Monoamine Oxidase Contains a Redox-active Disulfide*

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Mitochondrial monoamine oxidases A and B (MAO A and MAO B) are ubiquitous homodimeric FAD-containing oxidases that catalyze the oxidation of biogenic amines. Both enzymes play a vital role in the regulation of neurotransmitter levels in brain and are of interest as drug targets. However, little is known about the amino acid residues involved in the catalysis. The experiments reported here show that both MAO A and MAO B contain a redox-active disulfide at the catalytic center. The results imply that MAO may be a novel type of disulfide oxidoreductase and open the way to characterizing the catalytic and chemical mechanism of the enzyme.

Flavin-containing mitochondrial monoamine oxidases A and B (MAO A and MAO B) catalyze the oxidative deamination of neurotransmitters, such as dopamine, serotonin, and noradrenaline in the central nervous system and peripheral tissues. The enzymes share 73% sequence homology and follow the same kinetic and chemical mechanism but have different substrate and inhibitor specificities (1). Inhibitors of these enzymes are medically important antidepressants, but the rational design of new inhibitor drugs is hampered by the lack of the active site structure and by remaining controversies in the catalytic mechanism.

Chemical modification experiments provide evidence that a histidine residue (2, 3) is essential for the catalysis. There is also strong evidence that two cysteine residues are present in the active site of MAO (3–9). The inhibition of MAO by sulfhydryl reagents, first observed in 1945 (10), is well established (3–6), but a role for essential cysteine residues in the catalytic mechanism has not been identified. In the chemical mechanism, there is still controversy about whether MAO-catalyzed oxidative deamination proceeds via a radical mechanism, hydride transfer, or oxidation of a carbanion intermediate. In the most extensively tested hypothesis (11), the transfer of one electron from the amine to the enzyme (presumably to the flavin) is followed by the cleavage of the α-carbon-hydrogen bond to produce the amino radical. The substrate radical either passes a second electron to the flavin or combines with an unknown active site radical to give a covalent adduct that decomposes to the imine. However, no flavosemiquinone has ever been detected in the catalytic cycle (12–15). When flavosemiquinone is generated by reduction with dithionite, the weak epr signal observed (about half that expected) suggested that there might be coupling of FAD radical with an unknown protein radical (14). If such a putative non-FAD radical can be generated by reduction by dithionite, the number of electrons required to reduce the enzyme should be more than the two necessary for the FAD alone. The data presented in this paper demonstrate the presence of a second redox-active group in addition to the flavin in the active site of MAO.

EXPERIMENTAL PROCEDURES

Enzymes and Reagents—Human liver MAO A heterologously expressed in yeast and MAO B from beef liver were purified as previously reported (16, 17). The concentration of each sample was determined from the oxidized minus reduced extinction coefficient at 456 nm of 10,800 M⁻¹ cm⁻¹ for MAO A and 10,300 M⁻¹ cm⁻¹ for MAO B. Enzyme activity was determined spectrophotometrically using kynuramine (1 mM) for MAO A and benzylamine (3 mM) for MAO B.

Kynuramine dihydrobromide, benzylamine, and 3,4-aminphetamine hydrochloride were purchased from Sigma. 4,4′-Dipyridyl disulfide (DPDS) (Aldrich) was recrystallized once from ethanol. All the experiments were carried out at 20 °C in 50 mM sodium phosphate buffer, pH 7.2, containing 0.01% Brij-35.

4,4′-Dithio(bispyridine)-modified MAO A and MAO B were prepared by incubation of 10–15 μM of the enzyme with 50 μM DPDS. Aliquots of the reaction mixture were taken at intervals to measure the remaining enzyme activity. When the enzyme sample was inactivated by more than 95%, it was transferred into the anaerobic cuvette for dithionite titration.

Anaerobic Titrations—The titrations with sodium dithionite were carried out in a custom-made quartz anaerobic cuvette with a side arm, in an atmosphere of high purity argon. Dithionite standardized by titration of riboflavin was added via a gas-tight syringe attached to the cuvette. The spectra were recorded in a Shimadzu UV-2101PC spectrophotometer.

RESULTS AND DISCUSSION

Reductive Titration of MAO A—Human liver MAO A was made anaerobic by equilibration with argon and titrated with standardized dithionite to count the electrons required for full reduction of flavin cofactor. Fig. 1 shows the spectral changes occurring during the anaerobic titration with dithionite. The inset to Fig. 1 shows the changes in absorbance at 412 nm where the flavosemiquinone absorbs strongly and at 456 nm, the wavelength where bleaching is observed for both the one-electron reduction steps of a typical flavin. Full reduction required 2.0 ± 0.12 mol (n = 5) of dithionite per mol of flavin which is four electron equivalents (Fig. 1, inset), two more than expected for a flavin alone. The final spectrum was typical of the fully reduced enzyme (5). These data suggest that there is a redox-active functional group in the active site of MAO in close proximity to flavin, which (i) interacts with the flavin resulting in some stabilization of the flavosemiquinone, (ii) has a redox potential close to that for the flavin, so that both the flavin and this group are reduced simultaneously, and (iii) is able to accept two extra electrons upon the dithionite titration. The likely candidate for this group is a disulfide, because cysteines are known to be essential for the activity of MAO. The

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§ The abbreviations used are: MAO, monoamine oxidase; DPDS, 4,4′-dipyridyl disulfide.
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FIG. 1. Full reduction of MAO A requires four electron equivalents. Human MAO A overexpressed in yeast was purified as previously reported (16). MAO A (14.8 μM, in 50 mM sodium phosphate, pH 7.2, containing 0.05% Brij-35) was placed in the anaerobic cuvette with the side arm and made anaerobic by cycling 10 times between argon and vacuum using an all-glass gas-tight. Aliquots of dithionite solution (0.925 mM, standardized as described in Ref. 5) were added from the Hamilton syringe with a manual dispenser, anaerobically attached to the side arm of the cuvette by a gas-tight connector, and spectra were recorded after each addition in a Shimadzu UV-2101PC spectrophotometer. For clarity only selected spectra are shown, but all are plotted in the inset. The numbers on the traces indicate the volume of the dithionite solution added: 1, 0 μl; 2, 4 μl; 3, 8 μl; 4, 12 μl; 5, 16 μl; 6, 20 μl; 7, 24 μl; 8, 28 μl; 9, 32 μl. Inset, plot of absorbance changes at 412 nm and at 460 nm. The amount of electron equivalents in the dithionite added per 1 eq of the enzyme flavin was calculated as |2 × (molarity of dithionite) × (volume of dithionite added)/((volume of MAO A flavin) × (volume of enzyme))|. The oxidized-reduced difference extinction coefficient of MAO A flavin of 10.8 M⁻¹ cm⁻¹, as determined in Ref. 5, was used to calculate the molarity of FAD in MAO A.

Effect of the reductant (DPDS) on the reduction of MAO A flavin. MAO A (15 μg) with DPDS-modified MAO A (5 mM) was titrated anaerobically as described in the legend to Fig. 1. Full reduction was never achieved, but a two-electron reduction was required to generate the maximum absorbance at 412 nm. The amounts of standardized dithionite solution (1.875 mM) added were: 1, 0 μl; 2, 4 μl; 3, 8 μl; 4, 12 μl; 5, 16 μl; 6, 20 μl; 7, 24 μl; 8, 28 μl; 9, 32 μl. Inset, plot of absorbance changes at 412 nm and at 456 nm. The amount of electron equivalents added and the molarity of FAD in the MAO A sample were calculated as described in the legend to Fig. 1.

FIG. 2. Full reduction of MAO A modified with DPDS requires two electron equivalents. DPDS-modified MAO A was prepared by incubation of 6.6 μM of the enzyme with 50 μM DPDS. Aliquots of the reaction mixture, 3 μl, were taken at given time intervals to measure the remaining enzyme activity by the spectrophotometric assay with 1 mM nkyaramine (5). When the enzyme sample was inactivated by more than 95%, it was transferred to the anaerobic cuvette, made anaerobic, and titrated as described in the legend to Fig. 1. The amounts of standardized dithionite solution (0.55 mM) added were: 1, 0 μl; 2, 3 μl; 3, 4 μl; 4, 8 μl; 5, 10 μl; 6, 12 μl. Inset, plot of absorbance changes at 412 nm and at 456 nm. The amount of electron equivalents added and the molarity of FAD in the MAO A sample were calculated as described in the legend to Fig. 1.

FIG. 3. Dithionite titration of MAO A in the presence of the inhibitor, β-mercaptoethanol. MAO A (15 μg) with β-mercaptoethanol (5 mM) was titrated anaerobically as described in the legend to Fig. 1. Full reduction was never achieved, but a two-electron reduction was required to generate the maximum absorbance at 412 nm. The amounts of standardized dithionite solution (1.875 mM) added were: 1, 0 μl; 2, 4 μl; 3, 8 μl; 4, 12 μl; 5, 16 μl; 6, 20 μl; 7, 24 μl; 8, 28 μl; 9, 32 μl. Inset, plot of absorbance changes at 412 nm and at 456 nm. The inset shows the changes in absorbance at 412 nm and 456 nm as a function of the reducing equivalents added.

Effect of β-Mercaptoethanol—β-Mercaptoethanol, which reduces disulfides, inhibited MAO A (in agreement with Ref. 22) in a concentration- and time-dependent manner. The concentration required for 50% inhibition after 15 min in aerobic solution was 0.25 mM. The inhibition was fully reversible by dilution. Incubation of MAO A with β-mercaptoethanol (5 mM) under anaerobic conditions resulted in the reduction of the flavin cofactor. When oxygen is added to the reaction mixture containing reduced enzyme and β-mercaptoethanol, the flavin is reoxidized instantly. No reduction of free riboflavin was observed under the same conditions. This cannot be attributed to the difference in the redox potential between the free flavin in solution and the flavin in MAO because these are similar (19, 20). We speculate that β-mercaptoethanol reduces the active site disulfide, which, in turn, reduces the cofactor.

Effect of Ligand—Reductive titration of MAO A in the pres-
ence of the inhibitor (or pseudo-substrate), D-amphetamine (Fig. 3), gave completely different results from those obtained for the free native enzyme. It took two electron equivalents to reach the maximum absorbance at 412 nm, indicating the maximum formation of the semiquinone. No further significant spectral changes were observed even after the addition of more than eight electron equivalents and the incubation of the enzyme with dithionite for 5 h. There is no obvious explanation for why only the semiquinone of MAO is formed in the presence of the enzyme with dithionite for 5 h. There is no obvious explanation than eight electron equivalents and the incubation of the enzyme and dithionite for 5 h. There is no obvious explanation.

The redox stoichiometry for both MAO A and B is confirmed by reductive titration. Four electron equivalents were required to reduce the native enzyme and two after treatment with DPDS. The reductive titration of MAO B (not shown) gave results identical with those for MAO A (Figs. 1 and 2). Four electron equivalents were required to reduce the native enzyme and two after treatment with DPDS. The slopes for the enzyme alone were twice that expected (implying that 4 eq were required for full reduction, as demonstrated directly here) whereas those for the enzyme-substrate complex indicated the expected 2 equivalents.

Conclusions—The data reported here suggest that MAO contains a redox-active disulfide in the active site and, therefore, may be a new type of disulfide oxidoreductase. We speculate that when an amine is oxidized, electrons pass from the amine to the disulfide and then to the flavin. The formation in MAO of a carboxyl-imidazole-disulfide triad, similar to that demonstrated for disulfide oxidoreductases, would result in an increased positive charge on the disulfide which would facilitate electron transfer from the amine substrate. Similarities with the disulfide oxidoreductase family of enzymes, which includes glutathione oxidoreductase, lipoamide dehydrogenase, and thioredoxin reductase, may be useful in elucidating the mechanism. However, although there are structural analogies between the N-terminal region of MAO and the nucleotide binding region of lipoamide dehydrogenase (24), the dithiol sequence motif common to the disulfide oxidoreductase family (25) is not found in MAO.

REFERENCES

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