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Computer Modelling of Monoamine Oxidases

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Abstract—The article summarized results of long-term studies on active site structures of monoamine oxidase (MAO) performed in the Institute of Biomedical Chemistry (Russia) by computer modeling approaches. In mammals MAO exists in two highly homologous forms, MAO A and MAO B, distinguished by substrate specificity, inhibitor selectivity, and other characteristics. The development of approaches for active site modeling of these enzymes (with unknown three-dimensional structures) was originally based on analysis of inhibitory activity of selective inhibitors. It started from the analysis of relationships between the geometrical sizes of conformationally rigid molecules and their inhibitory activity. These studies resulted in molding of the active site structures of MAO A and MAO B. These molds reflect the sizes and shapes of active sites of these enzymes. These mold models have been used for virtual screening of molecular databases for new selective and non-selective MAO inhibitors. The generated models have been compared with three-dimensional structures of MAO A and MAO B, which appeared later.

Keywords: monoamine oxidase, MAO, computer modelling, active site, inhibitors

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INTRODUCTION

Introduction of computer-based technologies and molecular modeling in biochemical studies was one of the new trends that flourished during A.I. Archakov directorship in the Institute of Biomedical Chemistry (IBMC). In this context, monoamine oxidase (MAO, EC 1.4.3.4) has become one of the main objects used in the studies on the structure and properties of enzymes by methods of molecular modeling. MAO is a flavin-dependent enzyme that catalyzes the reaction of oxidative deamination of neurotransmitter amines in the central nervous system and peripheral tissues; it is the target of a wide range of pharmacological agents pooled in the group of monoamine oxidase inhibitors.

Studies of MAO have a long tradition in IBMC. They have started during V.N. Orekhovich directorship in the Laboratory of Biochemistry of Amines and Other Nitrogenous Bases under the leadership of corresponding member of the Russian Academy of Medical Sciences V.Z. Gorkin. Initially, studies performed in IBMC were focused on the biochemical characteristics of MAO in norm and pathology, as well as analysis of the effect of pharmacological agents exhibiting properties of MAO inhibitors. The Gorkin's team found that oxidative modification of MAO (A) was accompanied by alteration in the substrate specificity of this enzyme and sensitivity to specific inhibitors [1–3]. The oxidized enzyme also exhibited increased sensitivity to proteolytic enzymes [3–5]. Analysis of the MAO inhibitory activity of different groups of chemical compounds for possible development of new effective MAO inhibitors and studies of the mechanisms of action of existing pharmaceutical agents represented the other important aspect of research activity in the Gorkin's Laboratory. Studies performed in this Laboratory have shown that Russian antidepressants pyrazidol (pirlindole) and tetrindol selectively inhibit MAO A [6, 7].

MAO is an integral protein of the outer mitochondrial membrane; in mammalian organisms it exists in two forms, MAO A and MAO B, which are encoded by two different (but highly homologous) genes [8, 9]. These forms also differ by preferable substrates and sensitivity to diagnostic inhibitors [10, 11]. Changes in activity of these enzymes have been found in numerous neuropsychiatric disorders [10–13] and MAO inhibitors exhibit a pronounced therapeutic effect. MAO A inhibitors (e.g., moclobemide, brofaromin, pyrazidol, tetrindole) are used as antidepressants, while some MAO B inhibitors (e.g., deprenyl, rasagiline) are used for treatment of Parkinson's disease [14–16].

The study of structural features of the active site of MAO was seriously complicated by the fact that this hydrophobic enzyme could not be crystallized for a long time. Therefore, we have developed an original approach that employed information about the activity of MAO A and MAO B inhibitors for analysis of the active site structures and identification of differences in these enzymes.

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1. THE FIRST ANALYSIS OF RELATIONSHIP BETWEEN INHIBITORY ACTIVITY AND SIZES OF INHIBITORS

The starting point of our investigations was the observation that MAO inhibitory activity of conformationally rigid indole and isatin derivatives acting as competitive inhibitors of MAO (i.e., acting at the active center of this enzyme) depended on their geometrical dimensions [17]. This allowed us to propose an approach for pilot evaluation of the efficacy of inhibitors: when a rigid compound of interest can fit into a box of a certain size, it may inhibit this enzyme. In the case of indole and isatin derivatives the box size for MAO A was $14.2 \times 5.6 \times 1.8$ Å, while in the case of MAO B the box size was smaller: $8.5 \times 5.1 \times 1.8$ Å.

This analysis of indole and isatin analogues resulted in generation of the first, largely intuitive model of the active site of MAO [18]. This model predicted the active site structure as a sandwich, in which the inhibitor is located between the flavin oxazoline ring and the protein chain; however, subsequent comparison of this model with the spatial structure of MAO B showed that it was far from reality. Nevertheless, this model was consistent with the common concept (popular during that time) that an aromatic ring of MAO substrates and MAO inhibitors is accommodated in parallel to the flavin oxazoline ring and the complex is stabilized by $\pi-\pi$ interactions [19]. In this context, it should be noted that, in 1997, we demonstrated that the energetically similar complexes between the flavin moiety and MAO inhibitors could be formed when the inhibitor is perpendicular to the flavin cofactor [20]. This mutual localization was subsequently confirmed in studies of crystal structures of this enzyme.

The observed dependence between efficiency of MAO inhibitors and their sizes (capacity to be accommodated within the box) originally elucidated for indole and isatin analogs was later confirmed for pyrazinocarbazole derivatives [21]. However, this dependence was observed only for the entire set of MAO A inhibitors, while in the case of MAO B this was true only for rigid compounds with reduced mobility of substituents along the long axis of the molecules. For flexible inhibitors such dependence was not observed; the latter could be attributed to changes in the conformation of flexible molecules, which could be placed in the active site in the compact conformation (Fig. 1). This assumption was consistent with data from other laboratories [22, 23]. This observation we later used to formulate an important requirement to the compounds employed for such kind of research: adequate modeling of the active site of MAO can be carried out only with the use of reversible competitive inhibitors of MAO characterized by preferentially "rigid" (with limited conformational mobility) structure and a limited set of conformers.

2. 3D-QSAR AND CoMFA ANALYSIS

For more detailed study of active site structures of MAO A and MAO B we have performed 3D-QSAR and CoMFA analysis of pyrazinocarbazole derivatives, indole and isatin derivatives, and also carbobenzoxyethylamine derivatives [21, 24]. 3D-QSAR and CoMFA analysis of pyrazinocarbazole derivatives resulted in generation of more detailed models of active sites of MAO A and MAO B (Figs. 1a, 1b) [21]. According to these models, the region of MAO A active site accommodating C-8 substituents of pyrazinocarbazoles has an extended cavity (cleft), in which long flexible (Fig. 1a, top) or rigid (Fig. 1a, bottom) substituents can be tightly bound. In the case of MAO B this region is shorter (Fig. 1b) and therefore long rigid analogues cannot adopt reasonable conformation for "comfortable" localization at the active site and they are readily displaced by MAO B substrates (Fig. 1b, bottom). Flexible inhibitors can adopt compact folded conformation needed for tight binding at the active site of MAO B (Fig. 1b, top). This model explained higher efficiency of flexible MAO B inhibitors compared with their rigid analogues.

Comparison of this model with the spatial structure of MAO B has shows that the model correctly predicted limitation of inhibitors in length and so long flexible inhibitors need to adopt their conformation(s) for effective binding at the active site. The major difference between our computer-aided model and the actual active site structure of the MAO B crystal consisted the following: the model suggested a wide, short and sufficiently opened active site, while in reality (in the crystal) it represented a curved channel.

3. ACTIVE SITE MOLDING

The above described model and other 3D-QSAR and CoMFA did not fully characterized active site structures of MAO A and MAO B. So, for a more detailed description of active sites of MAO and for search of novel inhibitors we have proposed a new approach applicable for modeling of 3D structure of active sites of enzymes with unknown structures. We have defined this approach as an "active site molding" and the resultant model has been denominated as an active site mold [25].

This approach is based on the dogma that the active site of the target and its ligand should be characterized by steric complementarity. In this case, a set of ligand surfaces of the enzyme superimposed in a single three-dimensional model in biologically active positions and conformations can definitely reflect the structure of the inhibitor binding site in the active site.

The active site molding consists of two main steps. The first step includes the development of a pharmacophore and assessment of rules for alignment of inhibitors from various chemical classes. The second step is more difficult and less certain, as it involves

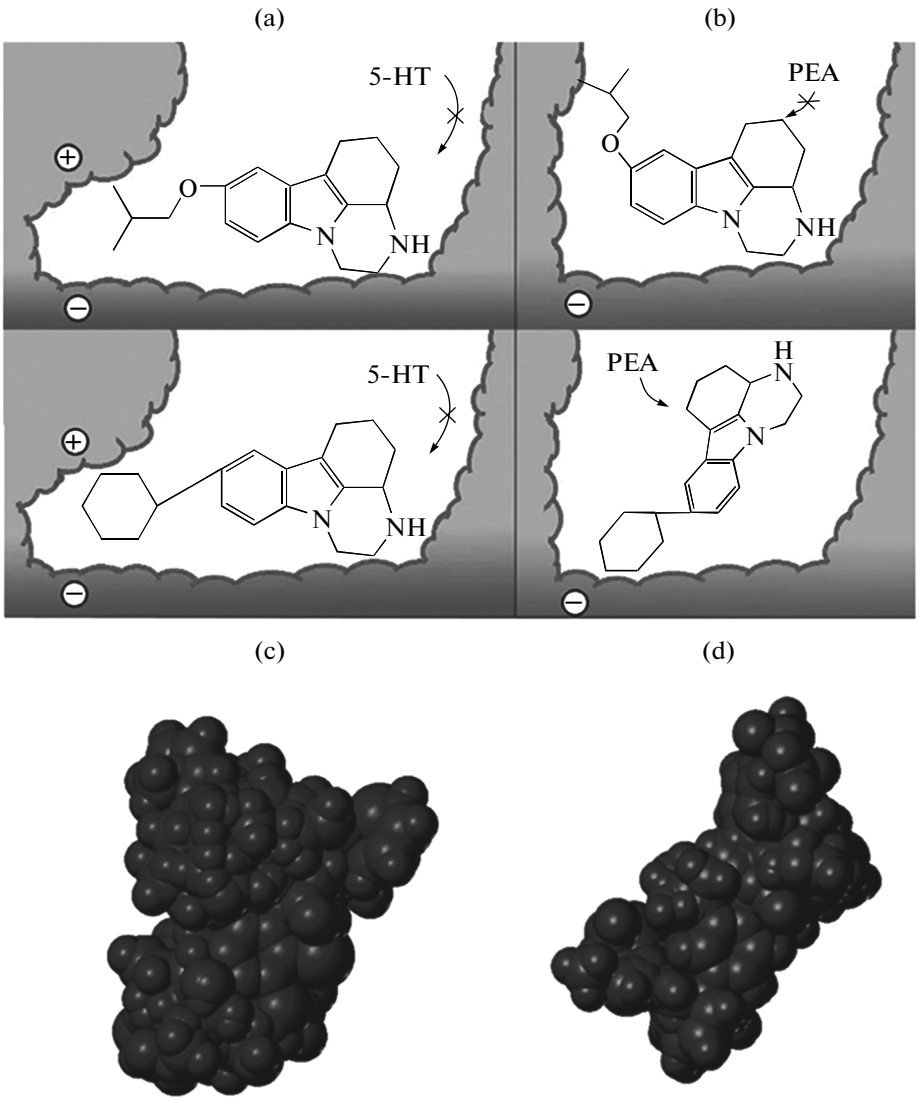


Fig. 1. Active site models of MAO A and MAO B. (a, b)—models constructed on the basis of 3D-QSAR + CoMFA models of pyrazinocarbazole derivatives (adapted from [21]); (c, d)—models of MAO molds (adapted from [25, 26]); (a, c)—MAO A, (b, d)—MAO B.

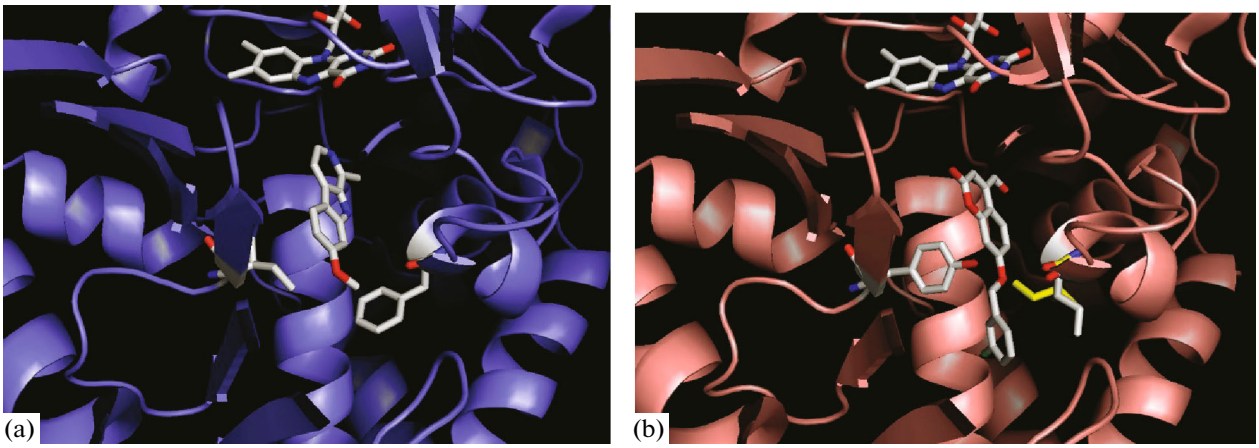


Fig. 2. The active site structure of MAO A and MAO B. (a)—MAO A complex with harmine (code PDB 2z5x), residues F208 and I333 are shown as a stick model; (b)—MAO B complex with inhibitor 7-(3-chlorobenzyloxy)-4-carboxaldehyde-coumarin (PDB code 2v60), I199 and Y326 residues are shown as a stick model (white), yellow shows the closed conformation of I199 in the complex with isatin (PDB code 1oja).

search of mutual spatial arrangement of substituents of different compounds.

This approach has been used to construct the active site molds of MAO A and MAO B [25, 26]. Comparison of the molds has shown that it is larger in MAO A than in MAO B (Figs. 1c, 1d); the latter well corresponded to the literature data [22, 27, 28].

In 2002, the first spatial structure of the MAO B complex with a covalently bound inhibitor pargyline appeared (PDB code 1gos) (Fig. 2) [29]. The active site of MAO B represented an elongated channel, curved near FAD. It was formed by aliphatic and aromatic, residues, which formed a hydrophobic environment consistent with earlier data obtained during analysis of the substrates and inhibitors of this enzyme [30–33]. The active site entrance of MAO B is located on the membrane side, it neighbors to the additional substrate binding site, hidden from aqueous environment by a loop. It is believed that this loop can act as a gating mechanism determining substrate entrance to the active site channel [29]. In turn, this loop contacts with the membrane, and so the state of the membrane may affect the efficiency of the enzyme.

Currently, 45 crystal structures of MAO A and MAO B complexes with the reversible and irreversible inhibitors are known. Some of them have been obtained occasionally. For example, the complex with 1,4-diphenyl-2-butene was obtained in an attempt to crystallize MAO B with amphetamine. However, instead of the expected complex, 1,4-diphenyl-2-butene was found in the crystal; it was released from polystyrene labware used for the crystallization of the enzyme. The complex with lauryl dimethylamine N-oxide was obtained in an attempt to crystallize the ligand-free MAO B structure, and lauryl-N-oxide was used as a detergent. Analysis of the crystal structure revealed that instead of the free form of the enzyme the crystal containing the detergent molecule in the active site was obtained. The Ile199 residue, which caused so much debates in context of its role [34, 35], plays a gate function; it subdivides the active site into the substrate binding region and a channel. The conformation of this residue depended on the length of bound inhibitor (Fig. 2b).

The emergence of the MAO B spatial structure was a good challenge for direct comparison of efficiency of the proposed molding method and correctness of the MOA B mold.

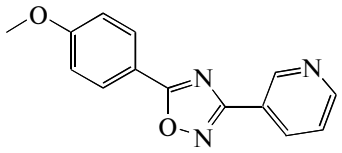
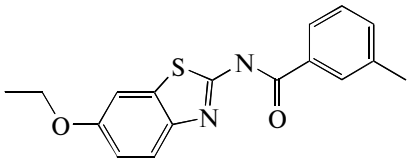
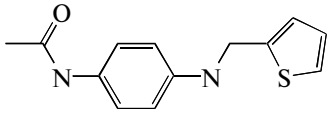
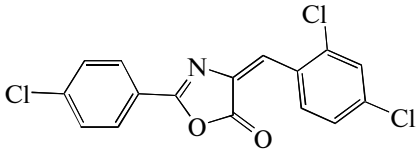
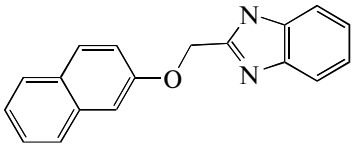
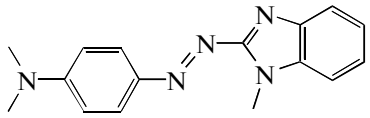
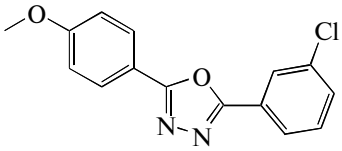
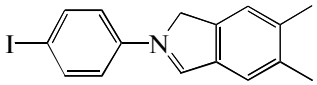
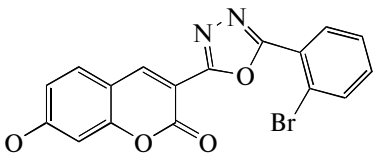
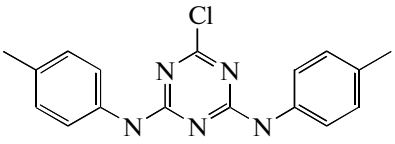
Comparison of the MAO B mold model with the active site of the enzyme shows that the model correctly predicts the basic structural features of the active site. For example, it correctly predicted the sizes of the active site cavity, its extended shape, bend around the flavin cofactor. The main difference of the mold from the active site (of the crystal) consisted in diameter of the active site "cylinder": it was larger in the model. There are several reasons for this discrepancy. The major reason obviously consists in the fact that the structure of the MAO active site B "adjusted" to the structure of the irreversible inhibitor, pargyline, with which the enzyme was crystallized. The interaction of the inhibitor with the active site influenced its structure, which became narrower. This suggestion is supported by the fact that many well-known effective MAO B inhibitors could not be accommodated in the active site cavity of the MAO B crystal structure (Veselovsky, unpublished data). Comparison of three-dimensional structures of MAO B with different inhibitors also suggests the relatively high lability of the active site of this enzyme. On the other hand, protein-ligand interactions can be accompanied by conformational changes of the ligand itself, while molding procedure used conformations calculated in vacuum. This may be another reason for the difference in the mold shape and a real crystal structure of the active site.

4. USE OF THE MAO A ACTIVE SITE MOLD FOR SEARCH OF NEW INHIBITORS

The constructed mold of MAO A has been used for search of new MAO A inhibitors in molecular databases by means of the docking procedure [36]. For this purpose we have generated a capsule around the MAO A mold and after that inhibitors forming the mold were removed and the resultant cavity was used for docking of potential MAO A inhibitors. Since this cavity reflected only the size and the shape of the active site, a rapid geometric docking (an original docking program DockSearch [37]) followed by subsequent additional analysis of selected compounds by other methods evaluating inhibitory effectiveness were considered as the most optimal approach. This study resulted in selection of 4 compounds for direct experimental evaluation of their MAO A inhibitory activity: the belonged to different chemical classes and had a rigid core structure and pharmacophore elements typical for MAO A. Although IC_{50} values characterizing inhibitory activity of these compounds ranged from 316 to 70 μM, all the compounds tested demonstrated selective inhibition of MAO A. In this context, it should be noted that the compounds of these chemical classes never considered as potential MAO inhibitors.

The emergence of spatial structures of MAO opened a new chapter in the study of this enzyme. It has enabled us to test our prediction structure of the active sites of these enzymes. The main results of these studies have already been reported above. In addition, the spatial structure of MAO B allowed us to search for new MAO B inhibitors by the methods of direct modeling. Docking compounds from molecular databases resulted in selection of 10 compounds for direct experimental evaluation of their MAO B inhibitory activity (table). Five of the selected compounds demonstrated MAO B inhibitory activity with IC_{50} values in the submicromolar range and the most potent MAO B inhibitor was characterized by the IC_{50} value of 10 μM.

Experimental values of IC_{50} (μM) for inhibition of MAO A and MAO B by compounds found during database search

Structure	IC_{50}, μM		Structure	IC_{50}, μM	
	MAO A	MAO B		MAO A	MAO B
	320	63		450	250
	⩾100	79		⩾100	250
	>1000	110		⩾100	35
	400	**10**		⩾100	⩾100
	200	60		⩾100	⩾100

Only one compound was inactive. All these compounds inhibited MAO A at higher concentrations. Thus, this work resulted in identification of new selective inhibitors of MAO B.

The next step included the search for reversible nonselective MAO inhibitors [38]. Interest in these inhibitors was stipulated by the fact that the first generation of irreversible inhibitors (e.g., iproniazid) inhibiting both types of the enzyme non-selectively were the most effective pharmacological agents of this type [39]. However, their wide clinical use was hampered by a risk of serious side effects [39, 40]. Therefore, it has been suggested that reversible non-selective MAO inhibitors may be equally effective as iproniazid, but devoid of side effects [39, 41, 42].

Previously, examining regulatory properties of isatin (endogenous MAO B inhibitor), we have found that this compound may also inhibit activation of soluble guanylate cyclase by nitric oxide [43]. Taking into consideration that deprenyl (selective irreversible inhibitor of MAO B) causes an increase in nitric oxide production in the tissues and vessels of the brain [44], we have suggested that some compounds, which activate soluble guanylate cyclase, may inhibit MAO activity. In this context, we have analyzed a series of soluble guanylate cyclase activators as potential inhibitors of MAO A and MAO B.

Four benzofuroxan derivatives, which stimulate guanylate cyclase activity due to release of nitric oxide [45, 46], have been examined.

Docking of the test compounds in the active site cavity showed that they can fit into the active site of MAO B, and three of them were characterized by high predicted binding energies. A comparison of the local-

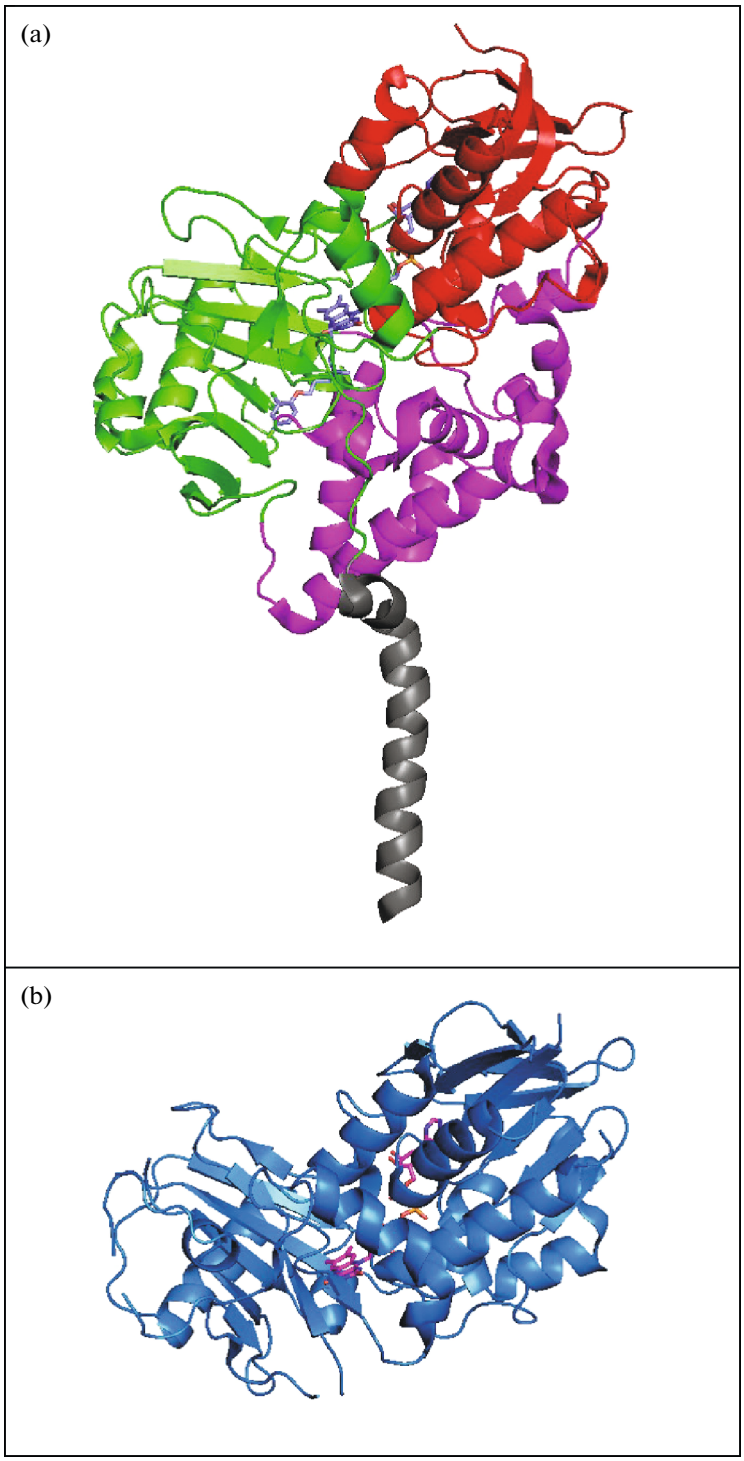


Fig. 3. Structures of human MAO B structure (a) and renalase (b). The FAD-binding sub-domain of MAO B is shown in red, the substrate-binding sub-domain is shown in green; membrane-binding sub-domain and the membrane helix are shown in pink and gray, respectively.

ization of these ligands with the spatial structure of MAO A has shown that they also can be accommodated in the active site of this enzyme as well. Experiments have shown that compounds with high predicted activity inhibited both forms of MAO, and benzodifuroxan and 7-nitro-1,3-benzotetrazin dioxide (7-NBTDO) caused almost the same reversible inhibition of MAO A and MAO B. Thus, computer modeling followed by experimental validation resulted in identification of new non-selective MAO inhibitors,

characterized by an inhibitory activity in the micromolar concentration range.

INSTEAD OF CONCLUSIONS: THE LATEST STUDIES OF MAO BY MEANS OF COMPUTER MODELING

In recent years, studies of MAO by means of molecular modeling techniques included docking of various inhibitors for prediction of their binding sites and features of their interaction [47] as well as molecular dynamics of the proteins and their complexes [48, 49]. The molecular dynamics studies have shown that the membrane plays an important role in the availability of the active site for substrates and substrate specificity is determined not only by amino acid residues of the substrate binding pocket, but also by residues located in the active site channel [48]. Attachment to the membrane also influences mobility of the protein globule. Analysis of the behavior of the protein globule by molecular dynamics showed that the MAO structure can be divided into three sub-domains: membrane, FAD-binding, and substrate-binding domains [49].

The movement the FAD-binding and substrate-binding domains were synchronized with each other, while the membrane sub-domain moved independently. Such asynchronous movement caused conformational changes leading to the opening of the active site channel of the enzyme required for entry of substrates and exit of the reaction products.

Interestingly, the two sub-domains (FAD- and substrate-binding) share similar spatial folding of the protein chain with a recently discovered FAD-containing protein renalase (see for review [50]), which was even denominated as MAO C [51] (Fig. 3). Despite the active study of the renalase structure (see reviews [50, 52]), its (catalytic) function remain poorly understood. But that is another story.

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