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A rational approach to elucidate human monoamine oxidase molecular selectivity

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ABSTRACT

Designing highly selective human monoamine oxidase (hMAO) inhibitors is a challenging goal on the road to a more effective treatment of depression and anxiety (inhibition of hMAO-A isoform) as well as neurodegenerative diseases (inhibition of hMAO-B isoform). To uncover the molecular rationale of hMAOs selectivity, two recently prepared 2*H*-chromene-2-ones, namely compounds **1** and **2**, were herein chosen as molecular probes being highly selective toward hMAO-A and hMAO-B, respectively. We performed molecular dynamics (MD) studies on four different complexes, cross-simulating one at a time the two hMAO-isoforms (dimer embedded in a lipid bilayer) with the two considered probes. Our comparative analysis on the obtained 100 ns trajectories discloses a stable H-bond interaction between **1** and Gln215 as crucial for ligand selectivity toward hMAO-A whereas a water-mediated interaction might explain the observed hMAO-B selectivity of compound **2**. Such hypotheses are further supported by binding free energy calculations carried out applying the molecular mechanics generalized Born surface area (MM-GBSA) method and allowing us to evaluate the contribution of each residue to the observed isoform selectivity. Taken as whole, this study represents the first attempt to explain at molecular level hMAO isoform selectivity and a valuable yardstick for better addressing the design of new and highly selective MAO inhibitors.

1. Introduction

Monoamine oxidases (MAOs) are flavoproteins regulating the level of biogenic amines (*e.g.* dopamine (DA), noradrenalin (NA), adrenaline (AD), 2-phenylethylamine (PEA) and serotonin (5-HT)) and dietary amines (*e.g.* tyramine) in mammals by catalyzing their oxidative deamination (Weyler et al., 1990 and Shih et al., 1999). Expressed in different peripheral tissues and in the brain, MAOs are outer mitochondrial membrane enzymes that exist in two known and fully characterized isoforms, namely MAO-A and MAO-B (Shih et al., 1999 and Westlund et al., 1985). 5-HT, neurotransmitter proved to be crucial in human depression, is primarily metabolized by MAO-A that prevails in catecholaminergic neurons, while MAO-B, predominating in serotoninergic neurons, reduces the levels of PEA preferentially. Both the isoenzymes are able to catabolize DA, AD and NA at similar rates (Youdim et al., 2006). The MAO well-established reputation as therapeutic target (Youdim et al., 2006 and Kumar et al., 2016) is closely related to the specific function of both isoforms: MAO-A selective inhibitors are clinically administered as anxiolytics and antidepressants (Casacchia et al., 1984; van Vliet et al., 1992 and Baldessarini, 1989) while MAO-B selective inhibition is typically used for the treatment of the Parkinson's disease (PD) early symptoms (Youdim and Bakhle, 2006). Furthermore, recent studies demonstrated the involvement of both MAO-A and MAO-B into the pathogenesis and progression of heart failure, being both isoforms responsible for an enhanced aldehyde metabolism, norepinephrine catabolism and ROS production (Kaludercic et al., 2010 and Kaludercic et al., 2013). A renewed interest toward MAOs is now growing in the field of anti-Alzheimer medicines, claiming for the putative efficacy of MAOs inhibition in reducing ROS toxicity and oxidative stress (Riederer et al., 2004; Pisani et al., 2011 and Farina et al., 2015). Unfortunately, the earliest MAO inhibitors showed low isoform selectivity, thus causing severe side effects associated to their activity in peripheral tissues such as liver, placenta, intestine and lung (Saura et al., 1996). In particular, hypertensive drug-induced crises have been ascribed to the increased effect of sympathomimetic amines such as tyramine (mainly present in red wine and cheese and hence triggering the so-called "cheese-effect") that is not scavenged by gastrointestinal MAO-

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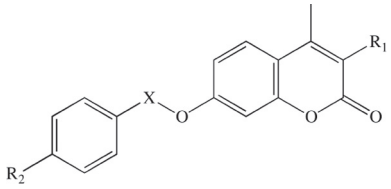
A when blocked by non-selective and irreversible MAO inhibitors (Anderson et al., 1993). Such important side effects can be strongly reduced by administrating reversible inhibitors with high MAO-isoform selectivity. This holds true both for antidepressants (MAO-A selective) and MAO inhibitors administrated to treat the early symptoms of PD (MAO-B selective). Indeed, a higher isoform selectivity of the inhibitor might consent the use of a lower therapeutic dose thus strongly diminishing possible adverse effects. Research efforts over last years allowed designing potent and more selective MAO-A and MAO-B inhibitors (Pisani et al., 2015, Carotti et al., 2002, Santana et al., 2006, Hassan et al., 2006, La Regina et al., 2007, Binda et al., 2007, Gökhan-Kelekçi et al., 2009, Matos et al., 2009, Matos et al., 2010, Karuppasamy et al., 2010, Pisani et al., 2016a and Wang et al., 2015). Nevertheless, a suitable model allowing to rationally approach the selectivity issue is still missing, although the structural information of both human isoforms in complex with inhibitors, which are available in the Protein Data Bank (PDB), unveiled some relevant dissimilarities between the two isoforms. In particular, besides differing in terms of primary sequence (72% sequence identity) (Bach et al., 1988), tissue distribution, and sensitivity to substrates and inhibitors, MAO-A and MAO-B show remarkable differences in the shape of their active sites (Kalgutkar et al., 2001). Unlike MAO-A showing a monopartite cavity (De Colibus et al., 2005 and Son et al., 2008), MAO-B has a bipartite active site so that the substrate/ligand has to negotiate a small entrance room before entering the second and larger inner cavity where FAD is accommodated (Hubálek et al., 2005). Two "gate-keeper" residues, namely Ile199 and Tyr326, are responsible for such bipartite shape that can be lost upon binding of specific ligands able to induce conformational changes altering the cavity structure (Hubálek et al., 2005). In MAO-A, Ile199 and Tyr326 are replaced by Phe208 and Ile335, respectively (the numbering is referred to human isoforms) and the substrate/ligand is thus not forced to cross a small entrance room to bind to the enzyme. MAO-A cavity is, in fact, characterized by a unique and wider hydrophobic pocket containing FAD (De Colibus et al., 2005 and Son et al., 2008). Taking advantage of such structural differences and aimed at designing isoform-selective inhibitors, our research group has put great efforts in the last years on the synthesis of several MAO inhibitors with the aim to elucidate the mechanistic rationale behind MAO-A and MAO-B selective block. In particular, 2*H*-chromene-2-one derivatives, better known as coumarins, have been thoroughly studied as selective and potent MAO-A (Pisani et al., 2013a) and MAO-B (Catto et al., 2006; Pisani et al., 2009; Pisani et al., 2013b and Pisani et al., 2016b) inhibitors. More specifically, the presence of a planar backbone allows an efficient lodgement into both MAO-A and MAO-B catalytic sites. Furthermore, 2*H*-chromen-2-one is a versatile heterocycle which can be easily functionalized with a high degree of chemical diversity. However, the molecular mechanisms underlying the observed isoform selectivity have not been fully elucidated yet. This was likely due to fact that our design was mostly assisted by molecular docking, a simulating technique very effective in providing reliable binding poses but in this case inadequate for deriving a trustable selectivity model. Such failure can be likely ascribed to the main limit of this computational technique: it totally (or almost) assumes a rigid protein structure. Nevertheless, it is acknowledged that a protein can experience specific conformational rearrangements upon ligand binding so that its dynamic behavior should be taken into account in order to have a realistic picture of the ligand binding mode (Nicolotti et al., 2009 and Nicolotti et al., 2008). To properly approach this point, in this work we carried out extensive MD simulations, nowadays considered the method of choice for investigating the dynamics of biomolecules (Karplus and McCammon, 2002 and Alberga and Mangiatordi, 2016). Two 2*H*-chromene-2-ones derivatives designed and prepared by our group (**1** and **2**, highly selective compounds toward MAO-A and MAO-B, Table 1) were employed as molecular probes for investigating the molecular mechanisms underpinning their binding specificity. Notice that biological assays were performed following an already published protocol (Pisani et al., 2016a). The comparative analysis of the

Table 1
Structures and inhibitory activity on hMAO-A and hMAO-B of compounds 1 and 2.



Ligand	R_1	R_2	X	hMAO-A[a]	hMAO-B[a]
1	H	NO_2	SO_2	3.4	2692
2	Cl	H	CH_2	135	0.85

[a] Expressed as IC_{50} (nM).

100 ns MD trajectories obtained from the resulting systems allowed us to get important insights into the specificity of the protein-ligand interactions in MAO-A and MAO-B. To the best of our knowledge, this study represents the first attempt to challenge the MAO isoform-selectivity by comparative MD simulations, a strategy already proved to be able to understand and interpret ligand selectivity toward other targets (Wang et al., 2005; Zeng et al., 2008; Martínez et al., 2009 and Hu and Wang, 2014). Moreover, for the first time, MD simulations have been carried out on the dimeric structures of the two MAO isoforms, embedded in the lipid bilayer, the natural environment of the two enzymes likely influencing the observed conformational changes. The results are discussed in the perspective of designing new and more isoform selective inhibitors.

2. Methods

2.1. Docking Simulations

Crystal structures of hMAO-A with Harmine (PDB code: 2Z5X (Son et al., 2008)) and hMAO-B with Isatin (PDB code: 1OJA (Binda et al., 2003)) were retrieved from the Protein Data Bank (PDB) as targets for preliminary docking studies. Protein structures were prepared using the Protein Preparation Wizard (Sastry et al., 2013) available from Schrodinger Suite v2015-4 (Schrödinger Release 2015-4, 2015) and allowing us: 1) to remove the co-crystallized ligands, 2) to add missing hydrogen atoms and 3) to determine the optimal protonation states for histidine residues at physiological pH. The obtained files were used for docking simulations performed by GOLD v5.2 (Jones et al., 1997) on compounds **1** and **2** on both crystal structures. Following protocols validated in our previous papers (Catto et al., 2006 and Pisani et al., 2009), GoldScore was selected as fitness function and eight water molecules were explicitly considered during the docking runs within the hMAO-B binding site. Furthermore, a spherical grid having a radius of 12 Å originating from the center of mass of the cognate ligands was used.

2.2. From X-ray Structures to Model Systems Preparation

The same crystal structures selected for docking simulations (2Z5X (Son et al., 2008) and 1OJA (Binda et al., 2003)) were chosen as starting point for building the model systems subjected to MD simulations. Notice that the crystal structure of hMAO-A contains only one monomer so that the dimeric structure for this isoform was obtained using the hMAO-B dimer as template for chains positioning. In addition, the full-length homodimer of hMAO-A contains 527 residues in each monomer while that of hMAO-B 520 residues. However, as far as hMAO-A crystal structure is concerned, the chain includes residues His12 to Leu524 while in the h-MAO-B crystal structure chain A includes residues Asn3 to Ile501 and chain B includes residues Asn3 to Ile496. Following the approach by Allen and Bevan (Allen and Bevan, 2011), missing C-terminal residues were added to the model systems and dihedral angles