Demonstration of Isoleucine 199 as a Structural Determinant for the Selective Inhibition of Human Monoamine Oxidase B by Specific Reversible Inhibitors*

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Several reversible inhibitors selective for human monoamine oxidase B (MAO B) that do not inhibit MAO A have been described in the literature. The following compounds: 8-(3-chlorostyryl)caffeine, 1,4-diphenyl-2-butene, and trans,trans-farnesol are shown to inhibit competitively human, horse, rat, and mouse MAO B with \( K_I \) values in the low micromolar range but are without effect on either bovine or sheep MAO B or human MAO A. In contrast, the reversible competitive inhibitor isatin binds to all known MAO B and MAO A with similar affinities. Sequence alignments and the crystal structures of human MAO B in complex with 1,4-diphenyl-2-butene or with trans,trans-farnesol provide molecular insights into these specificities. These inhibitors span the substrate and entrance cavities with the side chain of Ile-199 rotated out of its normal conformation suggesting that Ile-199 is gating the substrate cavity. Ile-199 is conserved in all known MAO B sequences except bovine MAO B, which has Phe in this position (the sequence of sheep MAO B is unknown). Phe is conserved in the analogous position in MAO A sequences. The human MAO B I199F mutant protein of MAO B binds to isatin \( K_I = 3 \mu M \) but not to the three inhibitors listed above. The crystal structure of this mutant demonstrates that the side chain of Phe-199 interferes with the binding of those compounds. This suggests that the Ile-199 “gate” is a determinant for the specificity of these MAO B inhibitors and provides a molecular basis for the development of MAO B-specific reversible inhibitors without interference with MAO A function in neurotransmitter metabolism.

Two isoforms of monoamine oxidase (MAO),1 MAO A and MAO B, exist in humans and are both 60-kDa outer-mito-

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The atomic coordinates and structure factors (code 2BK3, 2BK4, and 2BK5) have been deposited in the Protein Data Bank, Research Collaboratory for Structural Bioinformatics, Rutgers University, New Brunswick, NJ (http://www.rcsb.org/).

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1 The abbreviations used are: MAO, monoamine oxidase; CSC, 8-(3-chlorostyryl)caffeine; DPB, 1,4-diphenyl-2-butene; MMTP, 1-methyl-4-(1-methylpyrrol-2-yl)-1,2,5,6-tetrahydropyridine; r.m.s.d., root mean square deviation.

2 A. A. Khalil, B. Davies, and N. Castagnoli, Jr., submitted for publication.
In this report we compare the functional differences of inhibitor interactions between human MAO A and human, mouse, rat, horse, cow, and sheep MAO B. The crystal structures of human MAO B in complex with trans,trans-farnesol and that of the I199F MAO B mutant protein are also reported. These data identify structural determinants in MAO B that provide a molecular interpretation for the specificity of these reversible inhibitors.

MATERIALS AND METHODS

All reagents used in this study were purchased from commercial sources unless noted otherwise. Human recombinant MAO A and MAO B were expressed in Pichia pastoris and purified as described previously with the modification of the MAO B purification protocol by replacement of the polymer fractionation and differential centrifugation steps with a single chromatographic step using a Bio-Rad High Q anion exchange column and elution with a phosphate gradient (13, 14). Mitochondria from bovine, sheep, human, horse, rat, and mouse livers were isolated using a procedure described previously (15).

Construction of MAO A F208I and MAO B I199F Mutant Proteins—

The site-directed mutations were introduced into full-length MAO A and MAO B cDNA-containing pPIC3.5k vectors using the QuikChange XL kit (Invitrogen). The instructions from the kit were followed, and a single base mismatch containing pairs of complementary oligonucleotide primers (5′-CCACCCGGAATTCCTGTCACC-3′ and 5′-GGTGA-CAGGAGATTATCGAGGTCG-3′ for MAO A F208I and 5′-CAACAGA-ATCTTTCTGACAC-3′ and 5′-GGTGGCGAAGATTTGTTGG-3′ for MAO B I199F) were used. The presence of desired mutations in the constructs was confirmed by DNA sequencing. P. pastoris spheroplasts were prepared and transformed according to the Invitrogen protocol, and clones with the highest resistance to G-418 antibiotic were selected for fermentation as described earlier (13, 14).

MAO A and MAO B Activity Measurements—

MAO A activity was determined spectrophotometrically (316 nm) using kynuramine as a substrate in 50 mM potassium phosphate buffer, pH 7.5, containing 0.5% (w/v) reduced Triton X-100 at 25 °C. MAO B activity was also determined spectrophotometrically (250 nm) using benzylamine as a substrate in 50 mM HEPES buffer, pH 7.5, containing 0.5% (w/v) reduced Triton X-100 at 25 °C. MAO B activity in mitochondrial preparations was measured spectrophotometrically (420 nm) using 1-methyl-4-(1-methylpyrrol-2-yl)-1,2,3,6-tetrahydropyridine (MMP) as a substrate in 100 mM sodium phosphate buffer, pH 7.4, as described earlier (16). Any MAO A activity in these mitochondrial preparations was eliminated by a 30-min pretreatment with 1 μM clorgyline. Kinetic isotope effects for the MAO B mutant were determined by comparing K_m and k_cat values obtained for benzylamine and α,ω-dideuterobenzylamine under the conditions described above. Oxygen-saturated buffers were prepared by slowly bubbling oxygen gas through 50 mM HEPES buffer, pH 7.5, containing 0.5% (w/v) reduced Triton X-100 at 25 °C for 2 h.

**Scheme 1. Structures of inhibitors used in this study.**

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**RESULTS**

Comparison of MAO B Inhibition by CSC, DPB, and trans,trans-Farnesol in Liver Mitochondria Isolated from Different Species—MAO B from human, horse, bovine, sheep, rat, and mouse liver mitochondrial preparations were inhibited by CSC (K_i range from 0.1 μM for human liver MAO B to 1.4 μM for horse liver MAO B), DPB (K_i range from 0.3 μM for human liver MAO B to 8.3 μM for horse liver MAO B), and trans,trans-farnesol (K_i = 2.3 μM) as shown in Table II. MAO B from bovine and sheep liver mitochondria and recombinant human MAO A were not inhibited by these compounds. Isatin, a nonselective MAO A- and MAO B-reversible inhibitor, inhibited human MAO A (K_i = 3 μM), bovine MAO B (K_i = 6 μM), and human MAO A (K_i = 15 μM). These results point to interesting differences in MAO B from various species with regards to their sensitivities to these inhibitors and reflect differences in their respective active-site architectures.

Structure of the MAO B-trans,trans-Farnesol Complex—The structure of the MAO B-trans,trans-farnesol complex was determined to 1.8-Å resolution (Fig. 1 and Table I). The bound trans,trans-farnesol isoprenoid chain traverses both the entrance and the substrate cavities (Fig. 2) with Ile-199 in its “open” conformation as is observed in the 1,4-diphenyl-2-butene structure (6). No structural changes in the enzyme are observed relative to previous structures (root mean square 0.2 Å for 974 Ca atoms) (6). The OH moiety of the bound trans,trans-farnesol is located 3.4 Å from the C(4a) position of the flavin, and the 1-methylene carbon is 3.4 Å from the N(5) of the flavin. Additional enzyme-trans,trans-farnesol interactions are hydrophobic between the isoprenoid chain and the amino acid residues defining the cavities.

Amino Acid Sequence Alignments—Amino acid sequence alignments of the regions of the available MAO A and B sequences known to interact with 1,4-diphenyl-2-butene in human MAO B (6) point out to several key residues (Leu-164, Ile-199, and Tyr-326; numbering based on the human MAO B sequence) that are not conserved between MAO A and MAO B. Ile-199 is conserved in all known MAO B sequences with the exception of bovine MAO B where it is replaced with Phe.
The crystals belong to a C222 space group. Values in parentheses are for reflections in the highest resolution shell. $R_{\text{cryst}} = \Sigma F_{\text{obs}} - F_{\text{calc}} / \Sigma F_{\text{obs}}$, where $F_{\text{obs}}$ and $F_{\text{calc}}$ are the observed and calculated structure factor amplitudes, respectively. $R_{\text{cryst}}$ and $R_{\text{free}}$ were calculated using the working and test set, respectively.

### Table II

| Isatin, trans, trans-farnesol, CSC, and DPB inhibition of MAO B from horse, bovine, sheep, human, mouse, and rat liver liver mitochondria | $K_i$ (µM) |
|---|---|---|---|
| Horse liver MAO B | ND$^a$ | ND | 1.4 | 8.3 |
| Rat liver MAO B | ND | 5.0$^b$ | 1.1 | 6 |
| Mouse liver MAO B | ND | 2.4$^a$ | 0.2 | ND |
| Human liver MAO B | ND | 0.8$^a$ | 0.1 | 0.8 |
| Sheep liver MAO B | ND | ND | No inhibition | No inhibition |
| Bovine liver MAO B | 6 | No inhibition | No inhibition | No inhibition |
| Recombinant human MAO B | 3 | 2.3 | 0.2 | 0.7 |
| Recombinant human MAO A | 15 | No inhibition | No inhibition | No inhibition |

$^a$ ND, not determined.

$^b$ Taken from the work cited in Footnote 2.

Interestingly, all known MAO A sequences contain Phe in the analogous position (residue 208 in the human MAO A sequence). Ile-199 was previously identified as the “gate” residue in human MAO B, because it can exist in two different conformations; a closed conformation separating the substrate and entrance cavities or opened conformation fusing the two cavities (6). Because bovine MAO B as well as human MAO A are not inhibited by CSC, DPB, or trans, trans-farnesol, the substitution of Phe in the position of Ile-199 is predicted to abolish the binding of these inhibitors but not isatin binding in the active site of human MAO B. To demonstrate this prediction, the properties of the I199F human MAO B mutant protein were investigated.

**Human MAO B I199F Catalytic Properties**—A comparison of the kinetic parameters for the oxidation of benzylamine, 2-phe-nylethylamine, tyramine, and MMTP oxidation for wild type human MAO B and the I199F mutant protein is shown in Table III. The $K_m$ values for these substrates are higher for the I199F mutant as compared with wild type enzyme, and the mutant enzyme’s turnover numbers are ~50% those for wild type MAO B. Catalytic efficiencies ($k_{\text{cat}}/K_m$) for the substrates tested with the I199F mutant protein are in the range of 30–50% of those for wild type human MAO B. The $k_{\text{cat}}$ values for the purified mutant enzyme differ from those reported by Geha *et al.* (12) for the membrane-bound form of this mutant enzyme expressed in a baculovirus system. The rate-determining step in benzylamine oxidation by MAO B I199F is the C–H bond cleavage step (as in WT MAO B), because the kinetic isotope effect (the ratio of $k_{\text{cat}}/K_m$ for oxidation of benzylamine and $\alpha$-dideuterobenzylamine) is found to be 3.7, which agrees with the kinetic isotope effect determined for the wild type MAO B (14). MAO B I199F exhibits a similar affinity for the acetylenic inhibitor rasagiline ($K_i = 0.5$ µM) and for isatin ($K_i = 0.5$ µM).
CSC, DPB, and trans,trans-farnesol no longer inhibit the mutant enzyme. These results support the idea that Ile-199 is a crucial residue that allows binding of CSC, DPB, and trans,trans-farnesol to human MAO B but not to bovine and sheep MAO B and human MAO A. In this property human MAO B I199F is similar to bovine MAO B and human MAO A.

X-ray Structural Data of Human MAO B I199F Complexes with Rasagiline and with Isatin—To identify the structural details of the interfering effect of the I199F mutation on inhibitor binding to MAO B, the crystal structures of the mutant enzyme were determined in complex with the acetylenic inhibitor rasagiline and with the reversible inhibitor isatin (Figs. 3, A and B). In both cases, the binding of the inhibitors to MAO B I199F is identical to that of WT enzyme (respective r.m.s. deviations of 0.20 Å and 0.18 Å for 974 Cα atoms). Previous structural data on the covalent WT MAO B-N(5) flavocyanine complex with rasagiline have shown that Ile-199 is in its “open” configuration (8). In both mutant structures, the side chain of Phe-199 extends into the entrance cavity with essentially identical conformations (Fig. 4) rather than the “open/closed” conformations seen with Ile-199.

The increased size of the Phe aromatic ring relative to Ile (28.6 Å³) (25) prevents its occupation of the alternate conformations observed with Ile-199. The only available space for it to occupy is the entrance cavity of the enzyme. The presence of this bulky residue in the entrance cavity does not appear to alter the kinetic properties of the enzyme or the binding affinity of a small competitive inhibitor, but it does prevent the binding of molecules that must traverse the two cavities in their bound form. One question that arises is how this information relates to the functional and structural properties of human MAO A.

Human MAO A F208I Mutant Enzyme—To test whether the analogous mutation in MAO A would generate an enzyme capable of binding CSC, trans,trans-farnesol or DPB, the human MAO A F208I mutant enzyme was expressed in P. pasto-ris as described for MAO B I199F mutant protein. All data reported on human MAO A F208I were obtained using mitochondrial preparations isolated from P. pasto-ris, because the catalytic activity of the mutant was rapidly lost on solubilization from the membrane and subsequent purification. This mutant enzyme is capable of oxidizing MMTP (Km = 100 μM), and its activity was completely abolished on incubation with clorgyline in agreement with earlier results (12). No inhibition was observed on incubations of the membrane-bound form with CSC, trans,trans-farnesol, or DPB, which demonstrates that mutating Phe-208 to Ile does not allow the binding of these compounds. Although the mutant enzyme was catalytically active, it was no longer capable of binding isatin at the level of affinity observed with WT MAO A.

DISCUSSION

Due to their pharmacological importance, the molecular basis for understanding the respective substrate and inhibitor specificities of MAO A and MAO B has been under investigation for some time. Once it became possible to express recombinant MAO A and MAO B as functional enzymes, chimeras could be used (26, 27) as an approach to identify the sites for their functional differences. The high resolution crystal structures of human MAO B (6, 8) have provided needed structural information that suggests a molecular rationale for further investigation of the relative inhibitor specificities of MAO B. The structural and functional data presented in this study demonstrate that reversible inhibitors that occupy both the entrance and substrate cavities of MAO B exhibit a specificity for this isozyme and do not bind to MAO A. These data also point out that MAO B from different species does not exhibit the same inhibitor specificities. Therefore, prudence is advised when extrapolating the conclusions of studies on MAO B from differing species sources to the human enzyme.

Although the structure of bovine MAO B has not been determined, this source of enzyme has served as the standard for numerous MAO B studies, because it is readily available and can be isolated without contamination with MAO A. Although it does exhibit other functional properties in common with MAO B from other sources, the question arises as to why the bovine enzyme contains the Ile/Phe substitution that results in its loss of sensitivity to inhibitors such as trans,trans-farnesol. The similar behavior of the sheep enzyme suggests it also carries this substitution, although the sequence of sheep MAO B is yet undetermined. One common trait between bovine and sheep is that they are both ruminant animals and therefore may be exposed to higher levels of trans,trans-farnesol (isoprenoid levels vary considerably among plant species (28)). Therefore, their MAO B sequences might have evolved differently (both MAO A and MAO B are thought to have evolved from a common ancestral gene) than in other mammals to provide a protective mechanism against MAO B inhibition by components of their diets. This suggestion raises unanswered questions, because other animals (such as the horse), which also subsist on plant materials in their diets (although horses are not ruminants) have MAO Bs exhibiting properties similar to other mammals. Answers to these questions await further genome sequence determinations and further biochemical studies.

The structure of the trans,trans-farnesol-human MAO B complex is of interest from a mechanistic viewpoint. The polar OH group of trans,trans-farnesol is situated 3.4 Å from the flavin C(4a) position, and the 1-CH2 is positioned 3.4 Å from the N(5) of the flavin in the binding site of MAO B (Fig. 2B). The polar nucleophile mechanism for MAO catalysis (29) predicts substrate amine attack on the C(4a) position of the flavin.
in this complex Ile-199 adopts an "open" conformation (8). In superposition was done with the wild-type protein bound to rasagiline; approximately as in Fig. 2. All experiments were performed with air-saturated buffers. ND, not determined.

**FIG.4.** The shape of the substrate and entrance cavities in the complex between I199F MAO B and rasagiline.

as the initial step in the catalytic mechanism with a possible concerted α-CH bond cleavage occurring with the flavin N(5) functioning as the base. If the bound trans,trans-farnesol is considered as a substrate mimic, these structural data provide support for the polar nucleophilic mechanism. The lowered nucleophilicity of a hydroxyl group relative to that of an aminyl group would not lead to catalytic oxidation. Preliminary data show the aminyl analogue of trans,trans-farnesol does function as a substrate for MAO B.

An interesting aspect of this work is the finding that the reciprocal mutation in MAO A (F208I) does not lead to binding of this class of inhibitors. This mutation in human MAO A does not abolish catalytic activity but does alter its catalytic properties (12). In wild type MAO A, the gate between the entrance and substrate cavities is presumably formed by Ile-335 and Phe-208, which would be altered to a gate formed by two aliphatic side chains in MAO A F208I. Although MAO A F208I is catalytically competent, its active site is altered (with respect to wild type MAO A) as shown by almost complete loss of isatin binding capability. Thus, other secondary structural effects need to be considered in addition to alterations that would occur as a result of lowering the steric bulk of the amino acid side chain at position 208. The fact that clorgyline still inactivates mutant MAO A is in agreement with previous reports (30) that alterations in the structure of the catalytic site are subtle.

Bovine MAO B exhibits a higher catalytic turnover number (31) than the human I199F mutant enzyme, which merits some discussion. Although the mutation of Ile-199 to Phe results in a "gate" consisting of two aromatic residues (Phe-199 and Tyr-326) that would exhibit a higher rigidity, this cannot be the explanation for the observed differences in kcat, because the bovine enzyme also contains a Tyr residue at this position. It is likely that the bovine enzyme has evolved structural differences through other amino acid substitutions to achieve a catalytically more efficient enzyme. It is of interest that other studies have shown that the Y326I mutation in MAO B leads to an enzyme with substrate and deprenyl/clorgyline sensitivities more similar to MAO A and that the I335Y mutation of human MAO A results in a mutant enzyme with closer properties to MAO B (30). Structural data of human MAO B show that Tyr-326 is located near the junction of the entrance and substrate cavities and that the phenolic side chain of this residue forms one of the walls of the substrate cavity. Therefore, replacement of this bulky aromatic ring with an aliphatic side chain may relieve some of the steric constraints in the substrate cavity that have been documented with MAO B and found to be less constraining in MAO A by previous QSAR studies (5, 32). Although single amino acid substitutions can influence the respective individual properties of MAO A and of MAO B, their differences in substrate and inhibitor specificities are due to more complex structural alterations that probably result from contributions of multiple sequence changes.

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REFERENCES

1. Shih, J. C., Chen, K., and Ridd, M. J. (1999) Annu. Rev. Neurosci. 22, 197–217
2. Fowler, J. S., Logan, J., Wang, G.-J, Volkow, N. D., Telang, F., Zhu, W., Francheschì, D., Pappas, N., Ferriero, R., Shea, C., Garza, V., Xu, Y., Schlyer, D., Gately, S. J., Ding, Y.-S., Alexheff, D., Warner, D., Netusil, N., Carter, P., Jayne, M., King, P., and Vaska, P. (2003) Proc. Natl. Acad. Sci. U. S. A., 100, 11600–11605
3. Kumar, M. J., Nicholls, D. G., and Andersen, J. K. (2003) J. Biol. Chem. 278, 46432–46439
4. Chen, J.-F., Steyn, S., Staal, R., Petzer, J. P., Xu, K., Van der Schyl, C. J., Castagnoli, K., Castagnoli, N., Jr., and Schwarzschild, M. A. (2002) J. Biol. Chem. 277, 36040–36044
5. Miller, J. R., and Edmondson, D. E. (1999) Biochemistry 38, 13670–13683
6. Binda, C., Li, M., Hubalek, F., Restelli, N., and Edmondson, D. E., Mattevi, A. (2003) Proc. Natl. Acad. Sci. U. S. A. 100, 9750–9755
7. Hubalek, F., Binda, C., Li, M., Mattevi, A., and Edmondson, D. E. (2003) Acta Crystallogr. Sect. D 59, 1874–1876
8. Binda, C., Hubalek, F., Li, M., Herzog, Y., Sterling, J., Edmondson, D. E., and Mattevi, A. (2004) J. Med. Chem. 47, 1767–1774
9. Binda, C., Newton-Vinson, P., Hubalek, F., Edmondson, D. E., and Mattevi, A. (2002) Nat. Struct. Biol. 9, 22–26
10. Ma, J., Yoshimura, M., Yamashita, E., Nakagawa, A., Ito, A., and Tsuikiara, T. (2004) J. Mol. Biol. 338, 103–111
11. Tsugeno, Y. and Ito, A. (1997) J. Biol. Chem. 272, 14033–14036
12. Geha, R. M., Chen, K., and Shih, J. C. (2000) J. Neurochem. 75, 1304–1309
13. Li, M., Hubalek, F., Newton-Vinson, P. and Edmondson, D. E. (2002) Prot. Expr. Purif. 24,152–162
14. Newton-Vinson, P., Hubalek, F., and Edmondson, D. E. (2000) Prot. Expr. Purif. 20, 334–345
15. Kearney, E. B., Salach, J. I., Walker, W. H., Seng, R. L., Kenney, W., Zesotek, E., and Singer, T. P. (1971) Eur. J. Biochem. 24, 321–327
16. Yu, J., and Castagnoli, N., Jr. (1999) Bioorg. Med. Chem. 2, 231–239
17. Hubalek, F., Binda, C., Li, M., Herzog, Y., Sterling, J., Youdim, M. B. H., Mattevi, A., and Edmondson, D. E. (2004) J. Med Chem. 47, 1769–1766
18. Leslie, A. G. W. (1999) Acta Crystallogr. Sect. D 55, 1696–1702
19. Collaborative Computational Project, Number 4. (1994) Acta Crystallogr. Sect. D 50, 760–767
20. Murshedov, G. N., Vagin, A. A., and Dodson, E. J. (1994) Acta Crystallogr. Sect. D 50, 240–255
21. Jones, T. A., Zou, J. Y., Cowan, S. W., and Kjeldgaard, M. (1991) Acta Crystallogr. Sect. A 47, 110–119
22. Kleywegt, G. J., and Jones, T. A. (1994) Acta Crystallogr. Sect. D 50, 178–185
23. Kraulis, P. J. (1991) J. Appl. Crystallogr. 24, 946–950
24. Merritt, E. A., and Bacon, D. J. (1997) Methods Enzymol. 277, 505–524
25. Harpaz, Y., Gerstein, M., and Clohia, C. (1994) Structure 2, 641–649
26. Grimsby, J., Zentner, M., and Shih, J. C. (1996) Life Sci. 9, 777–787
27. Gottowik, J., Malherbe, P., Lang, G., Da Prada, M., and Cesura, A. M. (1995) Eur. J. Biochem. 230, 934–942
28. Sharkey, T. D., and Yeh, S. (2001) Annu. Rev. Plant Physiol. Plant Mol. Biol. 52, 407–436
29. Edmondson, D. E., Mattevi, A., Binda, C., Li, M., and Hubalek, F. (2004) Curr. Med. Chem. 11, 1983–1993
30. Geha, R. M., Rebrin, I., Chen, K., and Shih, J. C. (2001) J. Biol. Chem. 276, 9877–9882
31. Hassain, M., Edmondson, D. E., and Singer, T. P. (1981) Biochemistry 20, 595–600
32. Walker, M. C., and Edmondson, D. E. (1994) Biochemistry 33, 7088–7098