Characterization of MOCS1A, an Oxygen-sensitive Iron-Sulfur Protein Inolved in Human Molybdenum Cofactor Biosynthesis*

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The molybdenum cofactor in eukaryotic molybdoenzymes consists of a mononuclear molybdenum coordinated by the dithiolene moiety of a tricyclic pyranopterin, termed molybdop terin (MPT)1. In humans, defects in molybdenum cofactor biosynthesis lead to the pleiotropic loss of the molybdoenzymes sulfite oxidase, aldehyde oxidase, and xanthine dehydrogenase (2, 3). Affected patients usually die shortly after birth and show neurological abnormalities, such as attenuated growth of the brain, untreated seizures, and dislocated ocular lenses (4). The first step during human molybdenum cofactor biosynthesis is catalyzed by MOCS1A and MOCS1B, leading to the synthesis of precursor Z, an oxygen-sensitive 6-alkyl pterin with a cyclic phosphate, from a guanosine derivative, most likely 5′-GTP (5–7).

Analogous to other pteridine biosynthetic pathways, synthesis of precursor Z has been proposed to occur via a GTP cyclohydrolase-like reaction mechanism (5, 6). In contrast to these pathways, the C-8 atom of 5′-GTP is not released as formate but is retained and incorporated in a rearrangement reaction as the first carbon atom of the precursor Z side chain. In the second step of molybdenum cofactor biosynthesis catalyzed by MOCS3 (8) and MPT synthase (MOC52) precursor Z is converted into MPT (9–11). Finally molybdenum is incorporated into MPT by the multifunctional protein gephyrin (12, 13).

MOCS1A contains two highly conserved cysteine motifs (Fig. 1) proposed to be involved in iron-sulfur (FeS) cluster binding (14, 15), one is located near the N terminus (consensus sequence CX2CX2CX2 where X denotes any amino acid), and one is near the C terminus (consensus sequence CX1CX1CX1). Several mutations identified in molybdenum cofactor deficiency patients are located in these conserved cysteine motifs indicating their functional importance for protein activity (2, 3). Based on sequence similarities to proteins such as biontin synthase, pyruvate formate-lyase-activating enzyme, and anaerobic ribonucleotide reductase-activating enzyme, MOCS1A has been classified as a member of the superfamily of S-adenosylmethionine (AdoMet)-dependent radical enzymes (16). In this class of enzymes, AdoMet serves as the free radical initiator and undergoes cleavage to methionine and a 5′-deoxyadenosyl radical that in turn propagates radical formation by abstracting hydrogen atoms either from substrate molecules to form radical intermediates or from glycyl residues of enzymes to activate them for radical-based biochemistry (17–20). The source of the electron required for the cleavage of AdoMet is a reduced form of an FeS cluster. In MOCS1A the N-terminal cysteine motif is highly homologous to a motif in AdoMet-dependent radical enzymes (17–20).

The human proteins MOCS1A and MOCS1B catalyze the conversion of a guanosine derivative to precursor Z during molybdenum cofactor biosynthesis. MOCS1A shares homology with S-adenosylmethionine (AdoMet)-dependent radical enzymes, which catalyze the formation of protein and/or substrate radicals by reductive cleavage of AdoMet through a [4Fe-4S] cluster. Sequence analysis of MOCS1A showed two highly conserved cysteine motifs, one near the N terminus and one near the C terminus. MOCS1A was heterologously expressed in Escherichia coli and purified under aerobic and anaerobic conditions. Individual mutations of the conserved cysteines to serine revealed that all are essential for synthesis of precursor Z in vivo. The type and properties of the iron-sulfur (FeS) clusters were investigated using a combination of UV-visible absorption, variable temperature magnetic circular dichroism, resonance Raman, Mössbauer, and EPR spectroscopies coupled with iron and acid-labile sulfide analyses. The results indicated that anaerobically purified MOCS1A is a mononuclear protein containing two oxygen-sensitive FeS clusters, each coordinated by only three cysteine residues. A redox-active [4Fe-4S] cluster is ligated by an N-terminal CX2CX2C motif as is the case with all other AdoMet-dependent radical enzymes investigated thus far. A C-terminal CX1CX1CX1C motif that is unique to MOCS1A and its orthologs primarily ligates a [3Fe-4S] cluster. However, MOCS1A could be reconstituted in vitro under anaerobic conditions to yield a form containing two [4Fe-4S] clusters. The N-terminal [4Fe-4S] cluster was rapidly degraded by oxygen via a semistable [2Fe-2S] cluster intermediate, and the C-terminal [4Fe-4S] cluster was rapidly degraded by oxygen to yield a semistable [3Fe-4S] cluster intermediate.
The column was washed with 10 bed volumes of buffer A containing 20 mM imidazole and 1% (v/v) Triton X-100 followed by 10 bed volumes of buffer A containing 20 mM imidazole, and proteins were eluted with buffer A containing 500 mM imidazole. Brown-colored fractions were pooled, desalted by size exclusion chromatography on Sephadex G-25 equilibrated with buffer B (100 mM Tris/HCl, pH 9.0, 300 mM NaCl), concentrated to 10–15 mg/ml by ultrafiltration (Centriprep-30, Millipore), and stored at −80 °C.

Oligomeric State of MOCS1A—Fast protein liquid chromatography size exclusion chromatography with a Superdex 200 (Amersham Biosciences) column (1.0 × 30 cm) equilibrated with buffer B was used for size determination. The column was calibrated using aldolase (Mr = 158,000), bovine serum albumin (Mr = 67,000), ovalbumin (Mr = 43,000), chymotrypsinogen A (Mr = 25,000), and ribonuclease A (Mr = 13,700) as marker proteins.

Reconstitution of the FeS Cluster—Expression and purification of E. coli IscS was as described by Leimkuhler and Rajagopalan (21). All steps of the reconstitution procedure were made anaerobically as described above. Anaerobically purified MOCS1A (250 μM) in buffer B was incubated with 5 mM dithiothreitol for 30 min. Then a 10-fold molar excess of FeCl3 and L-cysteine and 200 μM pyridoxal phosphate in buffer B containing 5 mM dithiothreitol was added. After addition of 2.5–5 μM IscS the mixture was incubated for 4 h at 4 °C. Reconstituted proteins were desalted over Sephadex G-25 equilibrated with buffer B to remove pyridoxal phosphate and adventitiously bound iron and sulfide.

Electrophoresis and Immunoblot Analysis—Protein samples were analyzed by 12% SDS-PAGE, and gels were stained with Coomassie Brilliant Blue G-250. Immunoblotting on polyvinylidene difluoride membranes was carried out with primary polyclonal antibodies generated against recombinant MOCS1A (1.0000 diluted serum). The membranes were probed with alkaline phosphatase-conjugated secondary antibody, and bands were visualized with the 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium detection system (Promega).

Nitrate Reductase Overlay Assay—For functional complementation the E. coli moaA mutant strain KB2037 (moaA49deI) was transformed with the corresponding expression plasmids. Qualitative analysis of nitrate reductase activity was performed by a colony overlay assay (22).

In Vivo Assay for Precursor Z Synthesizing Activity—The E. coli moaD mutant strain MJ7chIM (DE3) was transformed with the corresponding expression plasmids and grown anaerobically in 50 ml of LB medium containing 0.4% (v/v) nitrate and 0.1 mM isopropyl-1-thio-β-D-galactopyranoside. Precursor Z was detected as compound Z in crude

Experimental Procedures

Expression and Purification of MOCS1A and Mutated Proteins—All expressions were conducted in LB medium at 30 °C in the E. coli strain BL21(DE3) or C100 (isc−) (DE3). Cultures were induced by the addition of 0.1 mM isopropyl-1-thio-β-D-galactopyranoside at an A600 of 0.1. After 8 h of growth, cells were harvested and stored at −80 °C. For cells grown for Mossbauer measurements, 5 mg/liter 57Fe, as ferric ammonium citrate, was added to the growth medium at induction. All purification steps were carried out either under anaerobic conditions inside a glove box (Coy Laboratories) in an argon atmosphere containing less than 2 ppm O2 at 4 °C or under aerobic conditions. Cells were thawed and resuspended in buffer A (50 mM Tris/HCl, pH 9.0, 300 mM NaCl, 10% (v/v) glycerol) containing 20 mM imidazole and 1% (v/v) Triton X-100. Hen egg white lysozyme (2 mg/ml) and a few crystals of bovine pancreas DNA were added, and the mixture was stirred for 30 min and sonicated. After centrifugation (60 min, 4 °C, 48,000g) MOCS1A was purified by Ni-NTA affinity chromatography (Qiagen, 1.5 ml of matrix/liter of culture volume) equilibrated with the same buffer. The column was washed with 10 bed volumes of buffer A containing 20 enzymes that ligates a [4Fe-4S] cluster, whereas the C-terminal motif is unique to MOCS1A and its orthologs.

Here we describe the purification of MOCS1A, the spectroscopic characterization of its FeS clusters, and site-directed mutagenesis investigations of the role of the N- and C-terminal cysteines. Single and triple Cys → Ser mutants were generated and analyzed in terms of catalytic activity and spectroscopic properties. The results showed that all six cysteines are essential for activity. The results also indicated that anaerobically purified MOCS1A is a monomeric protein containing two oxygen-sensitive FeS clusters, each ligated by only three cysteine residues. A redox-active [4Fe-4S]2+ cluster is ligated by the N-terminal CX3CX2C motif as in the case with all other AdoMet-dependent radical enzymes investigated thus far, whereas a [3Fe-4S]0 cluster is ligated by the C-terminal CX2CX1C motif. However, MOCS1A could be reconstituted in vitro to yield a form containing two [4Fe-4S]2+ clusters, and both clusters appeared to be degraded via [3Fe-4S]0 and/or [2Fe-2S]2 cluster intermediates on exposure to oxygen.

Multiple sequence alignments of the N- (A) and C-terminal (B) cysteine motifs of MOCS1A. Shown from top to bottom (GenBank™ accession numbers in parentheses) are: Homo sapiens (Hs) (CAC44527), Arabidopsis thaliana (At) (CAAS8107), Clostridium perfringens (Cp) (BAAB6928), Haemophilus influenzae (Hi) (P45311), Mycobacterium tuberculosis (Mt) (CAB08366), E. coli (Ec) (P30745), A. nicotinivorans (An) (CAAT779), Bacillus subtilis (Bs) (CAB09883), Staphylococcus carnosus (Sc) (AAC8338), Helicobacter pylori (Hp) (P56414), and Rhodobacter capsulatus (Rc) (Q9X53W).
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cell extracts by high pressure liquid chromatography as described previously (7).

Analytic Procedures—Protein concentrations were determined by the method of Bradford (23) standardized against bovine serum albumin. Protein-bound iron and acyl-labile sulfide were determined by the methods of Fish (24) and Fogo and Popowsky (25), respectively, standardized against either Fe(II) ethylenediammonium sulfate or ammonium sulfate.

Spectroscopic Methods—UV-visible absorption spectra were recorded using a Hewlett Packard 8453 diode array spectrophotometer. X-band (~9.6 GHz) EPR spectra were recorded on a Bruker ESP-300E EPR spectrometer equipped with an ER-4116 dual mode cavity and an Oxford Instruments ESR-B flow cryostat. Resonances were quantified using parallel conditions using a 1 mm Cu-EDTA standard. Resonance Raman (RR) spectra were recorded using an Instruments SA U1000 spectrometer fitted with a cooled RCA 31034 photomultiplier tube with 90° scattering geometry. Spectra were recorded digitally using photon-counting electronics, and improvements in signal-to-noise were achieved by averaging multiple scans. Band positions were calibrated using the excitation frequency and are accurate to ±1 cm⁻¹. Lines from a Coherent Sabre 10-watt argon ion laser were used for excitation, and plasma lines were removed using a Pellin Broca prism premonochromator. Scattering was collected from the surface of a frozen droplet of sample at 17 K using a custom-designed anaerobic sample cell (26) attached to the cold finger of an Air Products Diplex model CSA-202E closed cycle refrigerator. Bands originating from lattice modes of ice and a linear ramp fluorescent background have been subtracted from each spectrum shown in this work.

Variable temperature magnetic circular dichroism (VTMCD) measurements were carried out with an Oxford Instruments Spectromag 4000 split coil superconducting magnet mated to either a Jasco J715 or a Jaesco J730 spectropolarimeter using published protocols (27, 28). Variable temperature and variable field (VTVH) MCD saturation magnetization data were collected by monitoring MCD intensity at fixed temperatures as a function of the applied magnetic field. The data were corrected for temperature-independent contributions by extrapolating plots of MCD intensity versus inverse temperature to infinite temperature and subtracting a proportional correction at each field. Data are plotted as percent magnetization against $\beta B/2kT$ where percent magnetization is the percentage of the MCD intensity relative to magnetic saturation, $\beta$ is the Bohr magneton, $B$ is the magnetic field strength, $k$ is the Boltzmann constant, and $T$ is the absolute temperature. Theoretical VTVH MCD saturation magnetization data were constructed as published previously (29). Mössbauer spectra were recorded using spectrometers described previously (30). The zero velocity refers to the centroid of the room temperature spectra of metallic iron foil. Analysis of the Mössbauer data was performed with the program WM OSS (WEB Research).

RESULTS

Effect of ISC and SUF Proteins as Well as the GroES/EL Chaperonin System on the Expression of MOCS1A—MOCS1A contains a N-terminal extension of ~56 amino acids not found in any of its bacterial orthologs. Due to an increased stability in comparison with the full-length protein, MOCS1A without its N-terminal extension (MOCS1AΔ1–56, 39 kDa) was expressed with an N-terminal His tag. Deletion of the additional amino acids at the N terminus increased the in vivo precursor Z synthesizing activity by about a factor of 1.5 (data not shown).

MOCS1A and orthologous Moa proteins in eukaryotes and prokaryotes were characterized as FeS cluster-containing proteins (15, 31). The de novo synthesis and assembly of FeS clusters in E. coli is mediated by the ISC (iron-sulfur cluster) system (iscRSUA-hscBA-fdx) (32), while the SUF (sulfur) system (sufABCDAE) (33) has been proposed to be involved in the repair of oxidatively damaged FeS clusters and/or play a role in FeS biosynthesis under conditions of iron limitation (34). Expression of MOCS1A in the E. coli iscsM mutant strain CL100 (35) resulted in an inactive iron-free insoluble MOCS1A protein showing that MOCS1A is only stable in its holoform (data not shown). ApoMOCS1A is improperly folded due to the absence of the FeS clusters and tends to aggregate in inclusion bodies. Coexpression of MOCS1A with iscsRSUA-hscBA-fdx or sufABCDSE and the E. coli chaperonin system groES/EL in the E. coli strain BL21(DE3) increased the amount of soluble MOCS1A by a factor of 1.7–2.2, and MOCS1A could be purified by Ni-NTA chromatography with a yield of about 10 mg/liter of cell culture. However, coexpression of MOCS1A with the ISC or SUP proteins revealed no changes in the FeS cluster composition of purified proteins (data not shown). These studies showed that under overexpression conditions MOCS1A requires (i) the assistance of chaperones (GroES/EL) and/or (ii) proteins for efficient de novo biosynthesis of FeS clusters (ISC and SUF) for proper folding and/or insertion of the FeS cluster.

Spectroscopic Characterization of the FeS Cluster Composition of MOCS1A—MOCS1A was purified under aerobic conditions and under anaerobic conditions in an argon atmosphere. Both samples were brown, indicating the presence of FeS clusters, and the nature and properties of the FeS clusters in aerobic and anaerobic preparations were investigated using UV-visible absorption, RR, EPR, VTMCD, and Mössbauer spectroscopies coupled with iron, acid-labile sulfide, and protein analyses.

Aerobically purified samples of MOCS1A contained 3.6 ± 0.2 mol of iron and 3.9 ± 0.3 mol of acid-labile sulfide/mol of MOCS1A, and the absorption spectrum exhibited peaks/shoulders around 320–330 and 415 nm in the region between 500 and 600 nm (Fig. 2A). The spectrum is more typical of a [2Fe-2S]²⁺ cluster than a [4Fe-4S]²⁺ cluster (36–39); however, the iron content of almost 4 mol/mol of protein as well as an estimated ε₁₄₀ of about 13,500 M⁻¹ cm⁻¹ is more typical of [4Fe-4S]²⁺ clusters (ε₁₄₀ = 15,000 M⁻¹ cm⁻¹) than [2Fe-2S]²⁺ clusters (8000–10,000 M⁻¹ cm⁻¹) (36, 38). More definitive assessment of the FeS cluster content was provided by RR, EPR, and VTMCD, which indicated that aerobically purified MOCS1A primarily contains a mixture of [3Fe-4S]²⁻ and [2Fe-2S]²⁺ clusters. The RR spectrum (Fig. 3A) of the protein as purified comprises broad bands centered at 290, 338, 369, and 392 nm and is very similar to those reported for the [2Fe-2S]²⁺ clusters that are observed during oxygen-induced degradation of the [4Fe-4S]²⁺ clusters in other members of the AdoMet-dependent radical enzymes, e.g. anaerobic ribonucleotide reductase-activating enzyme (40), pyruvate formate-lyase-activating enzyme (41), biotin synthase (42), and the tRNA-methylthiotransferase MinB (39). EPR studies only showed a near isotropic S = 1/2 resonance centered near g = 2.01 (Fig. 4) with relaxation properties (observable only below 30 K) indicative of a [3Fe-4S]²⁺ cluster in addition to a large g = 4.3 resonance from adventitiously bound ferric ions. However, spin quantitation indicated that the g = 2.01 resonance is a very minor component accounting for only 0.01 [3Fe-4S]²⁻ clusters/MOCS1A monomer.

Parallel mode X-band EPR and VTMCD studies of aerobically purified MOCS1A revealed the presence of S = 2 [3Fe-4S]³⁺ clusters in addition to S = 0 [2Fe-2S]²⁺ clusters. A broad low field resonance centered near g = 9.4 was observed in the conventional perpendicular mode X-band EPR spectrum, and the resonance sharpened and intensified in parallel mode (Fig. 5). Similar integer spin EPR signals have been reported for a range of S = 2 [3Fe-4S]³⁺ clusters (43). Moreover parallel VTMCD studies (Fig. 6A and data not shown) showed a pattern of intense, temperature-dependent MCD bands that are uniquely characteristic of the excited state properties of cubane-type S = 2 [3Fe-4S]³⁺ clusters (43). On the basis of the intensity of the VTMCD spectrum, [3Fe-4S]³⁺ clusters are the major component of aerobically purified MOCS1A with between 0.5 and 1.0 [3Fe-4S]³⁺ clusters/monomer. [3Fe-4S]³⁺ clusters give rise to very weak RR spectra compared with [2Fe-2S]²⁺ clusters using a 457.9 nm laser (at least 20 times weaker (43)), and this presumably explains the absence of bands attributable to [3Fe-
4S$^0$ clusters in the RR spectrum shown in Fig. 3A. Reduction of aerobically isolated MOCS1A with dithionite resulted in partial bleaching of the visible absorption (data not shown), loss of the $g = 2.03$ resonance, and the appearance of a near axial $S = 1/2$ resonance, $g = 2.03$, 1.92, and 1.88 ($g = 2.03$ resonance), with relaxation properties (observable without substantial broadening only below 35 K) consistent with assignment to an $S = 1/2$ [4Fe-4S]$^{2+}$ cluster (Fig. 4). Spin quantitation of the $g = 2.03$ resonance indicated 0.14 [4Fe-4S]$^{2+}$ clusters/MOCS1A monomer. Parallel RR studies indicated the complete degradation of the [2Fe-2S]$^{2+}$ center as evidenced by the loss of the characteristic RR bands shown in Fig. 3A and showed only very weak bands that are best interpreted in terms of a [4Fe-4S]$^{2+}$ cluster (data not shown). In accord with the EPR results, VTMC$^d$ studies showed the characteristic temperature-dependent MCD bands of a [4Fe-4S]$^{2+}$ cluster (44–46) (Fig. 6B), and the intensity of the MCD indicates between 0.5 and 1.0 [4Fe-4S]$^{2+}$ clusters/monomer. Moreover the complete absence of MCD bands that can be attributed to $S = 2$ [3Fe-4S]$^{2+}$ clusters coupled with the loss of the $g = 9.4$ parallel mode EPR signal indicates that these clusters have been degraded or converted to [4Fe-4S]$^{2+}$. The pattern of VTMC$^d$ bands is indicative of significant contributions from an $S > 1/2$ [4Fe-4S]$^{2+}$ cluster (46), and this was further supported by VTMC$^d$ MCD saturation magnetization data, which cannot be fit based solely on an $S = 1/2$ ground state and indicate the presence of Kramers’ $S > 1/2$ component (Fig. 7). This is not particularly surprising as mixed spin [4Fe-4S]$^{2+}$ clusters appear to be the norm rather than the exception for [4Fe-4S]$^{2+}$ centers with one oxygenic ligand (45, 47). EPR and Mössbauer studies are currently in progress to identify the $S > 1/2$ spin state. Taken together with the RR data, we conclude that the low $S = 1/2$ spin quantitation (0.14 spin/monomer) is likely to be the consequence of incomplete reduction to the [4Fe-4S]$^{2+}$ state and a mixed spin ground state coupled with partial oxidative cluster degradation during purification. In summary, the spectroscopic results indicate that aerobically purified samples of MOCS1A contain a mixture of [3Fe-4S]$^{2+}$ and [2Fe-2S]$^{2+}$ clusters as purified. However, both the [3Fe-4S]$^{2+}$ and [2Fe-2S]$^{2+}$ clusters are degraded or converted to a [4Fe-4S]$^{2+}$ cluster following anaerobic reduction with excess sodium dithionite.

Aerobically purified samples of MOCS1A had increased iron and acid-labile sulfide contents of 5.0 ± 0.4 and 5.3 ± 0.3 mol/mol of protein, respectively, and an absorption spectrum that is more indicative of a [4Fe-4S]$^{2+}$ cluster as the dominant cluster type (Fig. 2A). In particular, the broad shoulder be-
between 500 and 600 nm had decreased, and the shoulder at 400 nm was more pronounced. Moreover the extinction coefficient at 410 nm ($\varepsilon_{410} \approx 16,000 \text{ M}^{-1} \text{ cm}^{-1}$) is indicative of approximately 1 [4Fe-4S]$^{2+}$ cluster/MOCS1A. Addition of dithionite resulted in a decrease of the absorbance in the visible region, demonstrating that the [4Fe-4S]$^{2+}$ cluster is redox-active (Fig. 2, inset). Evidence for the presence of [4Fe-4S]$^{2+}$ clusters in anaerobically purified MOCS1A is also obvious in the RR spectrum (Fig. 3B), which comprises weak bands at 255, 365, and 390 cm$^{-1}$ and an intense band at 338 cm$^{-1}$. The latter band is attributed to the symmetric breathing mode of the Fe$_4$S$_4$ cubane, and the spectrum is very similar to those reported for [4Fe-4S]$^{2+}$ clusters in AdoMet-dependent radical enzymes (39, 41, 42). The bands from the [4Fe-4S]$^{2+}$ cluster are superimposed on bands originating from the [2Fe-2S]$^{2+}$ cluster that is present in the aerobically purified protein as evidenced by the band at 290 cm$^{-1}$. However, the contribution from the [2Fe-2S]$^{2+}$ clusters is greatly overemphasized by RR due to the 5–10-fold greater resonance enhancement for [2Fe-2S]$^{2+}$ clusters compared with [4Fe-4S]$^{2+}$ clusters with 457.9 nm excitation (48). The observation of [2Fe-2S]$^{2+}$ clusters in the RR spectrum of the anaerobically prepared sample is likely to be a consequence, in whole or in part, of freezing and thawing of...
samples. Although frozen samples were transported for spectroscopic measurements and thawed under anaerobic conditions, UV-visible absorption and RR studies (data not shown) indicated that partial degradation of the [4Fe-4S]^{2+/H}_{11001} clusters accompanies freeze/thaw cycles.

Mössbauer studies of anaerobically prepared MOCS1A revealed the presence of [3Fe-4S]^{0} and [4Fe-4S]^{2+} clusters in distinct FeS cluster binding sites. Fig. 8C shows the 4.2 K spectrum (hatched marks) of anaerobically purified MOCS1A in a weak parallel, applied field of 50 mT. A central quadrupole doublet superposed with a broad paramagnetic spectrum was observed. The paramagnetic spectrum, which accounts for 60% of the total iron absorption, is identical to the spectrum of the [3Fe-4S]^{0} cluster characterized in the N-terminal triple Cys → Ser variant (see below) shown in Fig. 8B. Removal of the [3Fe-4S]^{0} contribution resulted in a quadrupole doublet (Fig. 8D) that is consistent with that of a [4Fe-4S]^{2+/H}_{11001} cluster (38, 49, 50). Since the N-terminal triple Cys → Ser variant contains a [3Fe-4S]^{0} cluster (see below), which therefore must be coordinated by the three conserved C-terminal cysteine residues, the observation of a [4Fe-4S]^{2+/H}_{11001} cluster in addition to a [3Fe-4S]^{0} cluster in MOCS1A suggests that the [4Fe-4S]^{2+/H}_{11001} cluster is coordinated by the conserved CX_{3}CX_{2}C N-terminal cysteine residues that are known to coordinate a [4Fe-4S]^{2+/H}_{11001} cluster in AdoMet-dependent radical enzymes. Taken together, the iron content of the Mössbauer sample (4.8 iron/monomer), the observation that the [3Fe-4S]^{0} cluster is close to full occupancy in the N-terminal domain (see below), and the analysis of the Mössbauer absorption that indicates that 60% of the iron is in [3Fe-4S]^{0} clusters and 40% of the iron is in [4Fe-4S]^{2+/H}_{11001} clusters indicates that the [3Fe-4S]^{0} cluster is present at full occupancy in the C-terminal domain and that the [4Fe-4S]^{2+/H}_{11001} cluster is present with ~50% occupancy in the N-terminal domain.

The presence of an S = 2 [3Fe-4S]^{0} cluster in as-prepared samples of anaerobically purified MOCS1A was confirmed by
other AdoMet-dependent radical enzymes such as pyruvate formate-lyase-activating enzyme and anaerobic ribonucleotide reductase-activating enzyme in which [2Fe-2S]^{2+} clusters are degraded or converted to [4Fe-4S]^{2+} clusters under reducing conditions (41, 51), the RR spectrum of dithionite-reduced anaerobically purified MOCS1A indicated degradation of [2Fe-2S]^{2+} clusters as evidenced by the loss of the 290 cm^{-1} band (Fig. 3C). Since [4Fe-4S]^{2+} clusters have negligible resonance enhancements with 457.9 nm excitation (48), the dithionite-reduced sample therefore affords the opportunity to investigate the RR spectrum of the [4Fe-4S]^{2+} cluster in isolation albeit with poorer signal-to-noise as a result of partial reduction. Hence the S = 1/2 spin quantitation is likely to be a substantial underestimate of the [4Fe-4S] cluster stoichiometry due to incomplete reduction and a mixed spin ground state. In summary, spectroscopic studies indicated that MOCS1A purified anaerobically contains predominantly substoichiometric amounts of [4Fe-4S]^{2+} clusters coordinated by the conserved cysteines in the N-terminal domain and near stoichiometric amounts of a [3Fe-4S]^{0} cluster coordinated by the conserved cysteines in the C-terminal domain. Reduction with dithionite resulted in degradation or conversion of the [4Fe-4S]^{0} and the trace amounts of [2Fe-2S]^{2+} clusters to yield partially reduced [4Fe-4S]^{2+} clusters as the sole type of cluster present in the protein.

**Stability of FeS Clusters in MOCS1A—Oxygen-induced [4Fe-4S]^{2+} cluster degradation via a semistable [2Fe-2S]^{2+} cluster intermediate has emerged as a common property of almost all of the AdoMet-dependent radical enzymes investigated thus far (37, 39–41). Hence the effects of oxygen on the FeS clusters in MOCS1A were investigated using RR and UV-visible absorption spectroscopies. The [4Fe-4S]^{2+} cluster in the anaerobically purified protein was almost completely degraded after exposure to air for 10 min at room temperature as evidenced by the loss or decrease in the intensity of the bands at 255 and 338 cm^{-1} in the RR spectrum (cf. Fig. 3, B and D). The resulting spectrum is almost identical to that of the aerobically purified protein within experimental error. Hence the [4Fe-4S]^{2+} in the N-terminal domain is shown to be rapidly degraded by oxygen via a semistable [2Fe-2S]^{2+} cluster intermediate.

In contrast, absorption studies indicated that the [2Fe-2S]^{2+} and [3Fe-4S]^{0} clusters in aerobically purified MOCS1A undergo a much slower oxygen-induced degradation with a half-life of 21 h after air exposure (Fig. 2B, upper panel) that does not occur under anaerobic conditions. This effect can be significantly delayed in the presence of 5'-GTP, 5'-GDP, and 5'-GTP occurs already with a 1:1 protein to 5'-GTP stoichiometry. Furthermore the effect of 5'-GTP, 5'-GDP, 5'-GMP, and 5'-ATP was analyzed. The half-life of the FeS cluster increased with an increase in phosphate groups (5'-GTP, 5'-GDP, 5'-GMP, 27 h; 5'-GMP, 24 h) perhaps indicating a stronger interaction. In contrast, 5'-ATP had no effect on MOCS1A stability as documented by a half-life of 18 h.

**Oligomeric State of MOCS1A—Size exclusion chromatography of aerobically purified MOCS1A showed two major peaks corresponding to the monomer with an apparent molecular mass of 40 kDa and dimer (~80 kDa) (Supplemental Fig. S1). Addition of up to 5 mM dithiothreitol to prevent oxidation of sulphydryl groups or to reduce already existing disulfide bonds did not significantly alter the aggregation behavior. The MOCS1A monomer and dimer show different iron contents and UV-visible absorption spectra (Supplemental Fig. S1, inset). The monomeric form has a higher cluster content based on iron analysis and absorption intensity (4.2 ± 0.2 mol of iron/mol of monomeric MOCS1A, $\varepsilon_{410}$...
The dimeric form has a decreased cluster content (2.8 ± 0.5 mol of iron/mol of monomeric MOCS1A, ε_{452} = 10,500 m\(^{-1}\) cm\(^{-1}\)/monomer). Purification of MOCS1A under anaerobic conditions led to the monomeric state (data not shown), indicating loss of iron during aerobic purification and subsequent dimerization possibly as a result of oxidative modification of free sulfhydryl groups.

In Vitro FeS Cluster Assembly in MOCS1A—As isolated, most AdoMet-dependent radical enzymes contain low amounts of iron as well as catalytically inactive [2Fe-2S]\(^{2+}\) clusters and in some cases [3Fe-4S]\(^{2+}\) clusters (17–20). However, reconstitution of apoproteins under anaerobic conditions with iron and sulfide creates a [4Fe-4S]\(^{2+}\) cluster in these proteins. To obtain MOCS1A with homogenous [4Fe-4S] clusters, MOCS1A was treated with EDTA under anaerobic reducing conditions to obtain the apoprotein. In agreement with the observed aggregation of MOCS1A in the iscS mutant strain, complete removal of the FeS cluster was accompanied by precipitation of the protein. However, after an IscS-catalyzed reconstitution of the anaerobically purified protein with a 6-fold molar excess of L-cysteine and FeCl\(_3\) in the presence of dithiothreitol, MOCS1A showed a broad absorption band centered at 410 nm that is typical of [4Fe-4S]\(^{2+}\) clusters (Fig. 2C). The reconstituted FeS clusters were extremely oxygen-sensitive and were rapidly degraded yielding polymeric iron sulfides as demonstrated by the appearance of absorption maxima at 420 and 610 nm (Fig. 2C). The iron content of anaerobically reconstituted MOCS1A (8.1 ± 1.2 mol of iron/mol of monomeric MOCS1A) and the extinction coefficient at 410 nm of ~30,000 m\(^{-1}\) cm\(^{-1}\) are both indicative of two [4Fe-4S]\(^{2+}\) clusters in reconstituted MOCS1A.

The conclusion that anaerobically reconstituted MOCS1A contains [4Fe-4S] clusters in both the C-terminal and N-terminal cluster-binding domains is supported by RR, VTMCD, and EPR studies. The RR spectrum (Fig. 3E) is characteristic of [4Fe-4S]\(^{2+}\) clusters (48) albeit with greater line widths that presumably reflect the overlap of the spectra from two similar, but not identical, [4Fe-4S]\(^{2+}\) clusters. The reconstituted samples showed no evidence for the 290 cm\(^{-1}\) band that is the hallmark of the [2Fe-2S]\(^{2+}\) cluster produced via oxygen-induced degradation of [4Fe-4S]\(^{2+}\) clusters. VTMCD studies showed only weak, predominantly temperature-independent bands (data not shown) consistent with the presence of only \( S = 0 \) [4Fe-4S]\(^{2+}\) clusters with no significant contribution from the intense transitions from the \( S = 2 \) [3Fe-4S]\(^{0}\) clusters that dominate the spectrum of the aerobically and anaerobically purified samples (Fig. 6A). EPR studies revealed a weak, slow relaxing radical signal centered around \( g = 2.005 \) (Fig. 4). Dithionite reduction led to the formation of [4Fe-4S]\(^{+}\) clusters with EPR and VTMCD properties (Figs. 4, 6B, and 7) quantitatively similar to those seen for anaerobically purified samples. The \( S = 1/2 \) EPR resonance (\( g = 2.03, 1.92, \) and 1.89) accounts for 0.28 spin/monomer, and the VTMCD spectra and VTVH MCD magnetization data indicate the presence of a mixed spin [4Fe-4S]\(^{+}\) cluster. Hence the low \( S = 1/2 \) spin quantitation is likely to be a consequence of the presence of \( S > 1/2 \) [4Fe-4S]\(^{+}\) clusters and incomplete reduction using dithionite.

**Characterization of Single Cys → Ser Mutants—**MOCS1A is characterized by two highly conserved cysteine motifs (Fig. 1), and to analyze the involvement of the six MOCS1A conserved cysteine residues in formation/stabilization of two FeS clusters and their importance for catalytic activity, all six cysteine residues of full-length MOCS1A (Cys\(^{80}\), Cys\(^{84}\), Cys\(^{87}\), Cys\(^{312}\), Cys\(^{315}\), and Cys\(^{320}\)) were individually changed to serine. The choice of cysteine substitution by serine was made to preserve as much as possible the H-bonding character and side chain geometry of the original cysteine residues. For functional characterization, the *E. coli* moaA mutant strain KB2037 was reconstituted with wild type or mutant MOCS1A, and reconstitution of molybdenum cofactor biosynthesis was determined by a nitrate reductase overlay assay (Fig. 9A). All six Cys → Ser mutants showed no complementation. Immunoblot analysis of
wild type MOCS1A and Cys → Ser variants revealed similar expression levels for the wild type protein and the mutants of the N-terminal cysteine motif (Fig. 9A). However, replacements of the cysteines of the C-terminal motif destabilized MOCS1A so much that only low amounts of protein could be detected in extracts (Fig. 9A). All six single Cys → Ser variants were purified as described for the wild type protein under aerobic conditions and characterized by UV-visible absorption spectroscopy and iron content analysis. While the mutants of the N-terminal cysteine motif could be purified in amounts comparable to wild type MOCS1A (Fig. 9B, upper panel), the yield of the mutants of the C-terminal cysteine motif was only about 20% (Fig. 9B, lower panel).

Mutants affecting the N-terminal cysteine motif (Fig. 9B, upper panel) as well as the C-terminal cysteine motif (Fig. 9B, lower panel) showed a decreased absorption in the whole visible region in comparison with wild type MOCS1A. Accordingly the iron contents of the N-terminal cysteines decreased by 30–50% of wild type levels: 3.4 mol of iron/mol of wild type MOCS1A, 2.0 mol of iron/mol of C80S, 2.4 mol of iron/mol of C84S, and 1.7 mol of iron/mol of C87S. The iron contents of the C-terminal cysteine variants ranged from 80 to 90% of wild type MOCS1A: 3.4 mol of iron/mol of wild type MOCS1A, 2.9 mol of iron/mol of C312S, 2.7 mol of iron/mol of C315S, and 3.1 mol of iron/mol of C329S. Although mutants of the C-terminal cysteine motif did not show such a significant effect as mutants of the N-terminal cysteine motif, these data are indicative for an involvement of all six cysteine residues in FeS cluster binding, suggesting two different FeS binding sites.

**Characterization of Triple Cys → Ser Mutants**—To obtain further evidence for two different FeS cluster binding sites, one ligated by the N-terminal CXXC motif and one ligated by the C-terminal CXXC motif, triple mutants (C80S/C84S/C87S and C312S/C315S/C329S) of MOCS1A–Δ1–56 were generated, purified under anaerobic conditions, and analyzed by UV-visible absorption spectroscopy as well as iron analysis. MOCS1A–Δ1–56–C80S/C84S/C87S, reflecting the proposed C-terminal FeS cluster, has an iron content of 2.9 mol/mol of protein and shows a UV-visible absorption spectrum with a shoulder at 390 nm and a broad weak absorption centered near 700 nm (Fig. 2D). The spectrum is readily reconciled in terms of a mixture of [3Fe-4S] and [4Fe-4S] 2+ clusters. [3Fe-4S] 0 clusters generally exhibit a broad ill defined shoulder centered near 400 nm and a weak broad band centered near 700 nm (43). The spectrum of [4Fe-4S] 0 clusters is greatly overemphasized in the RR spectrum due to much greater resonance enhancement.

Additional evidence for the dominant presence of [3Fe-4S] 0 clusters was provided by VTMC and EPR studies. EPR samples showed only a very weak signal centered at g = 2.004 (Fig. 4) and the characteristic low field resonance of the [3Fe-4S] 0 cluster centered at g = 9.4 that is enhanced in parallel mode (Fig. 5). The pattern, intensity, and saturation magnetization behavior of the bands in the VTMC spectrum (Figs. 6A and 7) can only be interpreted in terms of S = 2 [3Fe-4S] 0 as the dominant type of cluster in the anaerobically prepared MOCS1A N-terminal Cys → Ser variant. EPR studies have not provided evidence for ferricyanide oxidation of the [3Fe-4S] 0 cluster to yield an S = 1/2 [3Fe-4S] 1 cluster, indicating that the midpoint potential is likely to be greater than +420 mV (versus normal hydrogen electrode). However, reduction with dithionite resulted in the formation of a [4Fe-4S] 0 cluster as evidenced by the appearance of an S = 1/2 resonance (g = 2.03, 1.92, and 1.89) accounting for 0.21 spin/monomer that is very similar to those observed in dithionite-reduced aerobically and anaerobically purified MOCS1A samples (Fig. 4).

In accord with the results for the single mutants involving the C-terminal cysteines, MOCS1A–Δ1–56–C312S/C315S/C329S, reflecting the proposed N-terminal FeS cluster, showed low expression (~10% of wild type) consistent with a function of the C-terminal FeS cluster in maintaining the structural integrity of MOCS1A. This variant has an iron content of 2.6 mol of protein and shows a UV-visible absorption spectrum with a distinct maximum between 410 and 420 nm and a broad feature in the region of 500–700 nm more typical for [2Fe-2S] 2+ clusters (Fig. 2D). A remarkable decrease in protein solubility (~2–3 mg/ml) and the tendency to aggregate reflects the low soluble expression yields of this variant and prevented further spectroscopic characterization.
In summary, the results for the triple mutants of the N-terminal and the C-terminal cysteine motif provide clear evidence for two cluster binding regions. Each variant has approximately half the iron content of anaerobically purified wild type MOCS1A, and each shows different absorption characteristics indicative of different types of FeS clusters. The N-terminal triple Cys→Ser variant could accommodate a [4Fe-4S]** cluster, but this cluster was clearly rapidly degraded to yield a more stable [3Fe-4S]** cluster during purification. While more spectroscopic studies are required to fully characterize the cluster content of the C-terminal triple Cys→Ser variant, the absorption data are best interpreted in terms of [2Fe-2S]** clusters that are likely to be breakdown products of the [4Fe-4S]** cluster required for reductive cleavage of AdoMet.

**DISCUSSION**

In the present study, human MOCS1A was heterologously expressed in *E. coli* and purified as a soluble His-tagged protein under aerobic and anaerobic conditions. In accord with the studies of the homologous MoaA protein from *Arthrobacter nicotinovorans* (14, 15), MOCS1A was shown to be an FeS protein that requires two highly conserved cysteine motifs, one near the N terminus (CX_{1-2}CX_{2}C) and one near the C terminus (CX_{13}CX_{15}C). The mutagenesis results presented herein demonstrate that all six of the conserved cysteines in MOCS1A are required for catalytic activity and that the N-terminal and C-terminal cysteine-binding motifs ligate distinct FeS clusters. The type and properties of the FeS clusters in aerobically purified, anaerobically purified, and anaerobically reconstituted MOCS1A and the anaerobically purified N-terminal triple Cys→Ser variant were investigated using UV-visible absorption, RR, EPR, VTMCD, and Mössbauer spectroscopies coupled with iron and acid-labile sulfide analyses. Overall the results indicated that MOCS1A is a monomeric protein containing two very oxygen-sensitive FeS clusters, each ligated by only three cysteine residues.

A redox-active [4Fe-4S]** cluster that is readily degraded by exposure to oxygen and/or freeze/thaw cycles via a transient [3Fe-4S]** cluster and a semistable [2Fe-2S]** cluster intermediate is ligated by the N-terminal cysteine motif. Such cluster conversion behavior is characteristic of [4Fe-4S]** clusters in AdoMet-dependent radical enzymes, and both the electronic and vibrational properties of the [4Fe-4S]** and [2Fe-2S]** clusters are very similar to those previously reported for the equivalent clusters in enzymes of this protein family (17, 19, 20, 42). Hence we conclude that, analogous to AdoMet-dependent radical enzymes, the [4Fe-4S]**, [3Fe-4S]**, and [2Fe-2S]** clusters are all ligated by the three cysteines in the N-terminal motif and that the non-cysteinyl-ligated unique iron site of the [4Fe-4S]** cluster is essential for anchoring AdoMet (57–61) to induce reductive cleavage. Experiments designed to assess the ability of the [4Fe-4S]** cluster in MOCS1A to bind and reductively cleave AdoMet are currently in progress.

The functional form of the cluster ligated by the C-terminal cysteine motif is also likely to be a redox-active [4Fe-4S]** cluster based on spectroscopic studies of anaerobically reconstituted MOCS1A. However, the [4Fe-4S]** cluster was readily degraded by oxygen to yield a semistable [3Fe-4S]** cluster, and the spectroscopic results clearly demonstrated that the [3Fe-4S]** cluster is the dominant type of cluster present in this C-terminal cluster binding site in both aerobically and anaerobically purified samples of MOCS1A and the anaerobically purified N-terminal triple Cys→Ser variant. Hence we conclude that the C-terminal cysteine-binding motif ligates a [4Fe-4S]** cluster with only three cysteine ligands and that the non-cysteinyl-ligated iron is readily removed under mild oxidant conditions to yield a [3Fe-4S]** cluster. Indeed our inability to purify MOCS1A in a form corresponding to the anaerobically reconstituted form that contains two [4Fe-4S]** clusters, even under anaerobic conditions, may be a consequence of overexpression under aerobic conditions.

This study of MOCS1A constitutes the first successful attempt to characterize the type and properties of FeS clusters in a purified MoaA-type protein. In previous work, MoaA from *A. nicotinovorans* was heterologously expressed in *E. coli* and purified as a N-terminal fusion protein with either a glutathione S-transferase or a hexahistidine tag. Both proteins were reported to be brown albeit with “no significant features” in their UV-visible absorption spectra, and evidence for the presence of a [3Fe-4S]** cluster was based on iron and acid-labile sulfide determinations (approximately 4 iron and 3 acid-labile sulfides/MoaA monomer) and the observation of a weak isotropic g = 2.01 EPR signal that was present in the as-isolated but not the dithionite-reduced sample (15). However, these EPR signals were observed at 70 K and hence are likely to correspond to radical species since [3Fe-4S]** clusters exhibit fast relaxing S = 1/2 resonances that are not observable at 70 K (43). Indeed we observed weak (<0.01 spin/MOCS1A monomer), isotropic g = 2.01 resonances at 70 K with relaxation properties indicative of a radical species in both the aerobically and anaerobically purified samples of MOCS1A investigated in this work (data not shown). His-tagged *Rhodobacter capsulatus* MoaA was heterologously expressed as inclusion bodies in *E. coli*, and evidence for the presence of a [3Fe-4S]** cluster was based on the presence of a fast relaxing S = 1/2 resonance (g = 2.023, 2.015, and 2.004) of unknown concentration in the 2 K EPR spectrum of the washed insoluble pellet (31).

The important question that results from these studies is why MOCS1A requires two [4Fe-4S]** clusters. By analogy to other members of the AdoMet-dependent family of radical enzymes, the N-terminal [4Fe-4S] cluster is clearly implicated in reductive cleavage of AdoMet to generate the 5'-deoxyadenosyl radical. A purely structural or electron transfer role for the functional form of the cluster ligated by the C-terminal cysteine motif is also likely to be a redox-active [4Fe-4S]** cluster that binds AdoMet and an air-stable [2Fe-2S] cluster interme-
cannot be excluded, it seems very unlikely that the cluster bound by the C-terminal cysteine motif in MOCS1A functions as a sacrificial sulfate donor in MPT synthesis. However, we cannot completely exclude the possibility that MOCS1A is capable in providing one sulffer via its C-terminal FeS cluster analogous to biotin synthase and that the second sulfur is attached by MoaD via a mechanism analogous to Thf in thiamine biosynthesis to form the dithiolene group of molybdopterin.

Another more likely function is to invoke a role of the C-terminal FeS cluster in facilitating catalysis by binding and activating the substrate. The paradigm here is aconitase in which the active site comprises a [4Fe-4S] cluster ligated by only three cysteines with the non-cysteinyl-ligated unique iron site involved in the binding and activation of the substrate (72, 73). The formation of precursor Z involves a rearrangement reaction of the carbon atom at the 8-position of 5’-GTP (5, 6). The unique iron site of the C-terminal FeS cluster could be involved in temporary release of this carbon atom as formate. This would be analogous to a proposed GTP cyclohydrolase-like reaction mechanism (5, 6). GTP cyclohydrolases require zinc for their catalytic activity (74).

C-terminal cysteines constitute a new motif for the assembly of the unique iron site on the C-terminal [4Fe-4S] cluster of cyclohydrolases. A sacrificial sulfur donor in MPT synthesis. However, we cannot provide one sulfur via its C-terminal FeS cluster analogous to biotin synthase and that the second sulfur is attached by MoaD (3). Multiple studies show that the active site comprises a [4Fe-4S] cluster ligated in which the active site comprises a [4Fe-4S] cluster ligated by the dithiolene group of molybdopterin.

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66. Jameson, G. N., Cooper, M. M., Hernandez, H. L., Johnson, M. K., and Huynh, B. H. (2004) Biochemistry 43, 2022–2031.
Characterization of MOCS1A, an Oxygen-sensitive Iron-Sulfur Protein Involved in Human Molybdenum Cofactor Biosynthesis
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