

REVIEW ARTICLE

A tribute to sulfur

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Recent progress in a number of areas of biochemistry and biology has drawn attention to the critical importance of sulfur in the biosynthesis of vital cofactors and active sites in proteins, and in the complex reaction mechanisms often involved. This brief review is intended as a broad overview of this currently rapidly moving field of sulfur biochemistry, for those who are interested or are involved in one or the other aspect of it, a synopsis by one who has stumbled into this field from several directions in the course of time. Only for iron are metal–sulfur relationships discussed in detail, as the iron–sulfur subfield is one of the most active areas.

Keywords: Fe–S cluster biosynthesis; Fe–S cluster interconversions; sulfane sulfur; sulfurtransferases; sulfur insertion; adenosylmethionine.

Among the close to 12 nonmetallic elements essential for life, sulfur stands out because of its astounding chemical versatility. Within the past 5 years, several papers have appeared originating from quite different areas of biochemistry or biology, which have discussed or proposed unusual reaction pathways involving sulfur, apparently made possible by the specific properties of this element. In fact, as early as 1989 a review article appeared, entitled ‘Sulfane Sulfur in Biological Systems: A Possible Regulatory Role’ [1] which, from today’s viewpoint, almost appears visionary. There, the chemistry of sulfane sulfur is ably presented and the biological implications known at that time, are described. In the preface to a volume from a Symposium held in 1995 on the theme, ‘Transition Metal Sulfur Chemistry, Biological and Industrial Significance’ [2] we read: “The redox and reactive character of the sulfur combined with that of the transition metal, leads to versatile chemistry that has been exploited both industrially and biologically.” More recently attention has been drawn to disulfide, ‘Nature’s Transitory Covalent Bond’ [3], its significance in protein folding, and to ‘Protein Sulfenic Acids: Diverse Roles for an Unlikely Player in Enzyme Catalysis and Redox Regulation’ [4]; sulfur protonation in $[3\text{Fe}-4\text{S}]^{0,2-}$ clusters has been proposed [5]; and several authors have suggested a role for the μ_3 sulfide (μ_3 indicates the number of metal ions connected by a multivalent ligand) of Fe–S clusters in the catalysis of homolytic reactions, such as in pyruvate-formate lyase (PFL), anaerobic ribonucleotide reductase (ARNR), 2,3-lysine amino mutase (LAM), biotin synthase (BS) and the thioredoxin reductase (FTR): thioredoxin

(TRX) system; the significance of sulfur-delivering proteins for the synthesis of Fe–S clusters in proteins or of thiouridine has been recognized; and in the synthesis of thiamine, sulfur is made available for the closure of the heterocyclic ring via a thiocarboxylate formed in an ATP-dependent manner with the aid of several peptides; according to sequence similarities, a similar mechanism is probably also used in the biosynthesis of molybdopterin, while lipoic acid biosynthesis is thought to proceed in a way similar to that of biotin. In addition, we have learned from studies on microbes and eukaryotic cellular systems that there seems to be continuous synthesis, disassembly and rebuilding of Fe–S clusters in proteins; in eukaryotic systems this occurs in a compartment-specific manner, so that the building blocks, and in some cases even Fe–S clusters themselves or their precursor-structures may have to pass through membranes.

The present article will contain elements of all the areas mentioned with respective references. If it were only for the chemical aspects of sulfur or more specifically sulfane sulfur, there would be little to add to Toohey’s review [1] and the symposium volume on transition metal sulfur chemistry [2]. However, as far as understanding the biological implications, there has been unforeseen progress in the years since these papers appeared. This indeed verifies Toohey’s predictions, although with the emphasis less on regulation, than on the general significance of the subject. Thus, it seems timely to attempt a synopsis over the wide range of information now available in the various areas for the benefit of those on either side of the chemistry/biology boundary. About 50 years ago we awakened to the overriding importance of the ‘high energy phosphate’ in energy-providing pathways; are we now becoming aware of ‘reactive sulfur’, as one may call it for the sake of argument, in pathways that involve difficult bio-organic or inorganic syntheses?

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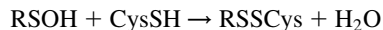
Abbreviations: AdoMet, adenosylmethionine; ARNR, anaerobic ribonucleotide reductase; BS, biotin synthase; C-DES, cysteine desulfhydrase; c, cytoplasmic; Fd, ferredoxin; FNR, fumarate, nitrate reduction (regulator); FTR, ferredoxin:thioredoxin reductase; IRE, iron responsive element; IRP, iron regulatory protein; LAM, 2,3-lysine aminomutase; m, mitochondrial; PFL, pyruvate-formate lyase; TRX, thioredoxin; UTR, untranslated region.

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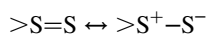
CHEMICAL ASPECTS

While phosphorous, the next neighbor of sulfur in the periodic system, accomplishes its tasks in biological systems by ionic reactions, in the form of its oxo-anions, sulfur, in contrast, in its most oxidized form, namely sulfate, is of limited use to higher organisms except for sulfation and detoxification reactions.

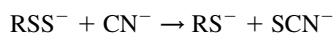
Rather, it is the chemical versatility of the lower oxidation states of sulfur that is vital in nature for anabolic reactions, with sulfenate (SO^-) maybe being at the upper limit of oxidation compatible with its use, as it can be readily reclaimed via the reaction:



Sulfenate has indeed been found to occur in proteins as a catalytically essential redox center or as an intermediate, sufficiently stable to be identified. This has been reported in NADH peroxidase and NADH oxidase, in peroxiredoxins and in nitrile hydratase. An excellent review on this topic is found in [4]. The main importance of the forms of sulfur discussed in the present review lies in their mobility, their use in metal cluster formation and initiation of radical reactions. In the context of our theme, the species to be considered are then S_8 (elemental sulfur), S^{2-} , $\text{R-S-S}_n\text{-S-R}$ ($n \geq 3$, polysulfide), RS_2H (sulfane), and $\text{S}_2\text{O}_3^{2-}$ (thiosulfate) and alkylthiosulfonates, $\text{RS(O}_2\text{)S}^-$. The sulfur in the four last-mentioned compounds is often called sulfur zero, S^0 ; however, this is true in a formal sense only and there are objections to this nomenclature, because zero-valent sulfur does not exist. Sulfur has an overriding tendency to self-associate and is not found as a single atom. This tendency goes so far that, when the chain is long enough that the ends can touch, rings of S_8 are formed. Elemental sulfur as such is not soluble and does not even seem accessible to microorganisms; the well-characterized 'sulfur' reductases, are in fact polysulfide reductases [6]. Sulfur or sulfides, however, are rapidly incorporated into polysulfide chains or into rings or exchanged between similar compounds. Clearly, juxtaposition of sulfur atoms leads to an activation of sulfur, so that it becomes more available for chemical reactions. We may look at activated sulfur as a resonance hybrid between the limiting structures:



with the zwitter-ion character predominating. If the thiosulfoxide form (left) could exist, it would be the starting point of a branched sulfur chain. However, elemental sulfur or polysulfides have never been found to form stable branched chains and there is no evidence that a stable thiosulfoxide or branched chains occur in biological sulfur compounds. However, from chemical studies on specifically selected models, there is evidence that the thiosulfoxide form can be sufficiently stabilized to be identifiable [7]. The prototypes of compounds containing reactive sulfur are those with the motive RS_nH ($n \geq 2$), where R can be a compound of low molecular mass or a protein and, as in all compounds of the type RSH , in aqueous solution at or above neutral pH, ionization can readily occur. RSSH or RSS^- is often called a persulfide, although this nomenclature is not officially recognized. The official name for any compound where the motif RSSH or RSS^- appears is 'sulfane'. While sulfanes in small molecules are fairly labile, when on a protein they can be protected and transported to sites of biosynthesis. The reactivity of sulfane sulfur with CN^- has become the criterion for sulfane sulfur, and operationally sulfane sulfur is defined as sulfur that reacts, at pH 8.5–10, with CN^- with formation of thiocyanate [8,9]:



Another type of compound must also be considered here, which contains cyanolyzable, i.e. reactive sulfane sulfur; these are compounds in which the sulfur-bonded carbon in a disulfide is adjacent to a double-bonded carbon such as in $\text{R-S-S-CH}_2\text{-CH}=\text{CH-R}$ or $\text{R-S-S-CH}_2\text{-CO-CO}_2\text{H}$. This may be important

in some of the reactions to be discussed. It must be pointed out here that the expression 'labile sulfide' (not sulfur) is in use for sulfur that is liberated in the form of H_2S on acidification of a solution containing Fe–S proteins or clusters. It is therefore preferable to use the term 'sulfane' sulfur for the cyanolyzable sulfur. As will be seen below, sulfane sulfur is the precursor of the sulfur that becomes incorporated into Fe–S proteins; it is the transport form for reactive sulfur and the 'currency', so to speak, for reactive sulfur as is ATP for 'high energy' phosphate.

ROLES OF SULFUR IN BIOLOGICAL SYSTEMS

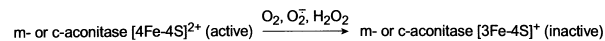
Taking now a somewhat closer look at the various examples mentioned in the introduction, we will search for underlying, unifying principles that one may recognize in the cases alluded to above. Only for organizing the material, two main categories of reactions can be differentiated: those involving metals (which will be called inorganic), by far the majority, and those involving carbon-bound sulfur (organic).

INORGANIC SYSTEMS

For the sake of brevity, the discussion will be limited here to systems involving iron only as the metal partner of sulfur, thus forgoing the consideration of fascinating metal–sulfur chemistry, mostly yet proposals, that occurs in Ni–, Mo–, and Cu–sulfur systems, on which many vital reactions depend, particularly in microorganisms [10–15].

FE–S CLUSTER SYNTHESIS AND DISASSEMBLY

The systems involving Fe–S clusters include those for the apparently continuous assembly, disassembly or rebuilding of Fe–S clusters, with the outstanding examples being cytoplasmic (c)-aconitase/iron regulatory protein (IRP) [16,17], FNR [18,19], and dihydroxyacid dehydratase [20]. As discussed in more detail in recent papers and reviews [21–23], the aconitases lose one Fe readily on exposure to O_2 , or even faster to O_2^- , with formation of the enzymatically inactive 3Fe form $[\text{3Fe-4S}]^+$, which, however, *in vivo* and *in vitro* can be reconstituted to form active $[\text{4Fe-4S}]^{2+}$ enzyme [21–23] (Scheme 1). In addition, c-aconitase alternates as aconitase and,



Scheme 1.

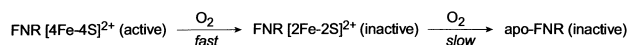


Scheme 2.

after complete loss of its Fe–S cluster, as 'iron-regulatory protein', IRP [24] (Scheme 2) which can bind to iron-responsive elements (IREs) in the untranslated regions of mRNAs and therewith regulate translation. While there is some spontaneous breakdown of the Fe–S cluster of c-aconitase, in the presence of substrate this probably proceeds at too slow a rate to be useful for regulation, except for subtle adjustments. Breakdown is more readily brought about by agents such as NO or H_2O_2 , but it is only *in vivo* that rapid resynthesis ensues from the product(s), whereas the reversal *in vitro*, or even the generation of fully active IRP, requires additional manipulations [25–28]. While it first seemed that iron responsive element (IRE)/IRP systems are unique to eukaryotes

[15], it has now been shown that the aconitases A and B of *Escherichia coli* [29], which are of the c-aconitase, not the mitochondrial (m)-aconitase type, are also able to bind to the 3' untranslated regions (UTRs) of mRNAs, and binding is most effective for the IRP-like apoforms. Of particular interest is the observation that these proteins can actually be bound to their own mRNAs, thus potentially effecting post-transcriptional control of their own synthesis [30]. Specific IREs have not yet been identified in these instances [30].

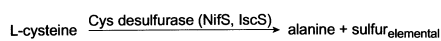
The global transcription regulator FNR of *E. coli* which governs the transition of the organism from anaerobic to aerobic metabolism, alternates between the active, O₂ sensing, holo [4Fe-4S]²⁺ form and the inactive [2Fe-2S]²⁺, and finally the apo-form during O₂ sensing [31] by means of its very O₂ sensitive Fe-S cluster (Scheme 3). Obviously, in all



Scheme 3.

these cases, there must be a continuous cycle of disassembly and reassembly or repair of clusters *in vivo*.

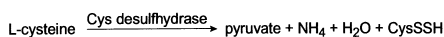
The pathway of Fe-S cluster synthesis has been outlined in the pioneering work of Dean and his collaborators [32] on the synthesis of the Fe-S clusters required for N₂ fixation by *Azotobacter vinelandii*. It had been known for some time [33] that Cys is the original source of the sulfide required. By analysis of the structure of the genes involved it was shown that a pyridoxal-phosphate-containing enzyme, a Cys desulfurase (Scheme 4), NifS, is the key enzyme in providing the sulfur in a



Scheme 4.

reactive form. In fact, NifS is itself the carrier for sulfur, which is present in a sulfane structure attached to a Cys residue of the enzyme in the form RSS*H. Similarly, a protein has been identified that is involved in building up a preliminary iron assembly on three Cys residues, called NifU [34]. NifS and NifU form a loose complex [34,35], and in the presence of additional Fe, e.g. as Fe citrate, a 2Fe cluster can be formed on the NifU protein.

NifS has become the prototype of sulfane sulfur carrier protein in Fe-S cluster synthesis, although enzymes such as rhodanese [36,37] or mercaptopyruvate sulfurtransferase [38] have long been known to be potential sulfane carriers and, as will be discussed below under 'Organic Systems', the function of rhodanese (thiosulfate-cyanide sulfurtransferase) is now seen in a new light. Homologs of *nifS* and *nifU*, which code for proteins less specialized than the Nif proteins are widely distributed in prokaryotes and eukaryotes [39-46]. These proteins, IscS and IscU in bacteria, or Nfs1p and Isu in yeast, are thought to produce and carry sulfane sulfur or provide Fe, respectively, for synthesis. An alternative to the NifS or IscS proteins has been found in *Salmonella* [47] and the cyanobacterium *Synechocystis* [48], in which cysteine-hydrogen disulfide (or persulfide), CysSSH, pyruvate and ammonia are formed from cyst(e)ine by an enzyme called cysteine desulfhydrase, C-DES (Scheme 5). There is evidence that in *Synechocystis* there may be as many as three proteins, including the desulfhydrase, which can provide sulfane sulfur [49]. A



Scheme 5.

homolog of NifS that is specific for selenocysteine has also been reported [50]. Recently, the 3D structure of a NifS homolog of the thermophilic bacterium *Thermotoga maritima* has been determined [51]. Like NifS, it is homodimeric and it was found that the active site Cys residue is located in the middle of a highly flexible loop of 12 amino acids, which may be able to adapt to varied geometries in acceptor proteins. The 3D structure of C-DES from *Synechocystis* has now also become available [52]. Contrary to the NifS type Cys desulfurases, C-DES does not have an active-site Cys of its own, but uses a cysteine residue of its substrate, cystine, to form the cysteine-hydrogen disulfide in a reductive cleavage reaction.

It seems possible that for some Fe-S proteins in single-cell organisms synthesis may proceed spontaneously, if a sulfane carrier associates with a NifU-like protein with free thiol groups in the right configuration, which may also have captured some iron atoms; however, it is more likely that here also specificity and regulation will eventually be found. Fe-S cluster biosynthesis becomes more complicated in eukaryotes, where there are intracellular organelles, which are only accessible through one or more membranes [53-57]. In this case the iron and the sulfur will have to be imported into specific compartments or alternatively, synthesis is completed in one compartment and the Fe-S cluster as such or in a precursor-form has to be exported. Thus, there is a whole set of auxiliary proteins required to synthesize the Fe-S clusters for the enzymes that are vital for survival of the organelle or the whole organism, such as the mitochondrial respiratory enzymes. This has been most thoroughly studied in yeast mitochondria, where at least 10 proteins have been identified (Table 1) [55]; among these are Fe and sulfane sulfur carriers, chaperonins, and an ABC transporter that all seem to be involved in membrane passage, and at least one electron donor protein that itself is a mitochondrial Fe-S protein, a ferredoxin (Fd) [59]. The requirement for a Fd, which appears to be exclusively synthesized within mitochondria, for the synthesis of Fe-S clusters, supports the notion that mitochondria cannot be formed *de novo*, but must arise from existing mitochondria [54,59]. Thus, at least for some proteins, Fe-S cluster synthesis has to be initiated or completed in the mitochondrion and the product has to be exported to the cytosol [54,59]. The channel that would allow export of an Fe-S cluster or a precursor form of a cluster has not been described thus far. Most likely Atm1 would be a component of such a channel [54]. It is, of course, a matter of great interest, in what form, if at all, an Fe-S cluster is transported through the channel. Clearly, a [2Fe-2S] cluster should be easier to feed through a channel than a cubane [4Fe-4S] cluster, maybe replacing its Cys ligands stepwise with temporary, weaker binding sites in the channel. As the [4Fe-4S] cluster can be easily rebuilt from the cuboid [3Fe-4S] cluster, and, if we take a hint from nature itself [60], we might imagine that the [3Fe-4S] cluster in its linear form [60], Cys₂FeS₂FeS₂FeCys₂, could pass as easily as a [2Fe-2S] cluster, and, as it is readily done *in vitro*, could be reconstituted to a [4Fe-4S] cluster.

An interesting phenomenon that has possible health implications is that Fe accumulates in mitochondria [41,44,45,54-57,59], and apparently in some unusable form, when the apparatus for Fe-S cluster synthesis is not functioning properly. It appears that iron import continues, irrespective of whether it is being utilized for synthesis or not, i.e. mitochondrial iron homeostasis fails under these conditions. This phenomenon is thought to be the basis for the hereditary disease named Friedreich's Ataxia [61,62].

Table 1. Components potentially involved in Fe–S cluster biosynthesis in yeast and bacteria. A compilation of proteins which have been shown or are supposed to have a function in the biosynthesis of Fe–S proteins in the yeast or in bacteria. For bacteria only the most common names are given. A prokaryotic homologue of Atm1p has been identified only in the obligate intracellular bacterium *Rickettsia prowazekii* [58]. ?, not known. Reproduced with permission from [55] and updated.

Yeast protein	Required for	Essential	Bacterial homologues	(Possible) function
Nfs1p	m, c	Yes	NifS, IscS	Cysteine desulfurase
Isa1p	m, c	No	NifA, IscA, HesB	
Isa2p	m	No	NifA, IscA, HesB	
Isu1p	m, ?	No ^a	NifU, IscU	Possible primary binding site of iron
Isu2p	m, ?	No ^a	NifU, IscU	
Nfu1p	m, ?	No	NifU	
Yah1p	m, c	Yes	Ferredoxin	Reduction of sulfur, iron or intermediate of Fe–S cluster formation
Arh1p		Yes	Ferredoxin reductase	NAD(P)H-dependent reductions of Yah1p
Ssq1p	m, ?	No	HscA	Chaperone of Hsp70/DnaK type
Jac1p	m, ?	Yes	HscB	Chaperone of Hsp40/DnaJ type
Bat1p	c	No	IlvE	Branched-chain amino-acid transaminase
Bat2p	c	No	IlvE	Branched-chain amino-acid transaminase
Atm1p	c	No	Atm1 of <i>Rickettsia prowazekii</i>	ABC transporter

^a Deletion of both *ISU* genes is lethal.

FE–S CLUSTERS AS PARTICIPANTS IN REACTIONS

A unique case seems to be the enzyme aconitase, where it has been shown that one specific iron atom of the Fe–S cluster enters into the enzymatic reaction catalyzed, i.e. isomerization of citrate and isocitrate via *cis*-aconitate (Fig. 1), by sequestering the substrate [63]. If an iron atom, while still engaged in electron spin-coupling with the other three iron atoms [64] and bonded to three cluster sulfides, is able to interact with external substances, it seems reasonable to expect that a μ_3 sulfide, bonded to three iron atoms, should also be able to interact with external substances; and this has indeed been proposed. After all, theoretical calculations have shown [65] that on oxidation or reduction the largest change in electron density takes place at the sulfurs, bridging and terminal, and not on the iron. Such involvement of sulfur has been postulated for a group of enzymes that has the task of accomplishing, under anaerobic conditions, homolytic bond cleavage of C–C, C–H, or C–S bonds via free radical intermediates.

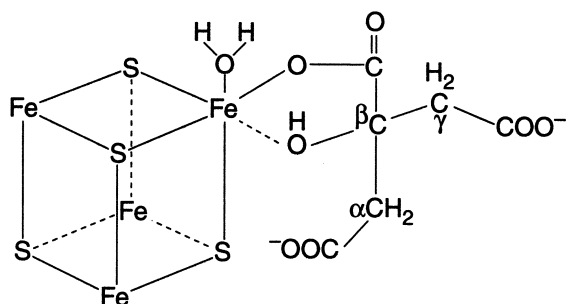


Fig. 1. The active site of m-aconitase with citrate bound. The iron atom that has no cysteine ligand becomes six-coordinate from four-coordinate by engaging into bonding with the beta-carboxyl and hydroxyl of citrate, while remaining electron-spin coupled to the Fe–S cluster. According to ENDOR and Mössbauer spectroscopy and crystal structures [21,63]. Note the analogy to Fig. 2.

They all depend on the cofactor adenosylmethionine (AdoMet), an evolutionary precursor of adenosylcobalamine [66]. These enzymes are pyruvate:formate lyase (PFL) [67,68], anaerobic ribonucleotide reductase (ARNR) [69], both of *E. coli*, lysine 2,3-amino mutase (LAM) from *Clostridium subliminale* [70], and biotin synthase (BS) from *E. coli* [71,72]. It was thought that radical formation is initiated for PFL, ARNR, LAM, and BS by the interaction of a [4Fe–4S] cluster μ_3 sulfide with AdoMet to form the adenosyl radical and methionine, as shown schematically in Fig. 2 [73]. With PFL and ARNR, this reaction actually requires two proteins, a separate, specific ‘activating enzyme’, which contains the Fe–S cluster involved and temporarily forms a complex with the protein (PFL or ARNR), in which a glycyl radical is to be generated. In LAM and BS, the Fe–S cluster is part of the protein itself. When purified, some of these proteins were obtained in oligomeric states, e.g. LAM as hexamer and the ARNR activating enzyme as dimer. In both activating enzymes and BS, [2Fe–2S] clusters

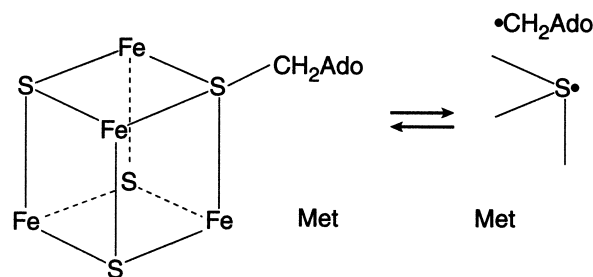


Fig. 2. Proposed reaction of one of the cluster sulfides of LAM with AdoMet, resulting in homolytic bond cleavage of AdoMet [73]. Note the analogy to Fig. 1. The enzyme, E-[4Fe–4S]⁺, interacts with AdoMet to form the active species designated as E-[4Fe–4S]/AdoMet, which may be reversibly converted to E-[4Fe–4S]/Met/Ado-CH₂, with dissociation of methionine [70], as shown in the figure. The deoxyadenosyl radical, once formed, is then thought to abstract a hydrogen atom from lysine in LAM to form a lysyl radical, or from glycine in PFL or ARNR to form a glycyl radical.

were originally found on purification. On reduction of such preparations, however, it was observed that [4Fe–4S] clusters were formed at the expense of the 2Fe clusters [68,74,75]. Thus, the idea arose that two monomers, each with one [2Fe–2S] cluster, become linked, forming a 4Fe cluster at their interface. However, in further work it has become clear that, when the PFL and ARNR-activating proteins are prepared under exclusion of oxygen, each monomer contains close to one 4Fe cluster, which only on exposure to air decays to a 2Fe cluster [67,69,76]. While for BS the notion that two 2Fe clusters are converted to a subunit-bridging 4Fe cluster has been debated [77], recent detailed analysis [78] has convincingly shown that the active enzyme can contain up to two [4Fe–4S] clusters per dimer; however, as in other cases (e.g. in LAM and PFL, 70,76), the potential cluster binding sites may not all be occupied. Thus, in all these cases, ultimately [4Fe–4S] clusters are the reactive species and the cluster has to be in the 1+ state to initiate the reaction. In LAM and ARNR, it has not been possible to reach the 1+ state with dithionite as the reductant. However, it was then observed that addition of AdoMet to the [4Fe–4S]²⁺ cluster dramatically raises the cluster's redox potential, so that reduction of the cluster becomes very facile [69,70]. However plausible, a mechanism, e.g. as sketched in Fig. 2, for the critical steps that may involve the sulfur has not been definitively established in any of these cases (see below). It seems that the same property of sulfur that facilitates difficult rearrangement reactions, namely its enormous versatility, also makes it an elusive target in chemical-analytical work.

Biotin synthase [71,72] is a special case inasmuch as a cluster sulfide not only participates in the reaction with AdoMet, but actually is removed from the 4Fe cluster and attached to dethiobiotin in a 'sacrificial' reaction. The step of interest in the context of this article is the insertion of sulfur into dethiobiotin (Fig. 3). It was shown by the use of isotopically labeled sulfur that the source of the sulfur actually is the Fe–S cluster of the synthase itself [80,81]. At this time it has not yet been possible to show catalysis, but at best stoichiometric conversion of dethiobiotin to biotin, and a mechanism of reassembly of the Fe–S cluster, which would be necessary for catalysis to occur, has not been established. In view of present knowledge about the proteins involved in cluster assembly (see above), one might expect that proteins analogous to the IscS/IscU type could be carrying out the cluster reassembly. According to sequence similarity, it is likely that lipic acid synthesis proceeds by a path similar to that of biotin [79,82].

An enzyme system that does not involve AdoMet, but was proposed to utilize a 4Fe cluster μ_3 sulfide in a homolytic

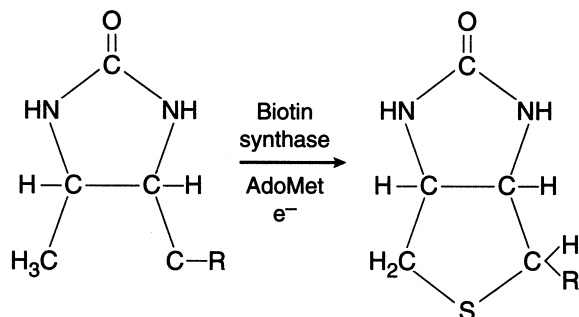


Fig. 3. Insertion of one of the sulfurs of the Fe–S cluster of biotin synthase into dethiobiotin, catalyzed by biotin synthase in an AdoMet dependent reaction.

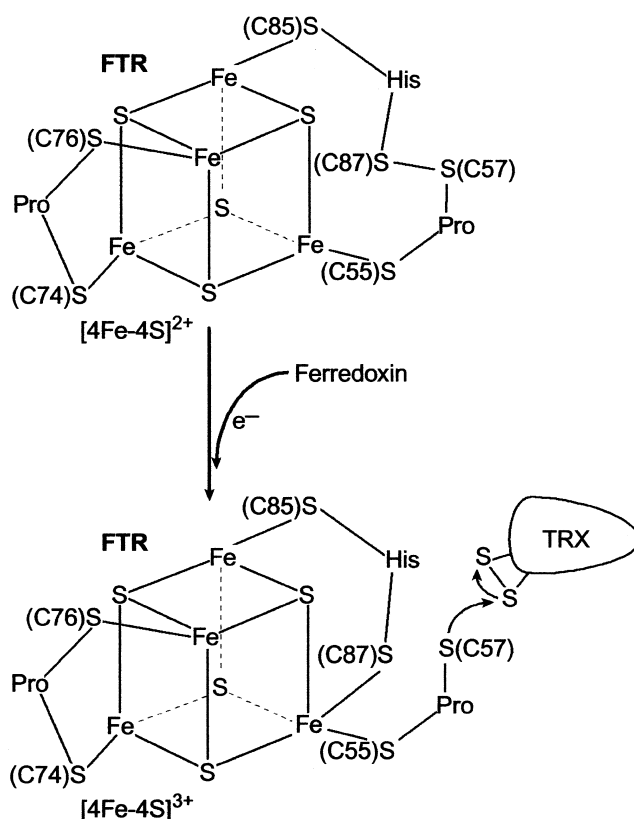


Fig. 4. Scheme proposed for the reaction of one of the iron atoms of the [4Fe–4S] cluster of FTR with the active site disulfide of FTR, preceding the reductive cleavage of the disulfide of TRX.

reaction, is that of FTR:TRX of spinach. In this instance, it was proposed that a μ_3 sulfide of the 4Fe cluster of the reductase makes a nucleophilic attack on the enzyme's active-site disulfide to make a –S–S(Cys) linkage between this μ_3 sulfide and one of the active site sulfurs [83]. However, a recently obtained X-ray structure of the FTR:TRX complex from *Synechocystis* makes it more likely that the link is not to a cluster sulfide but rather to a cluster iron. The sulfide is at a distance of 3.5 Å from the closest sulfur in the disulfide, whereas the iron is at 3.1 Å [84], (Fig. 4). Thus the idea that it is one of the cluster sulfurs, not sulfurs, which is the reactive cluster component, has gained appeal. In the same context, we may also want to recall here the proposal that it is the μ_2 cluster sulfides or Cys cluster ligands that become protonated on reduction of a [3Fe–4S] cluster [5] close to –0.7 V (standard hydrogen electrode). If verified, this might have important implications for Fe–S cluster involvement in proton transfer reactions and generation of proton gradients. However, in all this we must keep in mind that, according to Mössbauer spectroscopy [85] and theoretical calculations [65], the Fe–S cluster reacts as a unit, and while iron may be the exit port of electrons the events are conditioned by the electron distribution and its changes within the cluster as a whole.

ORGANIC SYSTEMS

Although we called these systems organic, some of them include the sulfane carrying IscS protein or an analog, which are also components of the assembly of Fe–S clusters, as discussed above under 'Inorganic systems'. IscS is, for instance, required for the *in vitro* biosynthesis of 4-thiouridine

in *E. coli* [86]. As we saw above, organic pathways may also involve sulfurs or irons that are part of an Fe–S cluster or may utilize the electron donating, accepting or polarizing ability of Fe–S clusters [85]. At least one pathway that does not involve Fe–S clusters at all appears to be that of thiamine biosynthesis. This pathway is complicated and not yet entirely understood. For details, a recent review may be consulted [71]. Thiamine synthesis involves at least six peptides (ThiF,S,G,H,I,J). Cysteine is the original source of the sulfur and the immediate donor, which completes the heterocycle synthesis, is a thio-carboxylate formed at the carboxyl terminus of ThiS in an ATP requiring reaction [71]. It has been reported recently that the ThiI peptide is a sulfurtransferase that shows sequence similarity to the well studied sulfurtransferase rhodanese [87]. Indeed, evidence for sulfur transfer from IscS to ThiI has recently been obtained [88]. Proteins of the rhodanese family appear to be ubiquitous in all forms of life [89]. While they are known for, and owe their name to the catalysis of thiocyanate formation from thiosulfate and cyanide, bovine liver rhodanese, for instance, has an affinity a thousand-fold higher for the reduced form of TRX than for cyanide [90]! A reaction analogous to that of sulfane-loaded rhodanese with TRX has been proposed as a critical step in the synthesis of thiouridine, for which the rhodanese homolog ThiI is also required for incorporation of sulfur [87]. A complete mechanism has been proposed for the incorporation of sulfur to form 4-thiouridine, and it is suggested that an extension of this mechanism may be applicable to the formation of the thiocarboxylate in thiamine biosynthesis [87]. From similarities in the structures of the peptides involved it is suggested that the synthesis of molybdopterin also involves a thiocarboxylate as the sulfur donor [91].

CONCLUDING REMARKS

As we are learning time and again, nature has found many and often unexpected ways to reach its goals. It is not the intention of this essay to single out the involvement of sulfane sulfur as the answer to unanswered questions that confront us, when we study reactions involving sulfur compounds. Sulfane sulfur certainly exemplifies the reactivity of sulfur and plays an important role in many biological systems. In addition, the reactive properties of a sulfane may be acquired by any structure RSSR, if activating groups, such as $-\text{CH}=\text{CH}-$ or $-\text{C}=\text{O}$ are close. The intention was to present in this essay examples from the realm of chemistry as well as biology, of the more unexpected observations or often even still proposals, such as the protonation of Fe–S cluster sulfide and/or ligand-cysteine sulfurs, the exchange of sulfide or elemental sulfur into similar sulfur containing structures, the use of Fe–S cluster sulfides for synthetic reactions or participation of cluster sulfurs or irons in homolytic reactions, the generation of various transport forms for sulfur and, last but not least, recent knowledge of the host of proteins evolved for the synthesis and maintenance of Fe–S proteins in higher organisms. Since submission of this article, a number of papers of significance to the above discussion have been published [92–95].

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