J Neural Transm (2013) 120:947–950
DOI 10.1007/s00702-013-0974-4

TRANSLATIONAL NEUROSCIENCES - SHORT COMMUNICATION

Structural comparison of the active site channels in rodent and primate vascular adhesion protein-1

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Received: 12 October 2012 / Accepted: 4 January 2013 / Published online: 18 January 2013

Abstract In this study, we have made homology models of mouse, rat, and monkey vascular adhesion protein-1 (VAP-1) to reveal basis for the species-specific ligand recognition of VAP-1. Based on the structural comparisons, rodent VAP-1s have a narrower active site channel than primate VAP-1s. The variable residues in mouse and rat VAP-1, Phe447 from arm I and the polar residues from the first α-helix of the D3 domain together with C-terminal residues are likely to affect ligand recognition and binding.

Keywords AOC3 · Human VAP-1 · Rodent VAP-1 · Molecular modeling · Structural comparison · Ligand recognition

Introduction

Human primary amine oxidase (AOC3), also called vascular adhesion protein-1 (VAP-1), belongs to the copper-containing amine oxidase/semicarbazide-sensitive amine oxidase (CAO/SSAO) enzymes. VAP-1 is a membrane-bound glycoprotein, which has both enzymatic and adhesive functions. It can use primary amines as substrates and, additionally, bind leukocyte-surface proteins (Salmi and Jalkanen 2012). CAOs are all tightly bound homodimers with a similar 3D structure consisting of D2, D3, and the catalytic D4 domain. The buried active site is highly conserved and has several common features: the cofactor topaquinone (TPQ) and the conserved catalytic aspartic acid residue are both involved in the catalytic reaction, and the three conserved histidines coordinate the copper ion involved in TPQ biogenesis.

Regarding human VAP-1, both Airenne (2005) and Jakobsson (2005) with coworkers concluded that the architecture of the active site cavity of human VAP-1 resembles the broad funnel in bovine serum amine oxidase (BSAO) and *Pichia pastoris* amine oxidase (PPLO), whereas the other known CAO structures have a smaller active site cavity. In human VAP-1, residues from D2 (85–88) and D3 (173–184, 206–212, 230–239) form one side of the cavity, while residues from the long β-hairpin arm I of the other subunit (444–450), together with residues from D4 (389, 393–397, 415–426, 467–469, 758–761, 762–763), form the other one. Overall, the majority of the residues are from the catalytic D4 domain.

Human VAP-1 is a potential drug target of anti-inflammatory therapy to treat acute and chronic inflammatory conditions like rheumatoid arthritis, psoriasis, atopic eczema, multiple sclerosis, diabetes, and respiratory diseases (Dunkel et al. 2011). It is currently known that the substrate and inhibitor binding properties of VAP-1 from various species differ considerably (Yu et al. 1994; Marti et al. 2004; Foot et al. 2012), but the underlying reasons for these differences are not well established. This would be of importance in the drug design of new ligands with a similar potency against rodent and primate VAP-1. Therefore, we have now generated homology models of *Mus musculus*, *Rattus norvegicus*, and *Macaca fascicularis* VAP-1 (hereafter referred as mouse, rat, and monkey VAP-1,

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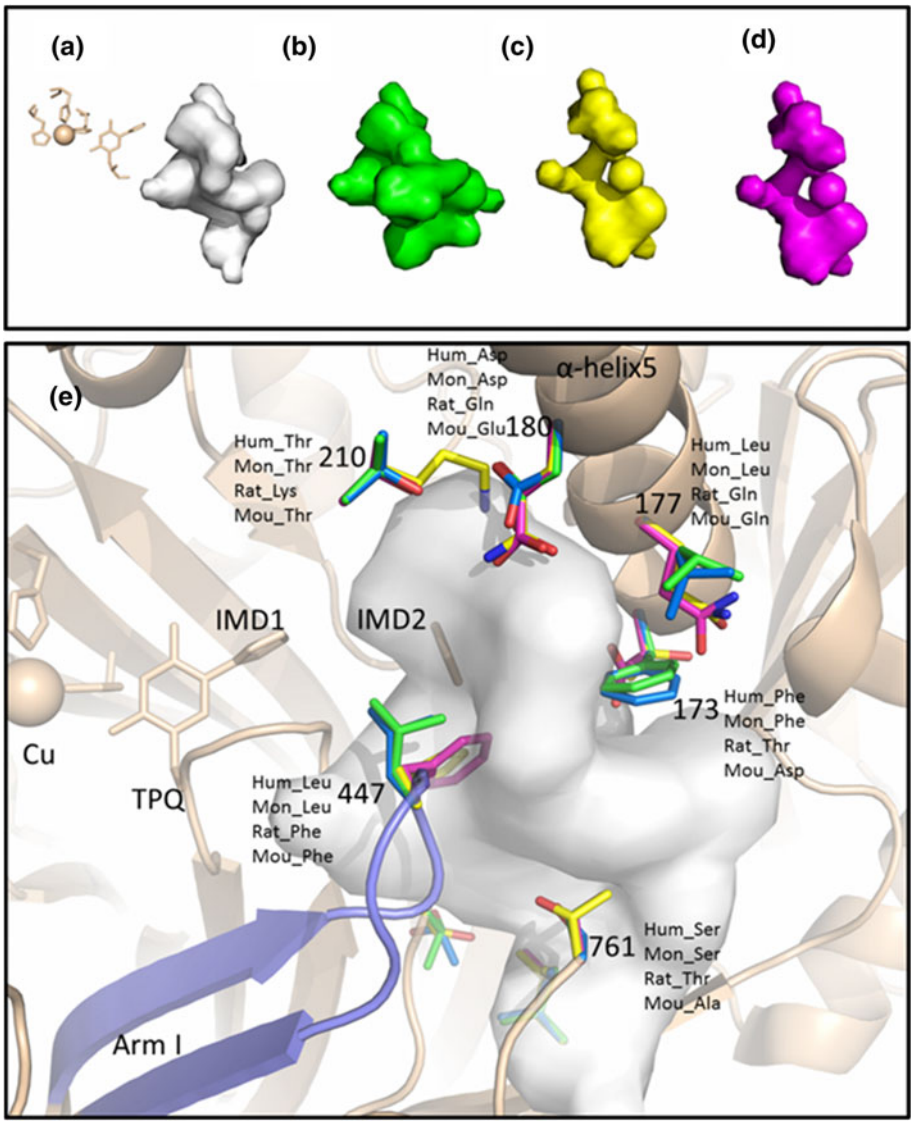

Fig. 1 The active site cavity in mammalian VAP-1. The shape of the cavity in **a** human, **b** monkey, **c** rat, and **d** mouse VAP-1. The cavity is narrower in rodent than in primate VAP-1. **e** The active site cavity in one chain of human VAP-1; *white surface* (PDB code 2Y74). The residues that are different between human (*blue*), monkey (*green*), rat (*yellow*), and mouse (*magenta*) VAP-1 are shown as sticks. Arm I (*blue*) from the other subunit of the dimer is lining the active site cavity

respectively) and compared them with human VAP-1 to identify residue substitutions that could have an impact on differences in ligand recognition and/or binding.

Materials and methods

The sequences of mouse (Accession number (AC) O70423), rat (AC O08590) and monkey (AC G7PUW6) VAP-1 were retrieved from the UniProt database. The Bodil modeling environment and MALIGN were used for sequence alignment and 3D visualization (Lehtonen et al. 2004). MODELLER 9.10 (Sali and Blundell 1993) was used to generate homology models using human VAP-1 (PDB code 2Y74) as the template structure. Out of the ten models generated, the model with the lowest energy according to the MODELLER objective function was chosen for further analysis. Additionally, the conformation of the residues lining the active site channel was analyzed in all ten models. The active site cavities were calculated with Surfnet (Laskowski 1995) using 1.6 and 3.0 Å radii for minimum and maximum gap spheres, respectively. The program Mask was used to find out residues lining the binding site. PyMOL (Version 1.3, Schrödinger, LLC) was used to prepare all the figures.

Results and discussion

Architecture of the active site channel

To identify differences in primate and rodent VAP-1, we made homology models and inspected the active site channels in detail. Since the sequences of mouse, rat, and monkey

VAP-1 are 83, 82, and 96 % identical to human VAP-1 sequence, respectively, their overall folds are likely to be very similar. The sequence identity between the rodent VAP-1s is also very high (93 %). The comparison of the overall size and shape of the active site channels in VAP-1 showed that they are similar in primates and resemble each other in rodents. On the contrary, the cavities of primate and rodent VAP-1 differ significantly, since the channel is much narrower in rodents than in primates (Fig. 1a–d).

Next, we analyzed residues forming the cavity walls to find out those resulting in different cavity architecture. In the channels of human and monkey VAP-1 all residues, except one ($Thr212^{human}/Asn^{monkey}$), are conserved and there are few differences between the channels in mouse and rat VAP-1. As primates and rodents are more distantly related, accordingly, the amino acids in their cavities differ more (Table 1). Based on our analysis, substitutions in positions 173, 177, 180, 210, and 761 from one subunit and 447 from the other one, substantially affect the characteristics of the active site channel.

Key substitutions in the active site channel

To find out the putative role of the variable residues in the species-specific ligand recognition, we studied their location in human VAP-1 structure and in the three homology models. Marti et al. (2004) have previously noticed species-specific enzymatic properties of VAP-1, but using the homology model of mouse VAP-1 they could not find any significant substitutions that would have explained the differences. Similarly, we could not find any prominent differences near TPQ. When we extended the analysis from the catalytic site to the recently identified secondary imidazole binding site in human VAP-1 (Elovaara et al. 2011), we could, however, find differences in the four species. Our models are based on the closely related human VAP-1 structure and thus likely to be more accurate than the models in the previous work done using *Hansenula polymorpha* and *Escherichia coli* VAP-1 structures as templates.

Table 1 Residues lining the active site channel of VAP-1 from human, monkey, rat, and mouse

Residue number	Human	Monkey	Rat	Mouse
Chain A				
173*	Phe	Phe	Thr	Asp
177*	Leu	Leu	Gln	Gln
180*	Asp	Asp	Gln	Glu
210*	Thr	Thr	Lys	Thr
212	Thr	Asn	Thr	Thr
239	Phe	Phe	Tyr	Tyr
388	Gly	Gly	Ser	Ser
395	Thr	Thr	Ser	Ser
425	Ile	Ile	Leu	Leu
761*	Ser	Ser	Thr	Ala
Chain B				
447*	Leu	Leu	Phe	Phe

Only residues that are different between the species are listed. The residues causing significant differences in the channel properties are marked with an asterisk. The residues are colored by hydrophobicity (blue = most hydrophobic and red = most hydrophilic)

Phe447 in mouse and rat VAP-1 is clearly limiting the width of the channel (Fig. 1e) and, therefore, we suggest that Phe447 functions as a porter residue in them. The corresponding residue in human and monkey VAP-1 is a smaller and non-aromatic leucine. Notably, this residue is from the β-hairpin arm I, which protrudes to the entrance of the active site channel from the other subunit of the VAP-1 dimer. The importance of the β-hairpin arm I in VAP-1 substrate binding has been reported early on by Wilce et al. (1997) and discussed by others thereafter. The fact that the rodents have larger residues in positions 177 and 180 additionally makes the channel narrower (Fig. 1e). Lys210 in rat could cause variations in ligand binding compared to primate and mouse VAP-1 with Thr210 (Fig. 1e). The substitution of two vicinal hydrophobic residues 173 and 177 in primate proteins by polar residues in rodents most likely has an effect on ligand binding properties. Together with residues 180 and 761, they lay right at the entrance to the active site and are solvent exposed and, therefore, might also contribute to ligand recognition. Based on the computational docking studies of PXS-4159A, a human VAP-1 inhibitor with a weak potency to rodent VAP-1, Phe173 in human VAP-1 is indeed interacting with the inhibitor (Foot et al. 2012). Residues 173, 177, and 180 are all located in the α-helix5 of D3 (Fig. 1e). Additionally, the last C-terminal residues differ between primate, rat, and mouse VAP-1s and, thus, might also affect ligand recognition. In this study, they were not modeled because of the lack of template structure. The C-terminal His762 and Asn763 in the primate VAP-1s correspond to Tyr762 and Lys763 in rat, while in mouse Tyr762, Arg763, Asp764, and Asn765 form the C-terminal part.

Conclusion

Here we have made a structural analysis of human, monkey, rat, and mouse VAP-1 and identified substitutions that may result in different enzymatic properties of rodent and

primate VAP-1. It is clear that the active site channel of VAP-1 in rodents is narrower and more polar than in primates indicating that human and monkey VAP-1 prefer bulkier and more hydrophobic ligands than rodent VAP-1. Altogether, our study gives an excellent starting point for future experimental and computational studies of species-specific ligand recognition. The results are of great importance for the development and design of drugs targeted to human VAP-1, as mouse, rat, and monkey are often used in the in vivo testing of candidate drugs.

Acknowledgments This work was supported by the National Doctoral Programme in Informational and Structural Biology (E.B.), the Centre for International Mobility (CIMO) scholarship (R.A.), Sigrid Juselius Foundation (T.A.S.), and the Academy of Finland (T.A.S. [132998]). We thank Prof. Mark Johnson for the excellent computing facilities at Åbo Akademi University. The use of Biocenter Finland infrastructure at Åbo Akademi (bioinformatics, structural biology, and translational activities) is acknowledged.

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