Identification of the Missing Component in the Mitochondrial Benzamidoxime Prodrug-converting System as a Novel Molybdenum Enzyme*

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Amidoximes can be used as prodrugs for amidines and related functional groups to enhance their intestinal absorption. These prodrugs are reduced to their active amidines. Other \(N\)-hydroxylated structures are mutagenic or responsible for toxic effects of drugs and are detoxified by reduction. In this study, a \(N\)-reductive enzyme system of pig liver mitochondria using benzamidoxime as a model substrate was identified. A protein fraction free from cytochrome \(b_5\) and cytochrome \(b_5\) reductase was purified, enhancing 250-fold the minor benzamidoxime-reductase activity catalyzed by the membrane-bound cytochrome \(b_5/NADH\) cytochrome \(b_5\) reductase system. This fraction contained a 35-kDa protein with homologies to the C-terminal domain of the human molybdenum cofactor sulfurase. Here it was demonstrated that this 35-kDa protein contains molybdenum cofactor and forms the hitherto ill defined third component of the \(N\)-reductive complex in the outer mitochondrial membrane. Thus, the 35-kDa protein represents a novel group of molybdenum proteins in eukaryotes as it forms the catalytic part of a three-component enzyme complex consisting of separate proteins. Supporting these findings, recombinant C-terminal domain of the human molybdenum cofactor sulfurase exhibited \(N\)-reductive activity in vitro, which was strictly dependent on molybdenum cofactor.

Numerous drugs and drug candidates contain strongly basic functional groups, such as guanidines, amidinohydrzones, and amidines. These groups, however, impair drug absorption from the gastrointestinal tract, because they are protonated under physiological conditions. Therefore, the prodrug principle was developed to enhance oral bioavailability (1). In the case of amidines, \(N\)-hydroxylation converts them to the corresponding \(N\)-hydroxamidines that are less basic and therefore unprotonated under physiological conditions, thereby enhancing intestinal absorption by diffusion (2). This prodrug principle was applied to a wide range of mainly antiprotozoal and antithrombotic drugs (2).

The wider application of amidoxime prodrugs requires the identification of the cellular \(N\)-reducing enzyme system. Previous studies described the ability of microsomes and mitochondria to reduce \(N\)-hydroxylated amidines (3–6), with the highest activities in the outer mitochondrial membrane (3, 4). For mammals, it was demonstrated that cytochrome (cyt)\(^2\) \(b_5\) and its FAD-containing reductase are involved in the reduction of hydroxylamines and \(N\)-hydroxylated amidines (7, 8). Both enzymes are present in a soluble and a membrane-bound form. The latter are components of an electron transport system associated with the mitochondrial outer membrane and the endoplasmatic reticulum in somatic cells (9), where they mediate electron transfer from NADH to a variety of final acceptors involved in lipid metabolism, such as fatty acid desaturase (10), sterol oxidases (11), and certain P450 enzymes (12). According to the physical electron transfer chain, an additional enzyme component is also postulated for drug metabolism. Kadlubar and Ziegler (7) described the oxygen-independent microsomal hydroxylamine reductase as a multicomponent enzyme system consisting of cyt \(b_5\), its reductase, and a third unidentified protein fraction that catalyzes the reduction of hydroxylamine and a number of its mono- and disubstituted derivatives. Later a similar microsomal enzyme system was described: a microsomal \(N\)-hydroxy reductase (8), consisting of the membrane-bound electron transfer chain described above and a postulated microsomal P450 isoenzyme (Fig. 1) as \(N\)-reductive protein that should be able to reduce \(N\)-hydroxylated derivatives of amidines, sulfonamides, and numerous other \(N\)-hydroxylated functional groups (8, 13, 14). The necessity of an additional \(N\)-reductive enzyme of the membrane-bound microsomal drug metabolism system was confirmed by Andersson et al. (3), although the identity of the third protein remained open. Recently, it was found that also the outer mitochondrial membrane harbored a \(N\)-reductive system (4) similar to the one of microsomes, and a third protein component was postulated to be necessary for \(N\)-reduction.

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‡1 The abbreviations used are: cyt, cytochrome; ABA3, A. thaliana molybdenum cofactor sulfurase; CHAPS, 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonic acid; CT, C terminus; HPLC, high performance liquid chromatography; MALDI, matrix-assisted laser desorption ionization; Moco, molybdenum cofactor; MS, mass spectrometry; MS/MS, tandem mass spectrometry; OMV, outer membrane vesicle; TOF, time-of-flight; HMCS, molybdenum cofactor sulfurase.
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Here the identification of this third N-reductive component in the outer mitochondrial membrane of pig liver is described. It turned out to be a novel molybdenum-containing protein hitherto not described in eukaryotes or bacteria sharing significant homology with the C-terminal domain of the molybdenum cofactor sulfursase (HMCS-CT).

EXPERIMENTAL PROCEDURES

Purification of the N-Reductive Mitochondrial Component—Pig liver mitochondria were obtained by differential centrifugation and an isotonic Percoll gradient (15) with modifications (4). The outer mitochondrial vesicle (OMV) fraction was purified using the steel disintegration method followed by two steps of sucrose density gradient centrifugation (16) with modifications (4). Frozen OMV fraction was thawed, pooled, and adjusted to 20 mM Tris-base, 0.1 mM EDTA, 0.1 mM dithiothreitol, 20% (m/v) glycerol, 0.9 mM Zwittergent® 3-14, pH 7.4. The detergent/protein ratio was 0.5. This solubilization mixture was stirred for 60 min on ice. The solubilized membrane fraction (∼13 mg of protein) was applied to a DEAE-52 column (2.5 × 10 cm) (DEAE 52-Cellulose Servacel®; Serva Electrophoresis, Heidelberg, Germany) and equilibrated with buffer (20 mM Tris-base, 0.1 mM EDTA, 0.1 mM dithiothreitol, 20% (m/v) glycerol, 0.9 mM Zwittergent® 3-14, pH 7.4). After sample application, the column was washed at a flow rate of 0.8 ml/min with two column volumes of the equilibrating buffer and was developed with a linear concentration gradient (250 ml) of 0–1.0 sodium chloride in equilibrating buffer. Fractions of 4 ml were collected and assayed. Active fractions were pooled and stored at −80 °C. All operations were performed at 0–4 °C. Protein was assayed using bichinchonic acid (17), according to the manufacturer’s directions (BCA protein assay kit; Pierce), using bovine serum albumin as a standard.

Purification of NADH Cyt b5 Reductase—NADH Cyt b5 reductase was purified from pig liver microsomes in a modified method of Laemmli (19), with a 5% stacking gel. Staining was performed with Coomassie Brilliant Blue R250 (Serva, Heidelberg, Germany). Standards and samples were pretreated with β-mercaptoethanol for 5 min at 90 °C.

Mass Spectrometry—Proteins were identified by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) peptide mass fingerprinting or nanocapillary liquid chromatography electrospray ionization tandem mass spectrometry (MS/MS). The analysis were performed by Planton GmbH (Kiel, Germany) and Richard Jones (National Center for Toxicological Research).

Immunoblot Analysis—Immunoblot analysis was performed by gel blotting protein-fraction A and B subjected to 12% SDS-PAGE using a primary polyclonal antibody raised against recombinantly expressed C terminus of Arabidopsis thaliana molybdenum cofactor sulfursase (ABA3-CT) (1:7000 dilution). The secondary horseradish peroxidase-conjugated anti-rabbit Ig (Sigma) was used in a 1:10,000 dilution, and chemiluminescence was detected using the ECL system (Amersham Biosciences).

nit-1 Reconstitution—Neurospora crassa nit-1 extract was prepared as described previously (20) and stored in aliquots at −70 °C. All reconstitutions were performed in nit-1 buffer (50 mM sodium phosphate, 200 mM NaCl, and 5 mM EDTA, pH 7.2) containing 2 mM reduced glutathione either in the absence or in the presence of 5 mM sodium molydate. The reconstitution assay was performed in a 40-μl reaction volume containing 20 μl of gel-filtrated nit-1 extract. Complementation was carried out anaerobically for 2 h at room temperature. After the addition of 20 mM NADPH and incubation for 10 min in the dark, reconstituted NADPH-nitrate reductase activity was determined as described (20). Importantly, the addition of sodium molydate to the reaction mix did not significantly enhance reconstitution of NADPH-nitrate reductase activity, indicating that all fractions tested contained molybdenum cofactor (Moco) rather than molybdenum-free molybdopterin.

Cloning of hmcs-CT—Polymerase chain reaction was performed with a human liver cDNA library (λ-Uni-ZAP XR, Stratagene) as template and by using primers HMCS-1429 (5′-GGA TAC ATG TCG ACG CTG GAT GAT-3′) and HMCS-stop (5′-TTA GGA GGT AAC ATC CTG GTG TTT CTC-3′) derived from GenBank™ entry AK000740, which represents a full-length hmcs-cDNA. By this procedure, a partial hmcs cDNA fragment of ∼1.3 kb was obtained, which contained the 3′-region of the hmcs open reading frame and whose correctness was confirmed by sequencing. Restriction sites for BamHI were introduced by polymerase chain reaction using primers HMCS-CT-start/BamHI (5′-ACC CAG GGA TCC ATG TCA GAG AAA GCT GCA GGA GTC CTG-3′) and HMCS-CT-stop/BamHI (5′-ACG GTG CAC CCT TGT ACG GTA ACA TCC TGG TGT TTC-3′), which amplified the last 966 bp of the open reading frame encoding for the putative C-terminal domain of HMCS. Simultaneous introduction of an ATG codon at the N-terminal end in frame with the vector-encoded His6 tag enabled cloning into the pQE80-plasmid (Qiagen, Hilden, Germany), thereby allowing expression of a His6-HMCS-CT fusion protein with an estimated molecular mass of 37.4 kDa. Cloning and expression of ABA3-CT for generation of anti-ABA3-CT polyclonal antibodies was performed as described earlier by Heidenreich et al. (21).

Expression and Purification of HMCS-CT—Routine protein expression was performed in freshly transformed Escherichia coli TP1000 cells (22). Cells were grown aerobically in Luria broth medium in the presence of 100 μg/ml ampicillin at 22 °C to a A600 of 0.1 before induction with 15 μM isopropyl-β-D-thiogalactopyranoside and the addition of 1 mM sodium molyb-
date. After induction, cells were grown for a further 20 h at 22 °C. Expression in E. coli strains RK5206 and RK5204 (23) was carried out likewise without the addition of sodium molybdate. Cells were harvested by centrifugation and stored at −70 °C until use. Cell lysis was achieved by several passages through a French pressure cell followed by sonication for 5 min. After centrifugation, His$_6$-tagged protein was purified on a nickel-nitrioltriacetic acid superflo matrix (Qiagen, Hilden, Germany) under native conditions at 4 °C according to the manufacturer’s manual. Eluted fractions were analyzed by SDS-PAGE. Molybdenum binding pterin bound to the purified proteins was detected and quantified by converting it to the stable oxidation product FormA-dephospho, according to Johnson et al. (24). Oxidation, dephosphorylation, QAE chromatography, and high performance liquid chromatography (HPLC) analysis were performed as described previously (25). FormA-dephospho was quantified by comparison with a standard isolated from xanthine oxidase for which the absorbitivity was $\varepsilon_{380} = 13,200 \text{ M}^{-1} \text{cm}^{-1}$ (24). Thereby, average saturation of HMCS-CT with MPT/Moco, determined as FormA-dephospho, was found to be about 41% (412 ± 96 pmol of FormA/nmol of protein; $n = 5$).

**NADH Cyt b$_5$ Reductase Activity**—Enzyme activity was determined by modification of the ferricyanide reduction assay (26).

**Heme Content**—Heme content was estimated by recording the sodium dithionite-reduced minus the oxidized spectrum (27).

**Assay for the Reduction of Benzamidoxime**—For determination of benzamidoxime reduction, incubations were carried out under aerobic conditions at 37 °C in a shaking water bath. Incubation mixtures of the subcellular fractions contained 56 μg (mitochondria) or 6 μg of protein (OMV fraction), 0.5 mM benzamidoxime (synthesized from benzonitrile and hydroxylamine as described in Ref. 28), and 1.0 mM NADH (or 0.4 mM NADH in the case of the OMV fraction) in a total volume of 150 μl of 100 mM potassium phosphate buffer, pH 6.0. After preincubation for 3 min at 37 °C, the reaction was initiated by the addition of NADH and terminated after 15 min (OMV fraction) or 20 min (mitochondria) by adding aliquots of methanol. If not otherwise stated, standard incubation mixtures of the reconstituted system contained 240 ng of purified mitochondrial benzamidoxime reductase almost abolished the activity of the outer mitochondrial membrane, exerting an IC$_{50}$ value of 8 μM. (iii) ion exchange chromatography of detergent-solubilized OMV fractions gave two pools (designated as fractions A and B) with benzamidoxime reductase activity (Fig. 2). Fraction A was devoid of any heme content (as tested by dithionite-reduced difference spectra) and of cyt b$_5$ reductase activity, whereas fraction B contained minor contents of cyt b$_5$ and its reductase. Therefore, fraction A was used for further analysis. Purification parameters are given in Table 1, and the results of SDS-PAGE analysis are shown in Fig. 3. The purified enzyme fraction A was stored at −80 °C, and the same activity in the reconstituted system was observed at least after one freeze-thaw cycle (data not shown).

**Enzymatic Characterization of Fraction A**—The reduction of benzamidoxime by NADH requires cyt b$_5$, its reductase, and an additional N-reductive protein contained in the purified mitochondrial protein fraction A. Fraction A alone was not able to catalyze the reduction of benzamidoxime by NADH (Table 2).
The low rate of benzamidoxime reduction obtained with the mitochondrial fraction A and purified NADH cyt $b_5$ reductase (Table 2) is caused by the contamination of the reductase with small amounts of cyt $b_5$. Similar results were obtained when replacing purified microsomal NADH cyt $b_5$ reductase by purified recombinant NADH cyt $b_5$ reductase (not shown). The $N$-reductive activity of the standard incubation mixture followed Michaelis-Menten kinetics (apparent $K_m$ 0.18 mM, $V_{max}$ was 12.25 $\mu$mol of benzamidine/min/mg of fraction A).

The specific activity of fraction B in the reconstituted system was slightly lower than the specific activity of fraction A (7.10 ± 0.08 $\mu$mol of benzamidine/min/mg (n = 4)). However, in contrast to fraction A, fraction B also reduced benzamidoxime without adding cyt $b_5$ and its reductase with a specific activity of 30 ± 1 nmol of benzamidine/min/mg (n = 4), demonstrating that it is contaminated with cyt $b_5$ and its reductase.

Composition of Fraction A—SDS-PAGE analysis of fraction A showed an almost electrophoretically pure protein with two dominant spots (35 and 66 kDa) in a Coomassie-stained SDS gel (Fig. 3B). Whereas the 66-kDa protein was identified as monooamine oxidase-B by MALDI-TOF, tryptic digestion and electrospray ionization-MS/MS sequencing of the 35-kDa protein and the C termini of HMCS and ABA3, which all displayed 100% identity to a putative protein from Homo sapiens hitherto referred to as “Moco sul-

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![Graph of benzamidoxime reductase activity and absorbance at 280 nm vs. NaCl gradient](Image)

**FIGURE 2.** Elution profile of solubilized OMV fraction. DEAE ion exchange chromatography with a linear NaCl gradient. Absorbance at 280 nm was recorded, benzamidoxime reduction activity (nmol/min/μl of DEAE fraction) was measured in the reconstituted system after the addition of cyt $b_5$ and NADH cyt $b_5$ reductase. Fractions 42-43 and 54-55 were pooled and designed as fractions A and B, respectively.

### TABLE 1
**Purification of mitochondrial $N$-reductive components**

| Stage                        | Total activity | Total protein | Specific activity | Purification | Yield |
|------------------------------|----------------|---------------|-------------------|--------------|-------|
| Swell disruption homogenate  | 36.5           | 2027          | 0.02$^a$          | 1            | 100   |
| Solubilized OMV fraction     | 30.3           | 13            | 0.23$^a$          | 13           | 8     |
| Fraction A                   | 1.9            | 0.19          | 10$^b$            | 556$^c$      | 5$^c$ |

$^a$ Specific benzamidoxime reductase activity of membrane preparations (μmol/min/mg of total protein).

$^b$ Specific benzamidoxime reductase activity of purified and cyt $b_5$/cyt $b_5$ reductase added. Therefore, all purification calculations are based on the activities using this assay system.

$^c$ Calculated to specific activity of the reconstituted system.
firmed the enrichment found by nit-1 analysis, since it showed a 17-fold enrichment of FormA-dephospho in fraction A (295.3 nmol of FormA/mg of protein) but no enrichment in fraction B (8.7 nmol of FormA/mg of protein) in comparison with the OMV fraction (17.4 nmol of FormA/mg of protein).

HMCS C-terminal Domain Is Able to Reduce Benzamidoxime—Since both the 35-kDa protein from fraction A and HMCS-CT share not only sequence homologies but also bind Moco, the question raised whether HMCS-CT could replace the 35-kDa protein enzymatically (i.e., whether it would be able to reduce benzamidoxime). As shown in Table 4, HMCS-CT was able to catalyze the reduction of benzamidoxime to benzamidine. The formation of benzamidine was also proven by liquid chromatography/MS. Interestingly, only HMCS-CT protein that was expressed in E. coli TP1000 and that therefore had Moco bound was capable of reducing benzamidoxime. Control incubations with HMCS-CT protein that was either binding molybdopterin, a molybdenum-free precursor of Moco, or that had no pterin bound due to expression in E. coli RK5206 and RK5204, respectively, showed no reductive activity, indicating that the molybdenum center is essential for the catalytic activity. In contrast to the mitochondrial protein, HMCS-CT is able to catalyze the benzamidoxime reduction without cyt b₅ and its reductase. A minor N-reductive activity was also detectable without adding NADH (Table 4). The reaction was linear with protein content and time over ~10 min (data not shown) and followed Michaelis-Menten kinetics. The apparent $K_m$ for the reaction was 15 μM, and $V_{max}$ was 0.24 nmol of benzamidine/min/mg.

DISCUSSION

Prodrug principles were developed to enhance oral bioavailability of those drugs that are charged under physiological conditions and therefore impaired in intestinal absorption (1). For the basic amidines, N-hydroxylation converts them to the less basic N-hydroxamidines that are more easily absorbed (2). Two cellular compartments have been identified to catalyze this reaction, the microsomes (5, 8) and the mitochondrial outer membrane (3, 4), harboring a multicomponent enzyme system consisting of cyt b₅, its reductase, and a third protein (Fig. 1). In this study, the missing protein was isolated from a mitochondrial OMV fraction free from cyt b₅ and cyt b₅ reductase, since these enzymes were previously claimed to be solely responsible for the reduction of amidoximes (32). This was accomplished by an improved purification protocol for mitochondrial outer membranes and a gentle solubilization process using Zwittergent® 3-14. The N-reductive activity of this protein fraction can only be determined in a reconstitution assay containing all three components of the N-reductive enzyme complex (i.e., cyt b₅ and its reductase have to be added). Here the well-defined purified microsomal proteins cyt b₅ (recombinantly expressed) and its reductase (biochemically purified from pig liver) were used. Microsomal cyt b₅ has a protein fold similar to cyt b₅ from the outer mitochondrial membrane (33) and was previously shown to enhance the N-reductive activity of the enzyme system located in the outer mitochondrial membrane fraction (4). The in vitro reconstitution assays required...
no additional phospholipids, probably due to Zwittergent® 3-14 present in fraction A, which is known to simulate the hydrophobic environment of native membranes (34).

The protein fraction free from cyt b₃ and cyt b₅, reductase contained monoamine oxidase-B and a 35-kDa protein with significant similarities to the C-terminal domain of the Moco sulfurase HMCS. Recombinant monoamine oxidase-B failed to reduce benzamidoxime in the reconstituted system (data not shown), so this enzyme was excluded from being the N-reductive component in fraction A.

Antibodies raised against the C-terminal domain of the Moco sulfurase ABA3 from A. thaliana revealed cross-reaction to this 35-kDa protein, indicating that they share structural similarities. Determination of the Moco-dependent nit-1 reconstitution activity showed an ~40-fold enrichment of nit-1 reconstitution activity and, simultaneously, of the N-reductive activity of fraction A. In common with the strong inhibitory effect of vanadate, an inhibitor of molybdenum enzymes (35), it is assumed that this 35-kDa Moco-containing protein represents the N-reductive component in fraction A. Since benzamidoxime was the model amidoxime used in this study, the 35-kDa Moco-containing protein was named mARC, which stands for mitochondrial benzamidoxime reducing component. However, other N-hydroxylated structures, such as N-hydroxyguanidines, N-hydroxyaminohydrazones, and hydroxylamines, will probably also be reduced by the enzyme system described here. Further studies to prove this assumption are in process. Preliminary results already demonstrated the mitochondrial reductive activity of other amidoximes and N-hydroxylated aromatic hydroxylamines as part of a detoxification system.

The discovery of a novel Moco-containing protein in the outer mitochondrial membrane is in agreement with the findings of Johnson et al. (36), who almost 3 decades ago detected considerable amounts of Moco associated with the outer membrane of rat liver mitochondria that did not derive from the known molybdenum enzymes sulfite oxidase or xanthine oxidase. At that time, the actual Moco source could not be identified. Hence, with the present work, it becomes likely that the Moco-containing fraction as described by Johnson et al. (36) corresponds to the mARC protein described here.

Since mARC is a homolog of the C-terminal domain of HMCS, the latter was taken for in vitro biotransformation studies. Although the physiological function of HMCS is different from N-reduction, HMCS-CT developed N-reductive catalytic activity. Most importantly, this activity was strictly dependent on Moco, since control incubations with HMCS-CT that was either binding molybdopterin or that had no Moco bound, showed no reductive activity. Thus, it was proven that the molybdenum center is essential for the catalytic activity. Such N-reductive potential has been described earlier for the molybdenum enzymes xanthine oxidase and aldehyde oxidase (37, 38), thereby supporting the assumption that Moco-binding enzymes play a fundamental role in reducing N-hydroxylated aromatic hydroxylamines as part of a detoxification system.

In summary, this study provided evidence that besides cyt b₅ and its reductase, a Moco-binding protein participates predominantly in the mitochondrial N-reductive enzyme system. This mARC protein represents a novel molybdenum enzyme hitherto not described in eukaryotes or bacteria. It is also unique, since it forms the catalytic part of a three-component enzyme complex. This is different from the other three molybdenum enzymes known in mammals, namely sulfite oxidase, xanthine oxidase, and aldehyde oxidase, where the single components of the electron transport chains are comprised within a single polypeptide chain. When comparing the arrangement of these electron transport chains, the N-reductive complex in the outer mitochondrial membrane is strikingly similar to eukary-

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**TABLE 3**

Identification of molybdenum cofactor by reconstitution of nit-1 NADPH nitrate reductase activity

Moco-dependent reconstitution of NADPH nitrate reductase activity was measured by co-incubation of N. crassa nit-1 extracts and the respective mitochondrial subfractions. Data are means ± S.D. of two determinations.

| Protein fraction | Activity (μmol NO₂⁻/min/mg) |
|------------------|-----------------------------|
| OMV fraction     | 0.06 ± 0.02                 |
| Fraction A       | 2.00 ± 0.24                 |
| Fraction B       | 0.15 ± 0.02                 |

**TABLE 4**

N-Reduction of benzamidoxime by HMCS-CT

The incubation mixture consisted of 135 μg of purified recombinant HMCS-CT as expressed in E. coli TP1000, RK5206, or RK5204, respectively, 0.5 mM benzamidoxime, and 1 mM NADH in 150 μl of 100 mM phosphate buffer, pH 6.0. The specific activity of HMCS-CT from TP1000 without co-substrate was significantly (p < 0.05) lower.

| E. coli expression strain | Co-substrate | Specific activity (nmol/min/mg) |
|--------------------------|--------------|--------------------------------|
| TP1000                   | 0.5 mM NADH  | 0.48 ± 0.04                    |
| TP1000                   | without NADH | 0.23 ± 0.01                    |
| RK5206                   | 0.5 mM NADH  | ND*                            |
| RK5204                   | 0.5 mM NADH  | ND*                            |

* ND, not detectable (limit of detection = 0.04 nmol/min/mg).
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otic nitrate reductases, occurring in plants, algae, and fungi, that consist of a single polypeptide chain with a cyt b₅ domain, a cyt b₅ reductase domain, and Moco-binding domain (39). Bacterial nitrate reductases, however, consist of separate proteins forming a multicomponent complex (40). Hence, the N-reductive complex in the outer mitochondrial membrane with its mARC protein represents the first case for a eukaryotic molybdenum enzyme consisting of separate proteins. The isolation of this reductase complex will help to test new N-hydroxylated compounds developed as prodrugs. In vitro assays with this complex could predict if a new prodrug would be reduced in vivo.

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