cluster was converted into a [4Fe–4S] cluster by insertion of a cysteine. Surprisingly, the enzyme was still functional, despite a considerably more negative midpoint potential for the new cluster (*24*). There is clearly much scope for further experiments to determine the rules that govern the assembly of iron–sulfur clusters, and the pathways of electron transfer in these complex proteins.

E. SPECTROSCOPY

All iron–sulfur clusters are colored, and the visible absorption and circular dichroism spectra give indications of iron–sulfur cluster type.

However, the absorption bands are broad and featureless, so the presence of the clusters may go unnoticed in the presence of other chromophores such as heme or flavin. Numerous other forms of spectroscopy are available for examining the iron–sulfur clusters in isolated proteins. Low-temperature EPR spectroscopy, introduced by Beinert and Sands (*25*) for the study of the mitochondrial respiratory chain, remains the most versatile method for characterizing iron–sulfur clusters in complex systems. EPR can detect most iron–sulfur clusters in appropriate oxidation states and has the advantage that other groups such as flavin, molybdenum, or nickel may also be observed.

The identification of iron–sulfur clusters by EPR is not always straightforward. Both [2Fe–2S] and [4Fe–4S] clusters typically give $g = 1.94$ signals in the reduced states, though the temperature dependence is a rough indication of cluster type. Signals due to $[4Fe–4S]^{1+}$ clusters are undetectable or extremely broad at temperatures higher than 40 K; the high-potential iron–sulfur protein (HiPIP)-type $[4Fe–4S]^{3+}$ cluster signals are detectable (but broadened) up to 60 K, whereas the $[2Fe–2S]^{1+}$ clusters are often, though not always, detectable at temperatures above 60 K (*26*). The spectra of some clusters in proteins are unusual or very difficult to detect, due to the existence of paramagnetic states with $S > \frac{1}{2}$ (described by Hagen, this volume) and to the presence of weak spin–spin interactions between adjacent centers.

When two or more paramagnetic centers are present in the same protein, exchange and dipolar interactions between them cause the appearance of additional lines in the spectrum, which do not occur in the spectrum of either center alone. For example, in the spectra of 2[4Fe–4S] ferredoxins there are additional lines in the center of the spectrum and in the wings. These are not true g factors, as was demonstrated by multifrequency EPR (*27*). Similar spectral features have been observed in the EPR spectra of proteins with multiple iron–sulfur clusters, for example, DMSO reductase (*19*). Extremely broad EPR spectra attributed to [4Fe–4S] clusters in succinate dehydrogenase, fumarate reductase (*28*), and [Ni–Fe] hydrogenase of *Desulfovibrio gigas* (*29*) have been attributed to interactions with reduced [3Fe–4S] clusters.

The spectra of oxidized $[4Fe–4S]^{3+}$ and $[3Fe–4S]^{+}$ clusters have average g factors greater than the free-electron g factor, $g_e = 2.0023$. The spectrum of HiPIP was the first of this type to be detected, and all spectra with $g > g_e$ were referred to as "HiPIP-type." In fact this was misleading. HiPIPs are unique types of soluble proteins, found so far only in a restricted range of bacteria (*30*). The other clusters that give EPR signals in the oxidized state at $g = 2.01$ have proved to be $[3Fe–4S]^+$ clusters. In some proteins, such as succinate dehydrogenase and fumarate reductase, the $[3Fe–4S]^+$ clusters can be observed in intact cells by EPR spectroscopy and appear to be functional (*31*). However, many [4Fe–4S] proteins undergo oxidative conversion to $[3Fe–4S]^+$ during isolation and yield substoichiometric signals around $g = 2.01$.

The reduced [3Fe–4S] clusters have a spin-even state, e.g., $S = 2$. Since this is a non-Kramers' state the EPR spectrum may be undetectable, or, if detectable, very broad. In some proteins it is observed as a broad signal around $g = 12$, e.g., in the partially reduced Ni hydrogenase of *D. gigas* (*32*). When the protein is fully reduced, interactions of the cluster with reduced [4Fe–4S] clusters cause the signal to disappear.

III. Classification of Iron–Sulfur Proteins

According to the International Union of Biochemistry (IUB) recommended nomenclature (*33*), iron–sulfur proteins have been classified into the following groups:

1. *Simple iron–sulfur proteins,* which have only iron–sulfur clusters. These include rubredoxins and ferredoxins, of which the only function is electron transfer, and other proteins, in which the iron–sulfur cluster(s) have a catalytic function.
2. *Complex iron–sulfur proteins,* which also contain other prosthetic groups and which generally have enzymatic activity. They are subdivided into iron–sulfur proteins containing flavin, molybdopterin, siroheme, and other prosthetic groups.

Table I includes a compilation, which is not exhaustive, of iron–sulfur proteins for which a function has been established or is strongly suggested. Comparisons of amino acid sequences help to identify families of proteins with similar structure but different enzymatic activities. The iron–sulfur proteins in Table I are loosely grouped in these families. It should be noted that there are also situations in which the same reaction is catalyzed by two different enzymes, coded by different genes, even in the same cell. In some cases, such as in fumarases and glycerol phosphate dehydrogenases from *E. coli,* there is one form, which is expressed under anaerobic conditions and which is an iron–sulfur protein, and another form, which is expressed under aerobic conditions and which is not an iron–sulfur protein. A possible reason is that the iron–sulfur protein versions may be catalytically more efficient, but more sensitive to oxygen.

When describing the functions of enzymes, particularly in bacterial metabolism, it is often helpful to distinguish between *assimilatory* and *dissimilatory* electron transfer processes. Assimilatory processes are intended to synthesize nutrients for growth, such as the reduction of nitrate to ammonium for amino acid biosynthesis. Dissimilatory electron transport generates energy for growth, such as the reduction of nitrate to N_2 gas in the denitrifying bacteria. There are of course some enzymes with functions that do not fall into either category, such as degradative reactions for disposal of unwanted compounds. Generally the dissimilatory enzymes will have a greater activity within the cell than do the assimilatory enzymes, which has assisted their isolation. The enzymes of the assimilatory pathways tend to be present in a wider variety of cells, and present all the time, whereas the dissimilatory pathways are more specialized and are induced by growth substrates. This is another reason why an organism may express two or more isoenzymes catalyzing the same reaction. Different versions of the enzyme may be adapated for specific pathways.

FERREDOXINS AND OTHER ELECTRON TRANSFER PROTEINS

Ferredoxins and rubredoxins are electron transfer proteins containing iron–sulfur clusters, without enzymatic function. Their functions are to some extent interchangeable, as seen, for example, in the range of ferredoxins used by mono- and dioxygenase systems and by oxoacid : ferredoxin oxidoreductases (see below). The variations of the sequences of ferredoxins are discussed in detail by Matsubara *et al.*, this volume.

1. Rubredoxins

Rubredoxins are characterized by having single iron atoms without labile sulfide. They are classed as iron–sulfur proteins since the iron is ligated entirely by cysteine sulfurs, four per iron atom (designated [Rd]). One of the few known functions of this protein is as intermediate electron carrier in ω-hydroxylation of fatty acids, for which a unique rubredoxin with two rubredoxin domains is used (*34*). In *Clostridium perfringens,* a function in electron transfer to nitrate reductase has been suggested (*35*).

2. [2Fe–2S] Ferredoxins

The [2Fe–2S] ferredoxins were first identified as photosynthetic electron transfer proteins in chloroplasts of plants, such as spinach, and in cyanobacteria. The chloroplast ferredoxins have been found to act as electron donors to several different enzymes, including nitrite reductase and glutamate synthase (*36*).

Other ferredoxins, which act as electron donors to cytochrome *P*-450, were isolated from animal tissues and bacteria. The naming of these proteins according to their source, such as adrenodoxin and putidaredoxin, has become established in the literature. The IUB Nomenclature Committee (*33*) prefers the terms "adrenal ferredoxin" and "*Pseudomonas putida* ferredoxin," and discourages the proliferation of further names. The homology of the sequences with the plant-type ferredoxins is very weak, but the [2Fe–2S] cluster-binding cysteines still occur in a similar arrangement. It is likely that these distantly related proteins have a similar folding pattern, as shown by recent two-dimensional NMR measurements (*37, 38*). The [2Fe–2S] ferredoxins of kidney and adrenal glands, like many eukaryotic proteins, undergo phosphorylation, which modulates their activity (*39, 40*).

A protein that is clearly homologous to the plant ferredoxins has been found in *Rhodobacter capsulatus,* although this organism does not have the type of photosynthetic NADP reduction that is found in plants (*41*). Its activity appears to be associated with nitrogen fixation. This purple photosynthetic bacterium has four ferredoxins, including two 2[4Fe–4S] ferredoxins and a [4Fe–4S][3Fe–4S] ferredoxin (*42–44*).

An unrelated [2Fe–2S] protein has been isolated from nitrogen-fixing *Clostridium pasteurianum* (*45*). The arrangement of cysteines in its sequence (Fig. 2) is so different from those in the plant-type ferredoxins (Fig. 2) that it probably has a completely different folding pattern (*46*). The function of this protein is unknown but it may resemble the [2Fe–2S] ferredoxin II from *A. vinelandii,* the function of which appears to be to bind to nitrogenase, protecting it from oxygen (*47*).

3. *Rieske Proteins*

Rieske proteins contain specialized [2Fe–2S] clusters with relatively high reduction potentials (0–300 mV). They were first isolated from mitochondrial complex III (*48*), but similar proteins were found in other types of membrane electron transfer chains. The [2Fe–2S] clusters of aromatic dioxygenases, which have somewhat more negative reduction potentials, appear to be similar (*49*). Spectroscopic studies have demonstrated that one of the iron atoms in the [2Fe–2S] cluster of the Rieske protein has two histidine nitrogen ligands instead of cysteines (*50*).

4. *2[4Fe–4S] Ferredoxins*

The 2[4Fe–4S] ferredoxins are primarily found in bacteria, though a similar ferredoxin has been found in *Entamoeba histolytica* (*51*). Different organisms use the 2[4Fe–4S] ferredoxins for different purposes, as low-potential electron carriers, particularly in anaerobic metabolism, and they have probably more different functions than any other iron–sulfur protein. The simple sequence of the 2[4Fe–4S] ferredoxins has almost twofold symmetry (Fig. 1), and may have been the evolutionary precursor of the [4Fe–4S][3Fe–4S], the [4Fe–4S], and the [3Fe–4S] ferredoxins (*12*), and similar sequences in many enzymes. Variations on the sequence are observed, such as insertion of a loop between the second and third cysteines, and the additional C-terminal extension in *A. vinelandii* ferredoxin I.

5. *[4Fe–4S] Ferredoxins*

The [4Fe–4S] ferredoxins can be considered as having evolved from the 2[4Fe–4S] type by the loss of two or more of the cysteines that bind the second cluster, Fig. 1c (*52*). The iron–sulfur clusters generally are similar in reduction potentials to those in the 2[4Fe–4S] ferredoxins and have a similar range of functions.

6. *[4Fe–4S][3Fe–4S] Ferredoxins*

The [4Fe–4S][3Fe–4S] ferredoxins require a minimum of seven cysteines for binding the clusters (Fig. 1b). This arrangement is found in ferredoxin from *Sulfolobus acidocaldarius* (*53*). Other 7Fe ferredoxins, such as the well-studied ferredoxin from *A. vinelandii,* contain more cysteines, in nonconserved positions, which do not bind the cluster (54, 55). Electrochemistry of *Desulfovibrio africanus* ferredoxin III indicated that on reduction it could incorporate an eighth iron ion to convert the [3Fe–4S] cluster to a [4Fe–4S] cluster. In this case, a noncysteine ligand is expected to bind the cluster, and an appropriately located aspartate was suggested (*56*) (see Armstrong, this volume).

7. *[3Fe–4S] Ferredoxins*

The [3Fe–4S] ferredoxins have been isolated and studied from a number of species. The most intensively studied is *D. gigas* ferredoxin II, for which the structure has been determined (*57*). The protein contains a total of six cysteines and appears to be an oxidized form of the *D. gigas* [4Fe–4S] ferredoxin I. The only proteins found to have the minimum three cysteines for the [3Fe–4S] cluster are the two ferredoxins of *Streptomyces griseolus*. Attempts to reconstitute the latter ferredoxins with [4Fe–4S] clusters were unsuccessful (*58*). *Desulfovibrio gigas* and *Pyrococcus furiosus* ferredoxins are prototypes for the [3Fe–4S] clusters, for which no chemical models have been synthesized.

Other metal ions, including zinc, cobalt, or nickel, may be incorporated into the reduced clusters, producing [3FeM–4S] clusters (*59–61*).

8. *High-Potential Iron–Sulfur Proteins*

HiPIPs, found in purple sulfur bacteria and some other species, are unusual in that their [4Fe–4S] cluster undergoes oxidation–reduction between the $[4Fe–4S]^{2+}$ and $[4Fe–4S]^{3+}$ oxidation states. As a result they have relatively high redox potentials and give EPR signals in the oxidized state. A long-standing anomaly in the EPR spectrum of the high-potential iron–sulfur protein of *Chromatium vinosum* was the appearance of several minor species that looked like other types of clusters or degraded forms. These occurred even in protein that was pure enough to be crystallized. These effects have now been explained (*62*) in terms of dimerized forms of the protein that occur in frozen EPR samples. This effect may also explain the EPR spectra of other ferredoxins that appear to contain more than one component, e.g., the ferredoxin of the benzene dioxygenase system (*63*).

IV. Membrane-Bound Iron–Sulfur Enzymes of Bioenergetic Systems

A. RESPIRATORY CHAINS

Membrane-bound electron transfer proteins that interact with quinones include hydrogenase, formate dehydrogenase, and succinate dehydrogenase, and, on the oxidizing side, nitrate reductase, fumarate reductase, and DMSO reductase. All of these proteins have a similar organization, 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 intermediary electron carriers, are situated between these two components. In the structure of glycerol phosphate dehydrogenase from anaerobic *E. coli,* there are two [4Fe–4S] clusters in the hydrophobic C subunit as deduced from the sequence of the *glpABC* operon (*64*).

Mitochondrial complex I (NADH : ubiquinone reductase), probably the most elaborate iron–sulfur protein, is encoded by more than 30 structural genes, many of which have been sequenced (*65*). Several of these subunits are encoded by the mitochondrial genome. Counterparts of complex I are found in bacterial respiratory chains. The protein not only transfers electrons, but pumps protons across the mitochondrial inner membrane (*66*). In purple photosynthetic bacteria, a similar enzyme operates in reverse for the energy-dependent reduction of NAD^+. Proteins homologous with the sequences occur widely, for example, in chloroplasts (*67, 68*). The protein may be considered as being assembled from two parts—a flavoprotein and a membrane-bound iron-sulfur protein (*69*). The flavoprotein subunit of the soluble NAD-binding hydrogenase of *Alcaligenes eutrophus* is homologous with the flavin mononucleotide (FMN)-binding flavoprotein subunit (*70, 71, 256*). Complex I contains at least four EPR-detectable iron–sulfur clusters and there are probably more that are EPR silent. The sequences of subunits indicate that the 51-kDa subunit contains FMN and a [4Fe–4S] cluster, and the NADH-binding site. Iron–sulfur clusters are also found in the 75-, 24-, and 23-kDa subunits (M_r of the bovine heart enzyme).

Complex III of the mitochondrial respiratory chain (ubiquinol : cytochrome *c* reductase) contains one iron–sulfur cluster in the Rieske protein. Similar proteins have been identified in plant mitochondria (*72*), in the cytochrome b_6f complex of chloroplasts and cyanobacteria, and in respiratory and photosynthetic bacteria (*73, 74*). The [2Fe–2S] cluster of the protein appears to interact with quinones, as indicated by changes in the line shape of the EPR signal when quinone, quinol, or quinol-type inhibitors are added (*75*).

B. PHOTOSYSTEM I

In the photosynthetic electron transfer chain of plants and cyanobacteria, the primary electron acceptor complex of photosystem I 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 (*76, 77*). In this complex, three iron–sulfur clusters have been identified by EPR spectroscopy.

The iron–sulfur cluster X (*78*) is proposed to be a [4Fe–4S] cluster bound between the two subunits ps1A1 and ps1A2 (products of the *psaA* and *psaB* genes). Each of these 83-kDa proteins contains two cysteines that are conserved in all species and that are thought to bind to the cluster. Interestingly, the cysteines are closely preceded in the sequence by a leucine zipper motif (*79*). This motif, consisting of regularly spaced leucine residues, is found in DNA-binding proteins and other proteins, where it helps to stabilize heterodimers (*80*). In photosystem I it is proposed that a leucine zipper helps to orient the cysteines in order to bind the [4Fe–4S] cluster. Cluster X has an exceptionally negative redox potential, −705 mV (*81*).

The terminal electron acceptors of photosystem I are iron–sulfur clusters A and B. These clusters have recently been identified with a

9-kDa subunit, encoded by the *psaC* gene (*82–84*). This protein shows a strong homology with the bacterial 2[4Fe–4S] ferredoxins. A number of iron–sulfur clusters appear to be encoded by the chloroplast genome, which has been sequenced from several species of plants. In the liverwort *Marchantia polymorpha* three open reading frames were identified. The *frxA* (identified with *psaC*) and *frxB* genes also occur in the higher plant tobacco (*Nicotiana tabacum*) chloroplast DNA; both have the characteristic 2[4Fe–4S] sequence type (Fig. 1). The gene *frxC* of *M. polymorpha* exhibits a high degree of homology with the *nifH* gene of nitrogen-fixing bacteria (*85*) (see Matsubara, this volume).

V. Soluble Proteins with Iron–Sulfur Clusters

Iron–sulfur clusters are single-electron carriers and they interact with other electron-carrying centers, such as heme or nonheme iron, molybdenum, nickel, and corrinoids. Redox reactions of iron–sulfur clusters with organic cofactors, such as flavin, quinone, or thiamin diphosphate, generally involve the formation of free radicals. The iron–sulfur proteins that interact with pyridine nucleotides (NAD or NADP) do so through the intermediary of a flavoprotein (*3*). The classic example is the reduction of NADP by the [2Fe–2S] ferredoxin in plant photosynthesis. The structure of the intermediary flavoprotein, ferredoxin : NADP reductase, was recently determined (*86*) and provides the prototype for the flavin-binding domains of some iron–sulfur flavoproteins (*87*). Amino acid sequence homologies have been observed between ferredoxin : NADP reductase and the flavoprotein components of NADPH : sulfite reductases of *E. coli* and *Salmonella typhimurium* (*88*).

A group of enzymes, the oxoacid : ferredoxin oxidoreductases, catalyze the oxidative conversion of pyruvate and 2-oxoglutarate to acyl-CoA and succinyl-CoA, respectively, with the elimination of CO_2. The 2-oxoacid : ferredoxin oxidoreductases of anaerobic bacteria contain [4Fe–4S] clusters and thiamin diphosphate (*89*). In methanogens, acetogens, and some photosynthetic bacteria, the enzyme operates in reverse for the fixation of CO_2 (*90*). Various types of ferredoxins or flavodoxins are used as electron acceptors or donors: the 2[4Fe–4S] in *Clostridium*; flavodoxin in *Klebsiella*; and [2Fe–2S] ferredoxins in *Halobacterium* (*91*), in cyanobacteria, and in the protozoon *Trichomonas vaginalis* (*92, 93*).

A. OXYGENASE SYSTEMS WITH IRON–SULFUR CLUSTERS

Many hydroxylation reactions in biology use the insertion of one or both atoms of the dioxygen molecule into a substrate, with a concomitant oxidation of NAD(P)H. Examples of different types of such oxygenases, of which many are now known, are given in Table I.

1. *Monooxygenases*

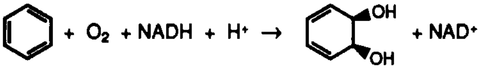
The monooxygenases catalyze the following reaction:

$$\mathrm{R \cdot H + O_2 + NAD(P)H + H^+ \rightarrow R \cdot OH + NAD(P)^+ + H_2O}$$

R · H may represent many types of aliphatic or aromatic molecules. These so-called mixed-function oxidases consist of a hydroxylase center, such as cytochrome *P*-450 or nonheme iron, and a short electron transfer chain that brings reducing equivalents from NAD(P)H. The electron transfer chain includes a flavin and often one or more iron–sulfur clusters of the [2Fe–2S] type. The iron–sulfur cluster may be either a separate ferredoxin, as in the *P*-450 systems, or part of an iron–sulfur flavoprotein that interacts with NAD(P) directly. In some cases the amino acid sequences of the iron–sulfur flavoproteins betray an ancestral relationship to the plant-type ferredoxins (*94*). The ferredoxins are usually [2Fe–2S] proteins, like adrenal ferredoxin or *Pseudomonas putida* ferredoxin. However, there are examples of ferredoxins with [4Fe–4S] and [3Fe–4S] clusters with this function. Two ferredoxins of the [3Fe–4S] type have been identified in the sulfonylurea monooxygenase systems in *S. griseolus* (*58*). A ferredoxin with a [4Fe–4S] cluster acts as the intermediary electron carrier to the steroid monooxygenase of *Bacillus megaterium* (*95*). [4Fe–4S][3Fe–4S] ferredoxins participate in the cytochrome *P*-450 of *Sacchoropolyspora erythraea* involved in erythromycin biosynthesis (*96*), and in cytochrome P-450$_{\mathrm{soy}}$ of *Streptomyces griseus* (*97*).

2. *Dioxygenases*

In the dioxygenases considered here, the substrate is aromatic and the product is a *cis*-dihydrodiol:



The structures of the bacterial aromatic dioxygenases have been reviewed recently (*98, 99*). Each has a large iron–sulfur dioxygenase component, in which the hydroxylation is carried out at a mononuclear nonheme iron site. The dioxygenase component also contains a [2Fe–2S] cluster that is similar in spectroscopic properties to those of the Rieske proteins (*99, 100*). The 4-methoxybenzoate-*O*-demethylase is usually a monooxygenase, but has some dioxygenase activities and structurally falls into this category (*101*).

The NADH-dependent reductase chains of these dioxygenases are heterogeneous in composition (Table I). They consist of either a flavoprotein or an iron–sulfur flavoprotein, and in some cases a ferredoxin. The EPR spectrum of the NADH-reduced iron–sulfur flavoprotein, phthalate dioxygenase reductase, shows a flavin radical and a rhombic signal resembling that of spinach ferredoxin (*98*). The structure of the protein has been determined (*98*). The iron–sulfur flavoproteins of the benzoate and toluate dioxygenases comprise an N-terminal sequence resembling the plant-type ferredoxins (*102a*), and a C-terminal sequence resembling other flavoproteins, including NADP: ferredoxin oxidoreductase (*100*). It is of interest that benzoate dioxygenase reductase is one of the few complex iron–sulfur proteins in which, like the simple ferredoxins, the iron–sulfur cluster has been assembled *in vitro* from iron salts and inorganic sulfide (*102*). The sequences of the ferredoxin components of benzene and toluene dioxygenases do not resemble the plant ferredoxins but show some similarities to the Rieske proteins (*102a–105*) (Fig. 2).

B. IRON–SULFUR PROTEINS WITH MOLYBDENUM AND TUNGSTEN

With the exception of Mo nitrogenase, all of the molybdenum-containing iron–sulfur proteins have their molybdenum in the form of the molybdopterin cofactor (*106, 107*). This cofactor contains a pterin, and in bacterial molybdo proteins it also contains a guanidine or cytidine nucleotide group (*108, 109*). The molydo-iron–sulfur proteins include oxidoreductases such as nitrate, formate, and DMSO reductases (*110*), and the molybdenum hydroxylases, which include xanthine dehydrogenase and aldehyde oxidase (*111*). The former proteins have multiple [4Fe–4S] clusters whereas the latter have two [2Fe–2S] clusters.

In the molybdenum hydroxylases, the molybdenum serves to introduce the —OH into the substrate; the oxygen derives from water rather than dioxygen. The sequences have been determined of the xanthine dehydrogenases from *Drosophila* (*112, 113*) and from rat liver (*114*). They are unusual in that the two [2Fe–2S] clusters appear to be bound in a relatively short, cysteine-rich peptide sequence near the N terminus. One group of cysteines conforms to the plant-type ferredoxin cluster-binding motif, but the other has different spacings. The latter has been tentatively assigned to the second cluster, Fe–S II, which has anomalous EPR behavior (*115*).

The formate dehydrogenases of bacteria contain, in various combinations, iron–sulfur clusters, molybdenum, tungsten, selenium, and flavin (*110*). These differences reflect their diverse functions. In *E. coli,* there are two alternative membrane-bound enzymes for the oxidation of formate to CO_2, one operating in the formate–hydrogen lyase pathway and in other respiratory pathways to fumarate and nitrate. In clostridia, formate dehydrogenases are soluble proteins and operate in the reverse direction, as a ferredoxin : CO_2 reductase, for fixation of carbon dioxide (*116*). The enzyme of *Clostridium thermoaceticum* contains tungsten instead of molybdenum (*117*).

C. NICKEL HYDROGENASES

Hydrogenases are enzymes that catalyze the oxidation or production of hydrogen with various electron acceptors or donors. The enzymes isolated from many bacterial species contain nickel and iron–sulfur clusters. Another class (see below) contains a specialized iron–sulfur cluster instead of the nickel center. The nickel-containing hydrogenases have been reviewed (*118–120*). In these enzymes, the nickel appears to be in a separate site, coordinated by two to four cysteine sulfurs, plus oxygen and nitrogen ligands from the protein. It is probably at this site that the hydrogen reacts. In some cases, selenium is also present in the enzyme, which changes some of the catalytic properties. It has been demonstrated in some enzymes that the selenium is present as selenocysteine and is a ligand to the nickel site (*121, 122*). The iron–sulfur clusters in the enzymes, and in some cases flavin or heme, serve to transfer electrons between the nickel site and donors or acceptors such as ferredoxin, NAD, deazaflavin, quinone, or cytochrome *c*. This leads to a considerable variety in the composition of the hydrogenases, though they all have a common basic structure with large and small subunits (see Voordouw, this volume).

D. SIROHEME IRON–SULFUR PROTEINS

Sulfite and nitrite reductases of plants and bacteria contain a specialized chlorin group known as siroheme and an iron–sulfur cluster. The NADPH-dependent assimilatory sulfite reductase of *E. coli* also has a flavoprotein subunit. Mössbauer and EPR spectra have been interpreted in terms of a heme iron antiferromagnetically coupled to a [4Fe–4S] cluster (*123*). A low-resolution electron density map indicated the presence of an iron–sulfur cluster at a distance of 0.44 nm from the heme iron (*124*).

The ferredoxin : nitrite reductase of plants is also a siroheme-containing iron–sulfur protein (*125, 126*). The sequence of the protein shows homologies with the *E. coli* sulfite reductase iron–sulfur siroheme subunit. The two proteins are also similar in their spectroscopic properties (*127*).

The assimilatory nitrite reductase from *E. coli* appears to be different. The sequence of this protein contains a cysteine-rich region and a flavin-binding motif, but does not show homologies to other nitrite and sulfite reductases (*128*). It was found to contain flavin and about four iron atoms/mole. Only a [2Fe–2S] cluster was detected by EPR (*129*), though the possibility of other clusters with high-spin states cannot be ruled out.

E. CATALYTIC IRON–SULFUR CLUSTERS AND MIXED-METAL CLUSTERS

1. *Nitrogenases*

Nitrogenases catalyze the reduction of dinitrogen to ammonium. They comprise a specialized group of proteins with unique types of iron–sulfur clusters. The biochemistry of nitrogenases has been extensively reviewed elsewhere (*130–134*). The molybdenum–iron protein of nitrogenase has been known for many years. Recently, "alternative" nitrogenases, one containing vanadium and iron, the other only iron, have been found. Comparison of these alternative nitrogenases with the Mo nitrogenase provides some informative insights (*135*). They all contain a specialized metal cluster that is probably the site of reduction of N_2 to ammonium. In the Mo nitrogenase, the Fe–Mo cofactor can be extracted and analysis has indicated a composition of $Fe_{5-7}MoS_{4-8}$ (*131, 133*). In the V nitrogenase the composition is $Fe_{5-7}VS_{4-6}$.

The structures of all three nitrogenases are similar, and all require a similar set of genes for cofactor biosynthesis. Probably all of the nitrogen-reducing cofactors involve the organic component homocitrate, although the structures have not yet been resolved. Spectroscopic measurements and consideration of the amino acid sequences of a variety of nitrogenases have indicated that only one cysteine is involved in binding the cofactor to the protein, the other ligands being oxygen and nitrogen. Three to four invariant cysteines in the α subunit and three in the β subunit are available for binding the P-cluster(s).

The nitrogenase $\alpha\beta$ components contain iron–sulfur clusters of unique properties, known as the P-clusters, comprising six to eight iron and labile sulfide atoms. The P-clusters were originally considered to be two [4Fe–4S] clusters with unusual redox behavior, but now it seems possible that they represent a single cluster with six to eight iron atoms. A low-resolution structure of the molybdenum–iron protein shows the

P-clusters as an elongated region of electron density, located about 1.9 nm from the Mo–Fe cofactor (*136*).

Nitrogenase reductase, or the iron protein, is the second essential component of the nitrogenase system. It acts as a low-potential, ATP-dependent electron donor to the dinitrogen-reducing protein. Analyses have indicated the presence of one [4Fe–4S] cluster per dimer of two identical subunits. Furthermore, site-directed mutagenesis experiments have suggested that two cysteines from each subunit are required to bind the cluster. The location of this cluster between the subunits has been confirmed by crystallographic studies of Rees, Georgiadis, and Chakrabarti (see Ref. *137*). A possible ATP-binding site has been located in a cleft between the two subunits. This type of electron transfer protein may have a wider application, as indicated by the homologous sequence of the *frxC* gene in the chloroplast genome of *M. polymorpha* (*85*). It seems likely that such an ATP-driven electron donor would be useful in other enzyme systems.

2. *Iron Hydrogenases*

The so-called [Fe] hydrogenases, which catalyze the production and consumption of hydrogen gas, also contain a specialized iron–sulfur cluster known as the H-cluster. (Their sequences and structure are discussed by Voordouw, this volume.) The relationship of the H-cluster to the active site clusters in nitrogenases, which are also capable of producing hydrogen gas, is at present unknown.

3. *Carbon Monoxide Dehydrogenases*

A number of different types of enzyme are referred to as "carbon monoxide dehydrogenases" (which is a misnomer, as CO does not contain hydrogen; a better term would be carbon monoxide oxidoreductase). They all catalyze the reversible oxidation of CO to CO_2. In aerobic, carboxydovoric bacteria a molybdenum-containing iron–sulfur protein with [2Fe–2S] clusters catalyzes the oxidation of CO.

A second type of CO oxidoreductase, isolated from acetogenic and methanogenic bacteria (*138, 139*), is probably better described as acetyl-CoA synthase. It catalyzes the reductive assembly of acetyl-CoA from a methyl-corrinoid protein, CoA, and either CO or CO_2 (*140*). The reaction is reversible; in acetogenic and some methanogenic bacteria it is used for the synthesis of acetate (*141*), and in acetoclastic methanogenic bacteria it is used for the consumption of acetate, which ultimately is converted to methane (*142*). The anabolic and catabolic acetyl-CoA synthetases differ in their molecular properties but appear to have a similar group of cofactors. EPR has detected the presence of several

[4Fe–4S] clusters and other, more unusual, species. At least one of the iron–sulfur clusters appears to have a spin state $>\frac{1}{2}$, and it has been suggested to be a [6Fe–6S] cluster (*142*). A third EPR-detectable component, seen in the CO-reduced enzyme from *C. thermoaceticum*, shows hyperfine splittings from the isotopes ^{57}Fe, ^{61}Ni, and ^{13}C, known as the Fe–Ni–C cluster (*143*).

In anaerobic photosynthetic bacteria such as *Rhodospirillum rubrum*, there is a nickel–iron–sulfur enzyme that catalyzes the oxidation of CO (*144*) and is probably coupled to the production of H_2, in a reaction analogous to formate–hydrogen lyase (*144a*). Like the acetyl-CoA synthases, the *R. rubrum* enzyme contains several regular [4Fe–4S] clusters, another cluster or clusters with spin states $>\frac{1}{2}$, and a species containing nickel and iron (*145*).

F. NONREDOX ENZYMES

Iron–sulfur proteins in which the iron–sulfur cluster has a nonredox function have recently been reviewed (*146–148*). The majority of them catalyze hydration/dehydration of double bonds. The first representative of this class to be recognized was aconitase. This isomerase was known to be activated by iron. Due to the efforts of Beinert, Kennedy, and co-workers, it was demonstrated that the inactive enzyme contains a [3Fe–4S] cluster that is converted to a catalytically active [4Fe–4S] cluster (*147*). Catalysis is believed to take place at the fourth iron atom, acting as a Lewis acid rather than a redox cluster.

Fumarase (fumarate hydratase) is a familiar enzyme of the citric acid cycle; its reaction is analogous to aconitase (aconitate hydratase). Recently two new fumarases, fumarases A and B, both of which are iron–sulfur proteins, have been recognized in *E. coli* (*149, 150*). They have been termed class I fumarases and bear little homology with the class II fumarase C of *E. coli* or those of aerobic organisms, plants, and animals. Fumarases A and B are thermolabile in comparison with the class II enzymes and are expressed under anaerobic conditions (*149*). They show some sequence homologies with aconitase, in the region of the cysteine residues (*151*).

Glutamine 5-phosphoribosyl-1-pyrophosphate (PRPP) amidotransferase, an enzyme involved in purine nucleotide biosynthesis, contains a [4Fe–4S] cluster. This cluster is very difficult to reduce (*152*) and no catalytic function has been established. The enzyme from *Bacillus subtilis* has been extensively studied. Since the [4Fe–4S] cluster is oxygen sensitive, like many iron–sulfur clusters of enzymes of anaerobic bacteria, it was suggested that a function of the cluster might be to inactivate the enzyme under aerobic conditions (*148*). If this is so, the function of the comparable cluster in human glutamine PRPP amidotransferase (*153*) remains to be established.

Endonuclease III from *E. coli* is an enzyme involved in the repair of various types of damaged nucleotides in DNA. The enzyme also contains a [4Fe–4S] cluster that is readily converted to a [3Fe–4S] cluster by oxidation (*154*). A possible function of the cluster might be in the β-elimination reaction catalyzed by the enzyme (*155, 156*). However, it is not known if the cluster has an accessible iron site as in aconitase; the ligands to the [4Fe–4S] cluster have not been identified in the sequence of the protein (*157*).

Nitrile hydratase, which catalyzes the conversion of nitriles to amides, contains an iron center. The iron is low spin and probably six coordinate. Recent extended X-ray absorption fine structure (EXAFS) and resonance Raman spectroscopic measurements indicate that it is coordinated by two to three sulfurs, the other ligands being oxygen and/or nitrogen. An optical absorption maximum at 710 nm was assigned as a ligand-to-metal charge transfer band (*158*). Therefore, in a broader definition this can be considered an iron–sulfur protein.

G. Iron–Sulfur Proteins in Gene Regulation

A new type of iron-binding protein has recently been isolated from human cells; this iron-responsive element binding protein (IRE-BP) regulates the synthesis of the iron-storage protein ferritin, and the transferrin receptor. The method of regulation is unusual in that the protein binds to the messenger RNA, rather than the DNA, at a sequence/structure motif known as the iron-responsive element (*159*). Excess iron causes an increase in the translation of ferritin mRNA and a decrease in the stability of transferrin receptor mRNA. It is likely that human erythroid 5-aminolevulinate synthase, involved in heme biosynthesis, is regulated in a similar way (*160*). The amino acid sequence of the IRE-BP shows homologies with aconitase; in particular, the three cysteines that bind the [3Fe–4S] cluster are conserved (*161*). Rouault *et al.* (*161*) have proposed that the iron binds to a [3Fe–4S] cluster in the protein, converting it to a [4Fe–4S] cluster, by analogy with the activation of aconitase (*162*). The resulting conformational change would cause the IRE-BP to dissociate from the mRNA. This discovery opens up new possibilities for the functions of iron–sulfur proteins.

Green *et al.* (*163*) have proposed a role for iron in the action of the FNR (fumerate/nitrate reductase) regulatory protein in *E. coli*. This protein acts at the DNA level and controls the anaerobic/aerobic switch of many enzymes (*164*). The sequence of the FNR protein comprises a DNA-binding domain at the C terminus and a cysteine-rich region at the N terminus. Specific mutagenesis has shown that cysteines 20, 23, 29, and 122 are necessary for its function. The form of iron binding to the protein, if any, is not known at present.

H. IRON–SULFUR PROTEINS OF UNCERTAIN FUNCTION

The "polyferredoxin" from methanogenic bacteria is a remarkable protein that was first identified from the sequence of an open reading frame, *mvhB*, in *M. thermoautotrophicum* (*165*). The sequence of polyferredoxin contains six tandem repeats of four cysteines, each resembling a bacterial 2[4Fe–4S] ferredoxin, and is predicted to contain 48 iron atoms in 12 clusters. The intervening sequence was predicted to be an amphipathic α-helix that could attach to a membrane or form a membrane pore. The observation that this gene is linked to the structural genes of the "methylviologen-reducing" hydrogenase, for which the acceptor has not been determined, suggests a function in electron transport in hydrogen-dependent formation of methane.

Two interesting nonheme iron proteins have been isolated from *Desulfovibrio* spp. One, named rubrerythrin by its discoverers, was isolated from *D. vulgaris* (*166*). It contains a rubredoxin-type iron center and a binuclear iron cluster that resembles the μ-oxo-bridged iron dimer in acid phosphatase (*167*). The protein has been recently found to have pyrophosphatase activity (*168*). The other, termed "desulfoferrodoxin," contains both a rubredoxin-type iron center and another iron center (*169*).

VI. Concluding Remarks

From consideration of the primary and tertiary structure of the iron–sulfur proteins determined so far, it is possible to discern a number of distinct structural themes. Most of the complex proteins are constructed from domains, some of which are related to the ferredoxin-like sequences and Rieske iron–sulfur proteins. These classes are indicated in Table I. Other proteins appear to be unrelated, including HiPIP, rubredoxins, Fe–S clusters of dehydratases, the H-cluster domain, the [4Fe–4S] clusters of siroheme-containing proteins, nitrogenases, and the nitrogenase reductases.

The present draft nomenclature of iron–sulfur clusters as [4Fe–

4S]$^{2+/1+}$, etc., has proved valuable in specifying the cluster types, avoiding the confusion that has befallen the hemoproteins, for example. It has been sufficiently flexible to encompass new types of clusters, such as the [3Fe–4S] cluster. It can cope with mixed-metal clusters such as [3FeNi–4S], although the terminology for clusters with noncysteine ligands needs clarification. The attempt to divide the conjugated iron–sulfur proteins into iron–sulfur flavoproteins, iron–sulfur molybdoproteins, etc., is less successful, as it leads to artificial divisions. As can be seen from Table I, the possible combinations of iron–sulfur clusters and other centers are numerous, and the resulting groupings are often unrelated to structure or function. Iron hydrogenase and aconitase have little in common with each other, apart from containing [4Fe–4S] clusters. The molybdenum nitrogenase has greater affinities with the vanadium and iron nitrogenases than with other molybdenum-containing proteins, such as xanthine dehydrogenase. In the latter protein it appears that the iron–sulfur clusters are acting as secondary electron carriers, whereas the flavin and molybdenum react with the actual substrates. Indeed, from recent experiments on mutants of *Drosophila* xanthine dehydrogenase, it seems that the enzyme can transfer electrons from xanthine to O_2 quite well without the iron–sulfur clusters (*170*). A more satisfactory classification would take into account (1) function, as in the Enzyme Commission (EC) classification of enzymes, (2) the protein family, and (3) the cofactor composition.

With new tools for detecting and characterizing iron–sulfur clusters, we are becoming more aware of the versatility of the iron–sulfur proteins. The iron–sulfur enzymes with specialized functions are especially prevalent among the bacteria, owing to their diverse metabolism. This is particularly the case for the strict anaerobes, wherein the sensitivity of iron–sulfur clusters to oxygen is not a disadvantage. Hence there are the iron–sulfur equivalents of enzymes such as fumarase, which in aerobes are not metalloproteins. These iron–sulfur proteins are especially difficult to isolate in the intact state. Gene sequences have revealed new ferredoxins and ferredoxin-like regions within larger proteins. Some of the cluster-binding regions are not readily detected from their cysteine motifs, and are only apparent when the tertiary and even quaternary structures of proteins are examined. Many new iron–sulfur proteins, and probably some surprising new functions, wait to be discovered.

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