Structural model of the catalytic domain of an enzyme with cell adhesion activity: human vascular adhesion protein-1 (HVAP-1)
D4 domain is an amine oxidase

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Human vascular adhesion protein-1 (HVAP-1) is a multifunctional protein having at least two different cellular roles, functioning both as a lymphocyte-endothelial cell adhesion protein and as an enzyme with monoamine oxidase activity. HVAP-1 is a 180 kDa homodimeric glycoprotein consisting of a membrane-spanning domain and three predicted extracellular copper-containing amine oxidase domains. In HVAP-1 the extracellular domains are composed of a large domain D4, containing the active site and forming the interface of the dimer, while the smaller D2 and D3 domains surround the D4 dimer near the entrance to the active site. The structural model of the catalytic D4 domain of HVAP-1 reveals that all components necessary for enzymatic monoamine oxidase activity are indeed present within the HVAP-1 and pinpoints residues that may be key to substrate entry through a channel to the active site and residues likely to be involved in substrate specificity as well as structural features critical to dimer formation. Proper glycosylation is required for the cell adhesion function of HVAP-1 and the predicted location of the sugar units at the solvent-exposed surface suits this function well.

Keywords: activated structure/copper amine oxidase/molecular modeling/structure and function/vascular adhesion protein-1 structure

Introduction

Human vascular adhesion protein-1 (HVAP-1) is a unique endothelial cell adhesion protein, which is mainly expressed in high endothelial venules of peripheral lymph nodes and vascular smooth muscle. HVAP-1 participates in lymphocyte recirculation mediating the initial L-selectin-independent interactions between lymphocytes and endothelial cells (Salmi and Jalkanen, 1992). The expression of HVAP-1 at the endothelial cell surface is induced at sites of inflammation in non-lymphoid tissues such as blood vessels of the tonsils, gut, skin and synovium (Salmi et al., 1993). In addition, it has been suggested that in both skin and synovium HVAP-1 plays a role in mediating increased lymphocyte entry into inflamed tissues (Arvilommi et al., 1996; Salmi et al., 1997).

In vivo, the 170 kDa HVAP-1 is a heavily sialylated glycoprotein (Salmi and Jalkanen, 1996). The proper sialylation of HVAP-1 is required for the adhesive function (Salmi and Jalkanen, 1996). Based on sequence predictions, 12 putative N-glycosylated and six putative O-glycosylated sites are located on the dimeric extracellular domain (Smith et al., 1998). Smith et al. (1998) have recently isolated the cDNA encoding HVAP-1 and Bono et al. (1998) have cloned the mouse homologue of human VAP-1. Surprisingly, HVAP-1 was found to have significant sequence homology to the copper-containing amine oxidase family (Smith et al., 1998).

Copper-containing amine oxidases (EC 1.4.3.6) (AOs) are enzymes that catalyze the oxidation of various primary amine substrates including many neurotransmitters, histamines and xenobiotic amines to their corresponding aldehydes with the subsequent release of ammonia and hydrogen peroxide. The substrate preference depends on the enzyme source. AOs have been studied extensively biochemically, spectroscopically and kinetically (Klinman and Mu, 1994) and they have been purified and characterized from many prokaryotic and eukaryotic organisms. In prokaryotes, these enzymes allow the organism to utilize amine substrates metabolically as sources of carbon and nitrogen. In higher eukaryotes, they are involved in cell differentiation and growth, wound healing, detoxification and signaling (Klinman and Mu, 1994; Fontecave and Eklund, 1995). However, little is known about their precise biological function.

Both human VAP-1 and mouse VAP-1 have been shown to have monoamine oxidase activity but their physiological substrate is unknown (Bono et al., 1998; Smith et al., 1998). Recently, this class of enzymes has been of special interest because they use a novel cofactor, a tyrosine residue that is post-translationally modified to 2,4,5-trihydroxyphenylalanine-quinone (TPQ) (Janes et al., 1992). The generation of TPQ occurs in a self-processing reaction that requires copper(II) and oxygen (Cai and Klinman, 1994; Ruggiero et al., 1997). HVAP-1 has been shown to have the covalently bound quinone too (Smith et al., 1998).

The crystal structure of a prokaryotic amine oxidase from Escherichia coli (ECAO), in both its inactive and active forms, was the first three-dimensional (3D) structure reported for this class of enzymes (Parsons et al., 1995). ECAO is a mushroom-shaped homodimer with four domains: a ‘stalk’ domain (D1) and ‘cap’ domains (D2, D3 and D4). Kumar et al. (1996) have reported the crystal structure of a eukaryotic amine oxidase from pea seedling (PSAO). The structure of PSAO differs from ECAO in having only the three ‘cap’ domains; the ‘stalk’ is missing from the ‘mushroom’. PSAO is glycosylated like most of the eukaryotic AOs. The central D4 domain, which contains the active site, is formed from a pair of extensive β-sandwiches and represents a unique protein fold. The active site copper ion is coordinated to three histidines in close proximity to TPQ. In the inactive form, TPQ is bound to copper, but in the active form, it is rotated away from the metal cation and oriented towards the putative catalytic base, an unprotonated aspartic acid side-chain. In both PSAO and
ECAO, the active site is deeply buried. The crystal structure of the complex of ECAO with a covalently bound inhibitor, 2-hydrazonepyridine, has revealed the identity of the catalytic base, the mode of substrate binding and a possible route for the substrate to enter the active site (Wilmot et al., 1997). In addition, there are two other metal-binding sites not located within the active site, which are occupied by calcium ions in the crystal structure.

HVAP-1 is a type-II transmembrane protein: in addition to the large extracellular amine oxidase ‘cap’ (domains D2, D3 and D4), each monomer of HVAP-1 has a single membrane-spanning domain located at the N-terminus which is not present in ECAO and PSAO. The membrane-spanning N-terminal domain was not modeled and the other two amine oxidase domains (D2 and D3) were not modeled because of their low sequence similarity with the corresponding domains of ECAO and PSAO. Here, we present a model of the D4 domain of human HVAP-1 in both its inactive and activated forms. The model is based on the sequence alignment of human and mouse VAP-1 (MVAP-1) with 15 AO D4 domains, the 3D structure of ECAO and additional structural data. The modeled structure is compared with ECAO, the ECAO–inhibitor complex and PSAO structures. The model reveals the overall and active site structure of the HVAP-1 D4 domain. The surface location of putative glycosylation sites necessary for the adhesive function could be pinpointed in addition to the location of the RGD cell attachment motif in HVAP-1.

Materials and methods

Molecular models were made on a Silicon Graphics O2 workstation using the commercial software package InsightII (Molecular Simulations) made available to us by the Center for Scientific Computing, Espoo, Finland. The model of the D4 domain of HVAP-1 was built using the high-resolution X-ray crystal structure of ECAO (Parsons et al., 1995) (PDB file code 10AC) as a structural template.

The sequences of 17 amine oxidase D4 domains were compared to assist in the identification of both structurally conserved and structurally variable regions in the proposed model: HVAP-1 = human vascular adhesion protein-1 (Smith et al., 1998); MVAP-1 = mouse vascular adhesion protein-1 (Bono et al., 1998); LSAO = lentil seedling copper-containing amine oxidase (Rossi et al., 1992); PSAO = pea seedling copper-containing amine oxidase (Tipping and McPherson, 1995); ATAO = Arabidopsis thaliana copper-containing amine oxidase (Moller and McPherson, 1995); KPAO = Klebsiella aerogenes copper-containing amine oxidase (Sugino et al., 1992); ECAO = Escherichia coli copper-containing amine oxidase (Azakami et al., 1994); AGAO = Arthrobacter globiformis copper-containing phenylethylamine oxidase (Tanizawa et al., 1994); AGHO = Arthrobacter globiformis copper-containing histamine oxidase (Choi et al., 1995); ARAO = Arthrobacter sp. (strain P1) copper-containing amine oxidase (Zhang et al., 1993); HPAO = Hansenula polymorpha copper-containing amine oxidase (Bruinenberg et al., 1989) ANAO = Aspergillus niger copper-containing amine oxidase (Frebort et al., 1996); HDAO1 = human copper-containing diamine oxidase-1 (Zhang et al., 1995); HDAO2 = human copper-containing diamine oxidase-2 (Zhang et al., 1995); RDAO = rat copper-containing diamine oxidase (Linguaglia et al., 1993); BSAO = bovine copper-containing amine oxidase (Mu et al., 1994); and HRAO = human retina copper-containing amine oxidase (Imamura et al., 1997). The sequence alignments were performed using the programs MALIGN and MALFORM (Johnson and Overington, 1993; Johnson et al., 1996).

The published structure-based sequence alignment of the ECAO and PSAO structures was used to refine the limits of the structurally conserved regions defined by the sequence alignment of the amine oxidases. The coordinates of the structurally conserved regions from the ECAO structure were used by the Homology Module within InsightII to build the corresponding structure for HVAP-1. The coordinates from ECAO for identical amino acids in the alignment of ECAO and HVAP-1 were transferred directly to the model. When the amino acids differed, the backbone coordinates were transferred and the side-chain atoms were automatically replaced to reflect the residue present in HVAP-1. The structurally variable regions were constructed using Homology’s Search Loop command, which searches for peptide segments from existing X-ray structures in the Brookhaven Protein Data Bank that met the defined geometric criterion to bridge the gap between each pair of adjoining structurally conserved regions. When all coordinates were assigned for the model, the optimum side-chain conformations were found for residues of HVAP-1 that differed in aligned positions from ECAO, using Homology’s Auto_Rotamer command.

The model was refined using Homology’s Splice Repair and Relax commands. Within the structurally conserved regions, only the mutated side-chain conformations were refined. Tyrosine Tyr471 in the initial model was then substituted with the coordinates of TPQ from the ECAO structure. The copper ion coordinates were also used directly since all of the residues interacting with the copper ion are highly conserved.

The structural model of the ‘active’ form of the HVAP-1 catalytic site was modeled using published details describing the structures of ECAO (Parsons et al., 1995) and the recently released structure of the ECAO–inhibitor complex (Wilmot et al., 1997) (PDB file code 1SPU) and the ‘active’ PSAO structure (Kumar et al., 1996) (PDB file code 1KSI). In the ‘active’ form, the main differences from the ‘inactive form’ concern the orientation of the modified tyrosine TPQ in the catalytic site. The other active site residues were also modified to correspond to the orientation of the active site residues in the ECAO–inhibitor complex structure. Tyr384 in the HVAP-1 active form has a similar orientation to Tyr381 in the ECAO–inhibitor complex structure, leaving the active site open.

The final HVAP-1 D4 model was compared with all of the currently available amine oxidase structures: ECAO, ECAO–inhibitor complex and PSAO structures by superimposing the structures with InsightII; only the Cα atoms of the key active-site residues were used.

The sequence alignment figures were generated using the program ALSCRIPT (Barton, 1993). Figures 3 and 4 were prepared using the program MOLSCRIPT (Kraulis, 1991), rendered using RASTER3D (Merritt and Murphy, 1994) and combined using ImageMagick 4.0.5. Figure 5 was prepared using the program MOLSCRIPT (Kraulis, 1991).

Results

Sequence alignment

The sequences of human and mouse VAP-1 were aligned with the catalytic domains of 15 other AOs (Figure 1). The latter include five bacterial sequences, one each from yeast and fungi, three plant and five mammalian sequences. The cDNAs of a human monoamine oxidase with a sequence identical with
Fig. 1. Sequence alignment of the D4 domains of 17 amine oxidases. The gap regions are shaded and the totally conserved residues in bold are boxed. Positions where only one sequence differs from the others are also in bold. Numbering is according to the HV AP-1 sequence.
HVAP-1 was described recently but the authors did not provide any functional data about the protein and incorrectly concluded that the cDNA encodes a secreted rather than a transmembrane protein (Zhang and McIntire, 1996). The two diamine oxidase sequences from human placenta are nearly identical, with the exception of 19 residues present in human diamine oxidase 2 (HDAO-2) that are missing from human diamine oxidase 1 (HDAO-1) (Zhang et al., 1995). Interestingly, this unique region is missing from all the other AOs including HVAP-1 and MVAP-1 (Figure 1). In the alignment of the D4 domains, there are only 22 fully-conserved residues, which represents about 5% of the 500 positions aligned in Figure 1. The structural and catalytic importance of these conserved residues will be discussed later in detail.

HVAP-1 has the highest sequence identity, 82.5 and 82.1% (Table 1), with the sequences of bovine amine oxidase (BSAO) and MVAP-1, respectively. Surprisingly, the sequence of HVAP-1 is more similar to the prokaryotic ECAO (26.9% sequence identity) and Klebsiella aerogenes (KPAO: 27.0%) sequences than to the AO sequences from the eukaryotic yeast Hansenula polymorpha (YAO: 22.0% sequence identity) and the filamentous fungus Aspergillus niger (ANAO: 21.1%). HVAP-1 is 26% identical with the sequence from Arabidopsis thaliana (ATAO), which is significantly higher than the level of identity shared by HVAP-1 and the other plant sequences (LSAO; lentil seedling) and PSAO, whose X-ray structure is also known. The level of sequence identity shared by HVAP-1 and ECAO (27%) and the identical location of key conserved residues in the alignment made it possible to model the HVAP-1 structure using the ECAO structure of the inactive form as a template structure.

Overall structure

The published structural alignment of PSAO and ECAO (Kumar et al., 1996) was used in model building in order to locate structurally conserved regions and to correct ‘errors’ in the sequence alignment shown in Figure 2. The HVAP-1 structure is based on this alignment, where 117 matched positions are identical (Figure 2), and on the 3D structure of ECAO [Brookhaven Protein Data Bank (PDB) code: 10AC] (Parsons et al., 1995). Thirty-nine C-terminal residues in the D4 domain of HVAP-1 could not be modeled because they do not exist in ECAO. In the ECAO structure, TPQ is in the ‘inactive’ form. However, Parsons et al. (1995) have reported also the structure of the ‘active form’ of ECAO, which was, unfortunately, not deposited with the PDB. Nevertheless, the overall structures of these two forms are nearly identical and the key difference is with the orientation of TPQ in the active site.

The model of the D4 domain of HVAP-1 includes two large, twisted, anti-parallel β-sheets, which form a β-sandwich domain in each monomer (Figure 3). The differences between the ECAO structure and the model of HVAP-1 primarily occur on the surface of the D4 domain and at the dimerization interface (Figure 3A). Two of the structurally variable regions (residues 579–585 and 607–620 in HVAP-1) are at the dimerization interface, forming contacts with the same regions in the other subunit. The first variable region is two residues longer in HVAP-1 than in ECAO. The second variable region is shorter in HVAP-1, where a short β-strand present in ECAO does not exist in HVAP-1. Other differences at the dimerization interface involving intersubunit hairpin loops are discussed below in more detail.

In ECAO and PSAO there is a small substructure that Parsons et al. (1995) termed the ‘Plug’, which consists of three short β-strands and an α-helix. The HVAP-1 ‘Plug’ structure (residues 496–518) is nine residues shorter than the ‘Plug’ in ECAO (residues 491–522). As a result, there is a surface loop in HVAP-1 instead of the short helical segment found in ECAO. In addition, there are several one- and two-residue insertions/deletions located on the surface of the HVAP-1 molecule. By comparing the entire structure of ECAO with the model of the D4 domain of HVAP-1, we observed that none of these variable regions occur at the D2–D4 or D3–D4 interfaces.

Glycosylation sites, disulfide bridges and RGD sequence motif

Three of the six possible N-glycosylation sites along the sequence of the monomer, at residues Asn592, Asn618 and Asn666, are positioned at the surface of the D4 domain and thus are potential glycosylation sites (Figure 4). Each of these sites is different from the three sites that are seen also on the surface of the D4 monomer of PSAO (Kumar et al., 1996) at positions Asn334, Asn364 and Asn558: Asn558 has one sugar residue attached in the crystal structure, while there is no clear evidence concerning the glycosylation of the other two sites. Interestingly, in the HVAP-1 D4 dimer the six potential glycosylation sites are all located on the top of the ‘mushroom’ forming ‘a circle of glycosylation sites’ (Figure 4). There are also three putative O-glycosylation sites per monomer (Smith et al., 1998). Two of these are located just after the transmembrane region and are not part of the D4 domain structure, but could serve to anchor HVAP-1 at the membrane boundary. The third site, Thr679, is located within the D4 domain but the model suggests that it is not at the domain surface and hence cannot be glycosylated. In total, there are five cysteine residues in the HVAP-1 D4 domain. Two of these are conserved in most AOs (ECAO and KPAO are the only exceptions, consistent with their roles as intracellular enzymes); the other three cysteines are located close to the C-terminal sequence of HVAP-1; the region not modeled here since it was not present in the structure of ECAO. As with the two cysteines present in the PSAO structure, it is likely that the corresponding cysteines, Cys404 and Cys430, can also form a disulfide bridge in HVAP-1; the model of HVAP-1 includes a disulfide bridge between them. It is likely that the other three cysteines in HVAP-1, also conserved in MVAP-1, BSAO and HRAO, form one intra-domain disulfide bond and one inter-domain bridge tying the domains together. Consistent with this possibility, the C-terminus of the present model lies at the HVAP-1 surface.

The HVAP-1 sequence contains an RGD tripeptide (residues 726–728) which has been found in fibronectin to be crucial for its adhesion to integrin (Ruoslati, 1986). The RGD motif has been found to play a role in cell adhesion in some other proteins too (D’Souza and Ginsberg, 1991). The RGD motif in HVAP-1 has been found to bind to a C-terminal sequence motif that is located right after the solvent-exposed C-terminus. Hence it must be on the surface of the molecule and could well be a cell attachment motif in HVAP-1. In the other AO sequences this site is not conserved but in MVAP-1 and BSAO the corresponding sequence is REG, which could possibly be a cell attachment site because a cyclic peptide containing the sequence RE has been shown to interact with same or overlapping region in α5β1 integrin as RGD (Koivunen et al., 1994). In order to establish whether the RGD motif in HVAP-1 really functions as a cell attachment site, the RGD site of HVAP-1 has been mutated and the functional testing of this variant is in progress.
Table I. The sequence percentage identity for the aligned AO sequences

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Fig. 2. Sequence alignment of HVAP-1 and ECAO D4 domain sequences on which the model is based. Structurally conserved regions are boxed and conserved residues are shaded. Helices and β-strands defined by the X-ray structure in ECAO are shown as cylinders and as arrows, respectively. Numbering: ECAO according to the structure coordinate file and HVAP-1 according to the sequence.

Fig. 3. (A) Stereo view showing the differences in the ECAO and HVAP-1 (top view). Structurally similar regions are colored red in one monomer and blue in the other. The structurally variable regions are colored green in ECAO and yellow in HVAP-1 in both monomers. (B) Stereo view of the overall fold of the HVAP-1 D4 dimer (side view). The monomers are colored red and blue. Helices are marked as spirals and stands as arrows. TPQ in the active site is presented in the inactive form.

The active site structure

The active site structure is highly conserved (Figure 5). The copper ion is coordinated by three histidines, His520, His522 and His684, in HVAP-1. In the sequence alignment, His520 and His522 are part of the HXH motif, common to AOs, where X is Thr521 in HVAP-1 (Figure 1). His522 is totally conserved in the sequence alignment while His520 is conserved
in all sequences except for ANAO (fungi), where a proline residue is found instead in Figure 1. His684 is totally conserved and is located near the C-terminal end of the AO in all cases.

In the alignment of the AOs (Figure 1), there is a conserved TXXNYD/E motif that contains the tyrosine that is modified to TPQ (X refers to a more variable position) (Mu et al., 1992). In HVAP-1, these residues are Thr467, Leu468, Leu469, Asn470, Tyr471 and Asp472. Two of these residues, Tyr471 and Asn470, lie within the active site cavity. Tyr471 is modified to TPQ in the active form and Asn470 is hydrogen bonded to it. The other conserved residues within the active site include Tyr372 and Asp386. In the active form structures of ECAO and PSAO, TPQ is close to an aspartate, Asp386, in HVAP-1, which functions as the catalytically important base (Parsons et al., 1995; Kumar et al., 1996; Wilmot et al., 1997). In the inactive ECAO structure, this aspartate is distant from TPQ and TPQ forms a bond with the copper ion (Parsons et al., 1995). Consequently, in order to activate the enzyme, TPQ in
the inactive Cu-binding conformation must rotate about the Cβ–Cγ bond, would no longer bind the metal and comes in close proximity to the catalytic aspartate. Tyr372 is hydrogen bonded to TPQ in both the active and inactive forms and plays an important role in stabilizing the position of the quinone–inhibitor complex, as seen in the structure of the ECAO–inhibitor complex (Wilmot et al., 1997).

In addition to these totally conserved residues, two additional residues within the active site might have an important role. One is a highly conserved tyrosine whose role is not clear. This residue, Tyr473 in HVAP-1, is conserved in all AOs except for PSAO and PSAO, where it is an asparagine (Figure 1). The other conserved residue is also a tyrosine in HVAP-1 and ECAO. In the ECAO–inhibitor complex structure this tyrosine, Tyr381, has moved to form a π–π ring-stacking interaction with the pyridine ring of the inhibitor. This residue, Tyr384 in HVAP-1, aligns with phenylalanine in the plant AOs and with an alanine in the AO from Arthrobacter globiformis (Kumar et al., 1996). In the predicted HVAP-1 structure all three

In AOs, the intersubunit interactions are formed by two long β hairpins, ‘arm-I and arm-II’, which extend out of the β-sandwich domain of one monomer and into the β-sandwich domain of the other monomer (Parsons et al., 1995; Kumar et al., 1996). Arm-I is three residues longer in VAP-1 (24 residues) than in the ECAO structure (21 residues). Within arm-I is located a highly conserved histidine residue, His443, that follows a conservatively substituted residue, which is arginine or lysine depending the source of the AO (Arg442 in HVAP-1 and Lys439 in ECAO). When these two important residues were aligned, a one-residue insertion before and a two-residue insertion after this conserved region were introduced into the HVAP-1 sequence relative to ECAO (Figure 2). In HVAP-1, as in the other AOs, these two residues participate in a conserved network of hydrogen bonds ranging from arm-I of one monomer to the vicinity of the active site in the other monomer. Arg442 of one monomer is hydrogen bonded to Asp403, Asp472 and Thr467 near the active site of the other monomer. Asp403 is totally conserved, whereas Asp472 is conservatively substituted with glutamate in YAO, ANAO and ARAA. Thr467 is conserved in all AOs except for the human retina sequence, where it is conservatively substituted by serine. His443 also participates in intermolecular interactions forming a hydrogen bond with Asp472 in the other monomer and an intramolecular hydrogen bond extends between Asp472 and Thr467. Because of the native homodimeric structure, these interactions are doubled in the AOs and exactly the same network of interactions occurs between Arg442, His443, Asp403, Asp472 and Thr467 to stabilize the dimer further.

The residues in the loop following the conserved R/KH pair are close to the active site entrance. In HVAP-1, these residues are His444, Ser445, Asp446, Leu447, Tyr448, Ser449, His450 and Tyr451, His444, Ser445 and Asp446 are inside the channel whereas the other residues are positioned on the surface. In ECAO, this loop is two residues shorter and glutamine, glutamate and methionine occupy the insides of the channel. In PSAO, the loop is two residues longer than in HVAP-1 and the residues in the interior of the channel are threonine, glutamate and asparagine.

The other arm, arm-II, is also three residues longer in HVAP-1 than in ECAO. A single residue gap at position 544 in the sequence alignment (Figure 1) is not found in the structural alignment of ECAO and PSAO (Kumar et al., 1996) and is not present in the alignment used for the model (Figure 2). The extra three residues in arm-II were built on the surface of the model between the two β-strands. Arm-II is located on the top of the dimer, the other β-strand being almost totally exposed. The role of this arm is mainly to stabilize the dimeric structure.

Discussion

The predicted structure of the catalytic D4 domain of HVAP-1 reveals both the overall structure and details about the surface and active site that are key to the multifunctional role of HVAP-1. Conserved and variant structural features were pinpointed by comparing the HVAP-1 model with the existing known structures, including the inactive form of ECAO (Parsons et al., 1995), the ECAO–inhibitor structure (Wilmot et al., 1997) and the active form of PSAO (Kumar et al., 1996). Like
the other amine oxidases, HVAP-1 is a dimeric enzyme with two long arms forming the interface and holding the two monomers together. The interactions of arm-I from one monomer with residues near the active site of the other monomer are highly conserved.

The model that we present does not include the membrane-spanning domain or domains D2 and D3. Structural details on the D2 and D3 domains of HVAP-1 are of great interest because the entrance to the active site lies between these two domains and the D3 domain forms the back of the substrate-binding pocket (Wilmot et al., 1997). In addition, residues from the D3 domain participate together with residues from D4 domain in forming the channel leading from the surface to the active site (Wilce et al., 1997). The modeling of the D2 and D3 domains is difficult for two reasons. First, the sequence conservation of these two domains with the currently known structures is low, the alignment will be unreliable and any resulting model will reflect these errors, which can be substantial. Second, the D2 and D3 domains occupy slightly different positions relative to each other and to the D4 domain in all of the currently known AO structures. Recently, Li et al. (1997) published a crystallographic study of the yeast copper-containing amine oxidase (YAO). The structure of YAO will provide additional structural information because its sequence is different from the sequences of all of the other currently known AO structures. Both the YAO structure and the AGAO structures are now available in PDB and will provide additional information useful in modeling the D2 and D3 domains of HVAP-1.

The active site structure of the D4 domain is conserved in all known structures of AOs, including the predicted HVAP-1 structure. First, an essential feature of these enzymes is the unusual cofactor TFP that is formed from a post-translational modification of a tyrosine residue, corresponding to Tyr471 in HVAP-1. Second, the active sites of all amine oxidases, with the exception of ANAO, contain three histidines that coordinate the essential copper cation: His522, His524 and His684 in HVAP-1. Third, a conserved aspartate residue functions as the catalytic base, key to the reaction catalyzed by amine oxidases: Asp386 in HVAP-1. Fourth, there are two additional conserved residues, an asparagine and a tyrosine, which form essential hydrogen bonding interactions with TFP in the active site: Asn470 and Tyr372 in HVAP-1.

The active site of the amine oxidases is buried and inaccessible to solvent (Kumar et al., 1996; Wilmot et al., 1997). Hence there must be an entry route to the active site for the substrate. Wilmot et al. (1997) have suggested that this entry point is located at the edge of the interface between the D3 and D4 domains, since the tip of the pyridine ring of the bound inhibitor in the ECAO–inhibitor complex structure is located at that point on the surface of the protein.

Wilce et al. (1997) recently published the crystal structures of the copper-containing phenylethylamine oxidase from Arthrobacter globiformis (AGAO) in three different forms: the holoenzyme in the active form, the holoenzyme in an inactive ECAO form and swinging in AGAO. The ‘gate’ residue is interacting with the pyridine ring of the inhibitor in the ECAO–inhibitor complex structure (Wilmot et al., 1997). Wilmot et al. (1997) could not find any correlation between having an aromatic residue in this ‘gate’ position and the corresponding AO preferentially having aromatic substrates. Hence it seems that the interactions between the ‘gate’ residue and the aromatic part of the substrate are not very specific.

The origin of the variance in the substrate preference of AOs is still an unanswered question. Wilce et al. (1997) suggested that the channel might have an important role in determining substrate preferences. In fact, this channel is different in all known structures although the active sites are highly conserved. By comparing the HVAP-1 structural model with the ECAO–inhibitor complex structure, we could identify two variable residues near the inhibitor-binding site. These residues are in the interior of the channel leading from the molecular surface to the active site and thus could affect the substrate preference of AOs. In HVAP-1, both residues are leucines whereas in ECAO they are valine and glycine. The importance of these two residues with regard to substrate preference will be tested by mutagenesis, but these studies will be complicated by the fact that the natural substrate(s) of HVAP-1 is at present unknown.

Three of the six possible N-linked glycosylation sites in the monomer were found to be within this catalytic domain and all of them are exposed at the surface and can potentially be glycosylated. In the dimer, they form a ring of six sites on top of the dimer (Figure 4). This is consistent with the HVAP-1 N-terminal region functioning to anchor the enzyme to the membrane and with the sugar residues extending out the most distal surface of the molecule for binding the corresponding HVAP-1 ligand on the comparatively enormous lymphocyte cell. The ligand of HVAP-1 is currently unknown but may be a lectin-like molecule, although it is known not to be a member of the selectin family. HVAP-1 clearly has multiple physiological roles because it has both adhesive and enzymatic activities. However, it is still unknown whether the adhesive function and enzyme activity are somehow connected. In order to determine which sites are glycosylated, all the possible N-glycosylation sites in HVAP-1 have been mutated and studies on these variants are in progress.

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