Archives of Biochemistry and Biophysics 498 (2010) 83–88

Contents lists available at ScienceDirect

Archives of Biochemistry and Biophysics

journal homepage: www.elsevier.com/locate/yabbi

A lysine conserved in the monoamine oxidase family is involved in oxidation of the reduced flavin in mouse polyamine oxidase ☆

Michelle Henderson Pozzi [a], Paul F. Fitzpatrick [b,*]

[a] Department of Biochemistry and Biophysics, Texas A&M University, College Station, TX 77843-2128, United States
[b] Department of Biochemistry and Center for Biomedical Neuroscience, University of Texas Health Science Center at San Antonio, San Antonio, TX 78229-3900, United States

ARTICLE INFO

Article history:
Received 22 March 2010
and in revised form 19 April 2010
Available online 22 April 2010

Keywords:
Flavoproteins
Enzyme mechanisms
Isotope effects
Enzyme kinetics

ABSTRACT

Lysine 315 of mouse polyamine amine oxidase corresponds to a lysine residue that is conserved in the flavoprotein amine oxidases of the monoamine oxidase structural family. In several structures, this lysine residue forms a hydrogen bond to a water molecule that is hydrogen-bonded to the flavin N(5). Mutation of Lys315 in polyamine oxidase to methionine was previously shown to have no effect on the kinetics of the reductive half-reaction of the enzyme (M. Henderson Pozzi, V. Gawandi, P.F. Fitzpatrick, Biochemistry 48 (2009) 1508–1516). In contrast, the mutation does affect steps in the oxidative half-reaction. The k_{cat} value is unaffected by the mutation; this kinetic parameter likely reflects product release. At pH 10, the k_{cat}/K_m value for oxygen is 25-fold lower in the mutant enzyme. The k_{cat}/K_{O2} value is pH-dependent for the wild-type enzyme, decreasing below a pK_a of 7.0, while this kinetic parameter for the mutant enzyme is pH-independent. This is consistent with the neutral form of Lys315 being required for more rapid flavin oxidation. The solvent isotope effect on the k_{cat}/K_{O2} value increases from 1.4 in the wild-type enzyme to 1.9 in the mutant protein, and the solvent inventory changes from linear to bowed. The effects of the mutation can be explained by the lysine orienting the bridging water so that it can accept the proton from the flavin N(5) during flavin oxidation. In the mutant enzyme the lysine amine would be replaced by a water chain.

Introduction

A large number of flavoproteins catalyze the oxidation of amines, with molecular oxygen as the final electron acceptor [1]. Based on their sequences and the three-dimensional structures of a growing number of flavoprotein amine oxidases, most of these enzymes can be grouped into two distinct structural families. D-Amino acid oxidase is the paradigm member of the structural family that also contains sarcosine oxidase, glycine oxidase, and dimethylglycine oxidase [2–5]. Monoamine oxidase is the most-studied member of the family that also contains polyamine oxidase, the histone lysine specific demethylase LSD1, and the L-amino acid oxidases [6–10]. The general reaction of flavoprotein amine oxidases can be divided into two half-reactions. The reductive half-reaction consists of the transfer of a hydride equivalent from the substrate to the flavin, producing reduced flavin and oxidized amine. This step is typically irreversible; therefore, these enzymes exhibit ping pong patterns in steady-state kinetic analyses [11]. While the mechanism of amine oxidation has been controversial [12–14], mechanistic and structural results are most consistent with direct transfer of a hydride from the amine substrate to the flavin [2,15–20]. In the less-studied oxidative half-reaction, two electrons are transferred from the reduced flavin to molecular oxygen, forming H_2O_2 [21,22].

Polyamine and spermine oxidases are involved in the catabolism of polyamines in a variety of eukaryotic cells. The mammalian polyamine oxidases (PAOs)[1] prefer N1-acetylated spermine and spermidine as substrates, oxidizing the substrate on the *exo* side of the secondary nitrogen (Scheme 1) [23]. Spermine oxidases catalyze a similar reaction, but are more active with non-acetylated spermine and spermidine [24,25]. In contrast, plant polyamine oxidases catalyze the oxidation on the *endo* side of the secondary nitrogen [26], a reaction also catalyzed by putrescine oxidases [27]. To date, no structure of a mammalian polyamine or spermine oxidase has been described. While there are structures of polyamine oxidases from maize and yeast, the former is more accurately a spermine oxidase based on its substrate specificity [28] and the latter is reported to catalyze the oxidation of N1-acetylspermine and spermine equally

☆ This work was supported in part by grants from the NIH (R01 GM058698) and The Welch Foundation (AQ-1245).

* Corresponding author. Address: Department of Biochemistry, University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Dr., San Antonio, TX 78229-3900, United States. Fax: +1 210 567 8778.
E-mail address: fitzpatrick@biochem.uthscsa.edu (P.F. Fitzpatrick).

[1] *Abbreviations used:* PAO, polyamine oxidase.

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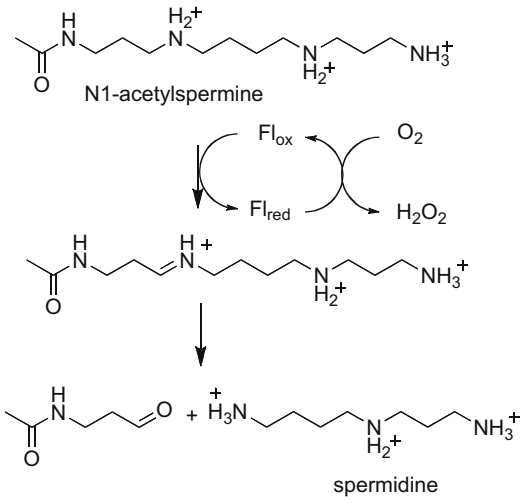
doi:10.1016/j.abb.2010.04.015



Scheme 1.

efficiently [29]. All of the published structures of proteins in the monoamine oxidase family show a conserved lysine residue in the active site [6–8,30,31]. In several of these structures there is a water molecule in an appropriate location to form hydrogen bonds to the lysine amino group and the FAD N(5). This is illustrated in Fig. 1 for maize polyamine oxidase. When this lysine in that enzyme is mutated to methionine, the rate constant for reduction of the flavin by spermidine is reported to be 1400-fold slower than the value for the wild-type enzyme, suggesting that this residue plays a critical role in amine oxidation [32]. Mutation of the corresponding lysine in LSD1, Lys661, to alanine yielded a mutant protein with no detectable activity in a gel-based assay [33], consistent with a critical role in that enzyme also. While the lack of kinetic data for the LSD1 K661A enzyme precludes determination of which steps in the reaction are affected by the mutation, amine oxidation is rate-limiting for that enzyme, at least with peptide substrates [19]. Alignment of sequences of mouse polyamine oxidase with other members of the monoamine oxidase family, including maize PAO and LSD1 (Fig. 2), shows that Lys315 in mouse PAO corresponds to the

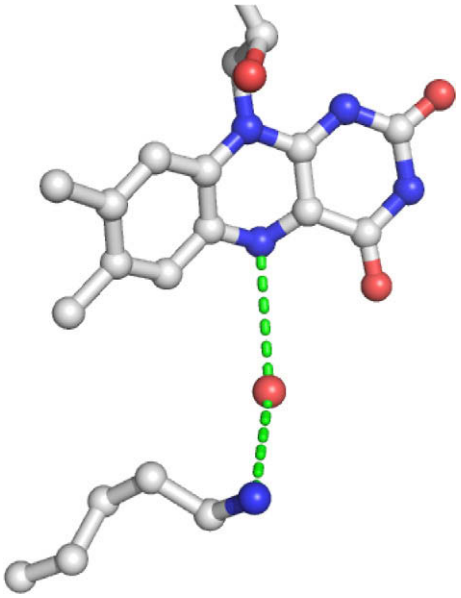


Fig. 1. The water-mediated interaction between the FAD and Lys300 in maize polyamine oxidase. The structure was drawn using pdb file 1h81 [49].

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Mouse PAO   FEPPLPAKKAEAIKKLGFGTNNKIFLEFEEPFW
MAO-B       FNPPLPMMRNQMITRVPLGSVIKCIVYYKEPFW
MAO-A       FRPELPAERNQLIQRLPMGAVIKCMMYYKEAFW
Maize PAO   FKPKLPTWKVRAIYQFDMAVYTKIFLKFPRKFW
Fms1        FQPPLKPVIQDAFDKIHFGALGKVIFEFEECCW
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Fig. 2. Multiple sequence alignment for mouse PAO, human MAO-B, human MAO-A, maize PAO, and yeast Fms1. Conserved residues are in bold. The lysine corresponding to Lys315 in mouse PAO is underlined.

conserved lysine residue. Surprisingly, mutagenesis of this lysine in mouse PAO to methionine has no effect on the rate constant for flavin reduction at the pH optimum or on the pH dependence of the reductive half-reaction [34], in contrast to the results with maize PAO and LSD1. We report here that mutagenesis of Lys315 in mouse PAO to methionine instead affects the oxidative half-reaction of the enzyme.

Experimental procedures

Materials

Spermine was purchased from Acros Organics (Geel, Belgium) and N1-acetylspermine was purchased from Fluka (Switzerland). Deuterium oxide was purchased from Cambridge Isotope Laboratories, Inc (Andover, MA). All other materials were of the highest purity commercially available. Wild-type and K315M PAO were expressed and purified as previously described [34].

Assays

Steady-state kinetic assays were performed using a computer-interfaced Hansatech (Hansatech Instruments) oxygen electrode [35]. All assays were initiated by the addition of enzyme. All buffers contained 10% glycerol; 50 mM PIPES, 50 mM Tris–HCl, 50 mM CHES and 50 mM CAPS were used for the pH ranges of 6.6, 7.1–8.6, 9.1–9.6, and 10, respectively. Solvent isotope effects were performed in buffers containing 50 mM CHES (pH 9 or pD 9.4) or 50 mM CAPS (pH 10, or pD 10.4), 10% glycerol. Glycerol buffers with a relative viscosity of 1.3 were prepared as described by Segur and Oberstar [36]. A concentration of 1 mM N1-acetylspermine was used in all assays. Due to the hygroscopic nature of N1-acetylspermine, its concentration was determined enzymatically [20].

Data analysis

Steady-state kinetic parameters were determined based on fits to the Michaelis–Menten equation using the program KaleidaGraph (Synergy Software). Data for the k_{cat}/K_{O2}-pH profile of wild-type PAO were fit to Eq. (1), which applies for a kinetic parameter that decreases at low pH due to the protonation of a single moiety; y is the k_{cat}/K_{O2} value at the specific pH being measured, c is the pH-independent value of the k_{cat}/K_{O2}, and K is the ionization constant for a residue that must be unprotonated. Eq. (2) was used to fit the k_{cat}-pH profile for the wild-type enzyme. This describes a kinetic parameter that decreases to a limiting value at low pH; Y_L and Y_H are the values of the kinetic parameter at the pH extremes and K is the ionization constant for the transition [37]. Eqs. (3)–(5) were used to fit the proton inventories for wild-type and K315M PAO. Eq. (3) describes a proton inventory arising from a single proton. Eq. (4) describes a proton inventory when two protons with identical fractionation factors contribute to the