J Mol Model (2009) 15:499–505
DOI 10.1007/s00894-008-0430-9

ORIGINAL PAPER

Analysis of a three-dimensional structure of human acidic mammalian chitinase obtained by homology modeling and ligand binding studies

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Received: 24 September 2008 / Accepted: 17 November 2008 / Published online: 16 December 2008
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Abstract The three-dimensional (3D) model of the human acidic mammalian chitinase (hAMCase) was constructed based on the crystal structure of the human chitotriosidase (EC 3.2.1.44, PDB code 1HKK) by using InsightII/Homology module. With the aid of molecular mechanics and molecular dynamics methods, the last refined model was obtained and further assessed by Profile-3D and Procheck, which confirms that the refined model is reliable. Furthermore, the docking results of the ligands (allosamidin and NAG_2) into the active site of hAMCase indicate that allosamidin is a more preferred ligand than NAG_2, and that Glu119 forms hydrogen bond with allosamidin, which is in good agreement with the experimental results. From the docking studies, we also suggest that Trp10, Glu49, Asp192, and Glu276 in hAMCase are four important determinant residues in binding as they have strong van-der-Waals and electrostatic interactions with the ligand, respectively.

Keywords Acidic mammalian chitinase · Docking · Homology modeling

Introduction

Chitin, a linear polymer of β-(1,4)-linked N-acetylglucosamine (NAG) (Fig. 1), plays a key role in the life cycles of several classes of human pathogens, such as fungi, nematodes, protozoan parasites, and insects [1–4]. Chitinases (EC 3.2.1.14) are endo-β-1,4-N-acetylglucosaminidases that can fragment chitin and have been identified in several organisms varying from bacteria to humans [5–6]. Chitinase inhibitors have generated a lot interest given their chemotherapeutical potential as insecticides, fungicides, and antimalarials [7–9]. Biotechnological exploitation of chitinases, as well as design of inhibitors with sufficiently high selectivity and affinity, requires detailed knowledge of the structures and enzyme-substrate interactions.

The chitinase from human stomach and lung (hAMCase) consists of 476 amino acids, has a molecular mass of 50 kDa. Recent studies suggest that hAMCase is a functional chitinase and the experiment showed that hAMCase contributes to Th2-mediated inflammation through an IL-13-dependent mechanism, and inhibition of AMCase decreases airway inflammation and airway hyper-responsiveness [10–12]. Thus, hAMCase is a potential therapeutic target for anti-inflammatory therapy in Th2-mediated diseases such as asthma [13]. However, up to now no report has been found about the three-dimensional structure of the hAMCase, and thus theoretical studies on the binding mode of the hAMCase with its inhibitors are necessary to reveal the interaction occurring in the active site. Although the investigation of ligand binding mechanisms have been performed by biochemical methods recently, till now, there are no theoretical efforts being made and the three-dimensional (3D) structure of hAMCase remains to be elucidated in detail. Knowledge of the 3D structure is, however, essential for understanding the relationship of enzyme function and structure.

To our best knowledge, the homology modeling is an efficient method for the 3D structure construction of protein [14–16]. In this paper, we tried to obtain a reliable 3D structure of hAMCase based on human chitotriosidase (EC

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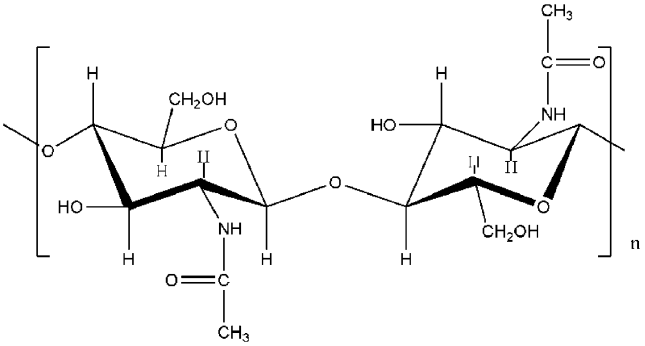

Fig. 1 The structure of Chitin

3.2.1.44, PDB code 1HKK) by the InsightII/Homology method and molecular mechanics (MM) and molecular dynamics (MD) simulations [17]. The model structure was then used to search the active site and carry out binding studies by flexible docking with the NAG_2 and allosamidin ligands. The docked complex would be used to identify the key residues for revealing further the ligand reaction mechanism, in particular identifying the binding residues with the NAG_2 and allosamidin and identifying the more preferred ligand.

Theory and methods

All simulations were performed on SGI O3800 workstations using InsightII software package developed by Accelrys Inc [18]. Primary sequence of hAMCase was obtained from the UniprotKB-Swiss-Prot (http://ca.expasy.org/sprot/, Accession No. Q9BZP6). The consistent-valence forcefield was used for energy minimization and MD simulations.

3D model building

The homology module [19] was used to build the initial model of hAMCase.

The first step was searching a number of related sequences to find a related protein as a template by the BLAST program [20] (http://www.ncbi.nih.gov) and then the sequences were imported into the ClustalW program (version 1.83) [21]. Program Modeler was performed to build the 3D structure of hAMCase. Modeler is an implementation of an automated approach to comparative modeling by satisfaction of spatial restraints [22–23]. For the remaining side chains, library values of rotamers were adopted. Through the procedure mentioned above, an initial model was thus completed.

The initial model was improved by energy minimization. After 300 steps of conjugate gradient minimization performed, the MD simulation was carried out to examine the quality of the model structures by checking their stability via performing 300 picoseconds (*ps*) simulations at a constant temperature 298K. To account for the solvent effect, the homology solvent model was solvated in layers of TIP3P water molecules with 15 Å. Finally, energy minimization of full protein was performed until the root mean-square (rms) gradient energy was lower than 0.001 kcal mol^{-1} $Å^{-1}$. All calculations mentioned above were accomplished by using Discover3 software package [24]. In this step, the quality of initial model was improved.

After the optimization procedure, the structure was checked using Profile-3D [25] and Procheck [26]. The Profile-3D method measured the compatibility of an amino acid sequence with a known 3D protein structure. This is especially useful in final phase of the protein structure modeling.

Binding-site analysis

The Binding-site module [27] is a suite of programs in InsightII for identifying and characterizing protein active sites, binding sites, and functional residues from protein structures and multiple sequence alignments. In this study, ActiveSite-Search was used to identify protein active sites and binding sites by locating cavity in the hAMCase structure. When the search was completed, the largest site was automatically displayed on the structure. And then, by using Asite-Display, other sites were also obtained. The results can be used to guide the protein-ligand docking experiment.

Docking ligands to hAMCase

Affinity, which uses a combination of Monte Carlo type and simulated annealing procedure to dock, is a suite of programs for automatically docking a ligand (guest) to a receptor (host) [28]. By means of the 3D structure of NAG_2 and allosamidin which were obtained from Gaussian03 program [29], the automated molecular docking was performed by using docking program Affinity. A key feature is that the "bulk" of the receptor, defined as atoms which are not in the binding site specified, is held rigid during the docking process, while the binding site atoms and ligand atoms are movable. The potential function of the complex was assigned by using the consistent-valence forcefield and the cell multiple approaches was used for nonbonding interactions. To account for the solvent effect, the centered enzyme-ligand complex was solvated in layers of TIP3P water molecules with 5 Å. Finally, the docked complex of hAMCase with NAG_2, and allosamidin was selected by the criteria of interacting energy combined with the geometrical matching quality. The complex was used as the starting conformation for further energetic minimization

and geometrical optimization before the final model was generated. The global structure with the lowest energy was chosen for computing intermolecular binding energies. The Ludi [30] program was used to characterize the affinity and the binding preference of a ligand to the protein.

Results and discussion

Homology modeling of hAMCase

A high level of sequence identity should guarantee more accurate alignment between the target sequence and template structure. In the result of the BLAST search, the high sequence identity between the hAMCase and 1HKK is 56% which allows for rather straightforward sequence alignment (see in the Fig. 2). It was reported that the sequence of hAMCase is that of the precursor protein which appears to have a signal peptide sequence. By using SignalP software [31], we can predicate that a possible cleavage site for a signal peptidase is present between the amino acid residue Ala21 and Tyr22. In this paper, the model is made up of residues starting from residue 22. Automated homology model building was performed using protein structure modeling program Modeler. All the side chains of model protein were set by rotamers. With this procedure, the initial model was completed.

The refinement of the homology model was obtained through MM optimization and MD simulations, and then the final stable structure of hAMCase was displayed in Fig. 3a. From Fig. 3a, we can see that this enzyme has 12 helices and 13 sheets. The conformation with the lowest energy was chosen and the 3D structure was superimposed with 1HKK using the homology module [19]. Their root mean square deviation (RMSD) value is 1.23 Å (Fig. 3b), indicating a good overall structure alignment with 1HKK. The final structure with the lowest energy was checked by Profile-3D (Fig. 4) and the self-compatibility score for this protein is 169.24, which is higher than the low score 75.48 and the top score 167.73. Note that compatibility scores above zero correspond to 'acceptable' side chain environment. From Fig. 4, we can see that all residues are reasonable. And then, the structure of hAMCase was evaluated using Procheck. Among these residues, no residue was found in the disallowed regions of Ramachandran plot (Fig. 5). The statistical score of the Ramachandran plot shows that 75.2% are in the most favored regions, 22.8% in the additional allowed regions, and 1.9% in generously allowed regions. The above results indicate that the homology model is reliable.

Identification of binding site in hAMCase

In order to investigate the interaction between hAMCase and the ligand, the binding pocket was defined as a subset that contains residues of any atoms within 5 Å from ligand. The active sites were obtained using Binding-site module. It was reported that family 18 chitinases have a conserved $(\beta/\alpha)_8$ fold, where a surface groove contains exposed aromatic residues, used for binding the chitin substrates [32]. In hAMCase, the conserved α/β-barrel motif forms a deep pocket, and the hAMCase shares a most prominent DXXDXDXE motif and includes the glutamate involved in catalytic reaction, which is highly conserved in the sequences of chitinases proteins from animals, plant, and bacteria. It is obvious that the chosen site is in good agreement with the conclusion drawn by van Aalten et al. [32]. This site is composed of 20 residues (Thr8, Gln12-Tyr13, Glu49, Glu77-Trp78, Gly81-Thr82, Pro84, Glu119-Tyr120, Arg124, Asp192, Tyr246, Glu276, Ile279, Ala281, Trp339-Ala340, and Leu343). Based on the experiment and our theoretical predicted results, in this study this site was chosen as the more favorable binding site to dock the ligand.

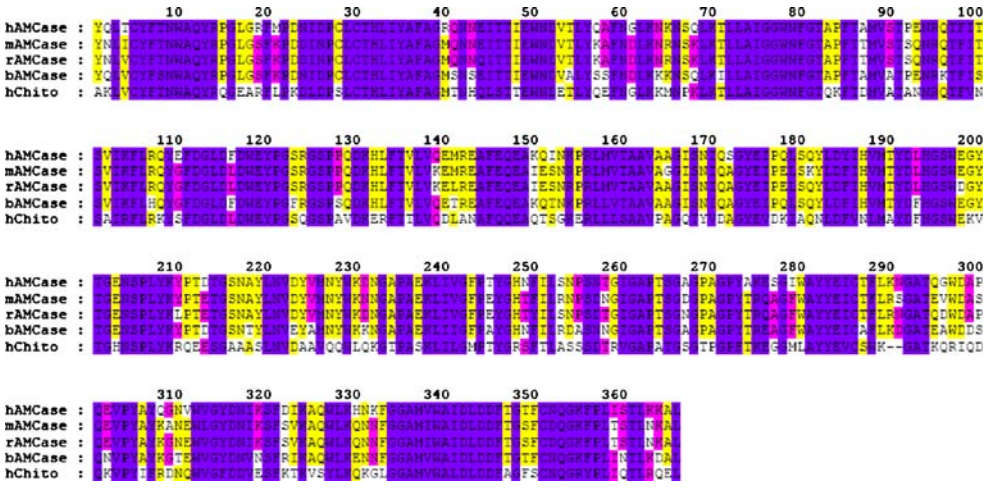

Fig. 2 Sequence alignment of hAMCase and 1HKK

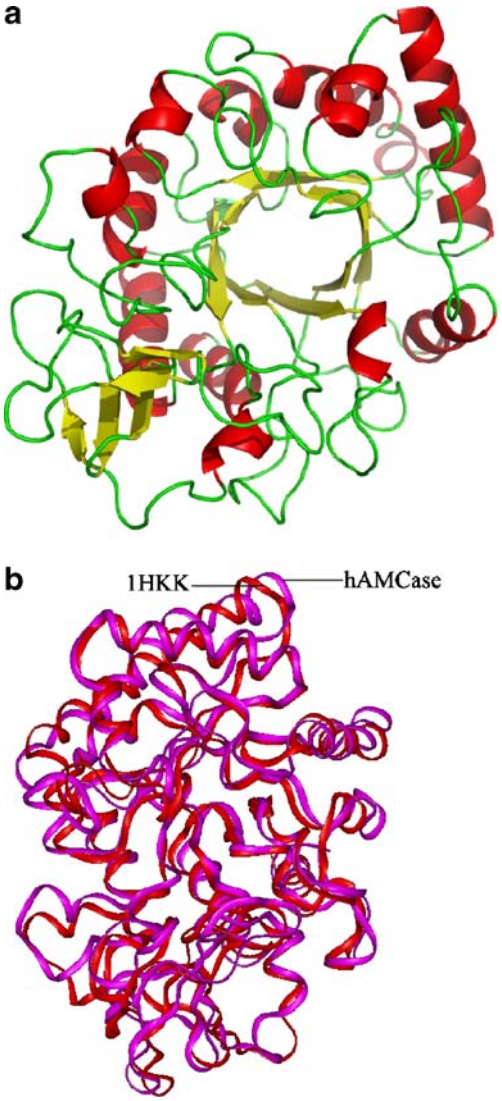

Fig. 3 (**a**) The final 3D-structure of hAMCase. The α-helix is represented by red color, the β-sheet is represented by yellow color and the loops are represented by green color. (**b**) Comparison of refined hAMCase model with its template 1HKK. Magenta ribbon representation of hAMCase. Orange ribbon representation of 1HKK

Docking study

All reports suggest that the pseudotriasccharide allosamidin can inhibit all family 18 chitinases with K_i values in the nanomolar to micromolar range [33–34]. NAG_2, one of the endogenous inhibitors, is of interest because of its overt structural overlap with Allosamidin [35]. Yi-Te et al. have demonstrated the inhibiton of hAMCase by NAG_2. In the following discussion, the interactions of the ligands with the receptor in the modeled complexes are investigated, and we shall compare the inhibition ability of hAMCase by allosamidin with that by NAG_2. The 3D structures of allosamidin and NAG_2 are shown in Fig. 6.

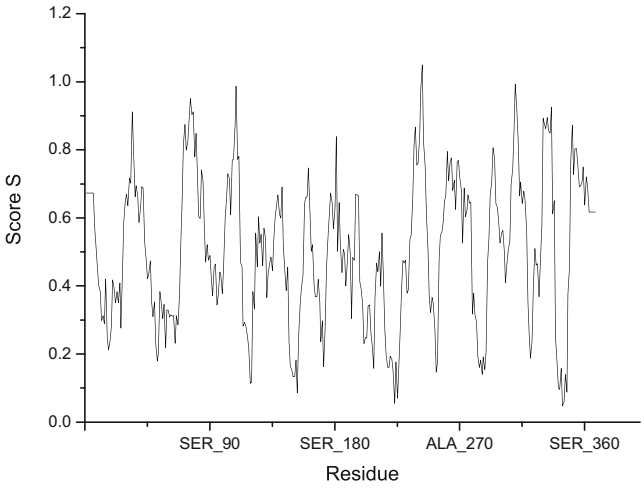

Fig. 4 The 3D profiles verified results of hAMCase model, residues with positive compatibility score are reasonably folded

Docking of the inhibitor into the active site

To understand the interaction between hAMCase and allosamidin, allosamidin- hAMCase (A-h) complex was generated by Affinity module and the binding 3D conformation of the complex is described in Fig. 7. As is well known, hydrogen bonds play an important role for structure and function of biological molecules, especially for the enzyme catalysis. There are seven hydrogen bonds between hAMCase and allosamidin: the carboxyl O of Glu276 interacts with the OH5 and amino H of sugar A, which has two hydrogen bonds (2.16Å, 1.53Å). The allosamizoline moiety of allosamidin is bound to carboxyl O of Glu119 by a hydrogen bond (2.28Å) and the OH1 of sugar B forms two hydrogen bonds with the amino H and carboxyl O of Glu276 (2.02 Å, 2.28 Å), respectively. It should be pointed out that two pairs of hydrogen bonds are also formed between hAMCase and allosamidin with the aid of H_2O. One is formed between carboxyl O of Glu192 and OH7 of

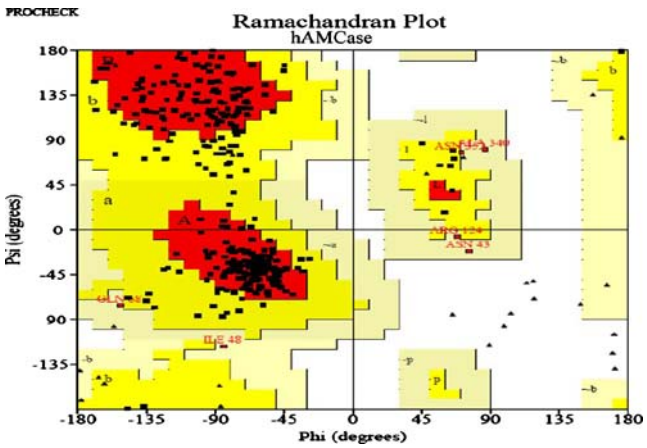

Fig. 5 Ramachandran plot of hAMCase obtained by PROCHECK

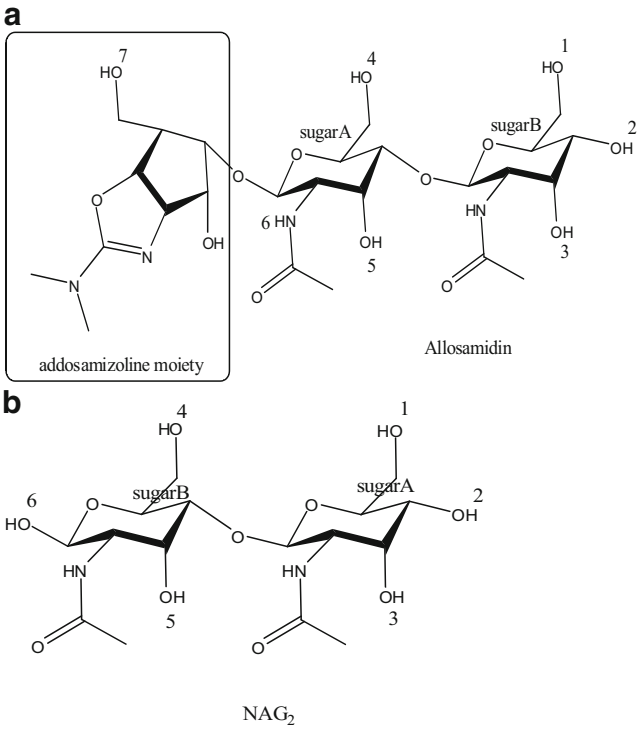

Fig. 6 The structure of the substrates: (**a**) allosamidin, (**b**) NAG_2

allosoamizoline group by taking H_2O as a bridge. Another one is formed between the amino H of Gln12 and OH3 of sugar B. These hydrogen bonding interactions enhance the stability of the complex. Among these hydrogen-bonding interactions, we can think that the Glu276 is the main contributor to the A-h complex because this residue forms four hydrogen bonds with hAMCase.

To determine the key residues that comprise the active site of the model, the interaction energies of the substrate with each of the residues in the active site were calculated. Significant binding-site residues in the models were identified by the total interaction energy between the ligand and each amino acid residues in the enzyme. This identification, compared with a definition based on the distance from the substrates, can clearly show the relative significance for every residue. Table 1 gives the interaction energies including the total, van-der-Waals and electrostatic energies with total energies lower than -1.0 kcal mol^{-1}. These interactions determine a stable binding mode for enzyme-substrate complex (E_{vdw}=−35.54 kcal mol^{-1}, E_{ele}= −112.77 kcal mol^{-1}, E_{total}=−148.31 kcal mol^{-1}). Through the interaction analysis, we can know that Glu192 (−59.41 kcal mol^{-1}), Glu276 (−52.00 kcal mol^{-1}), Glu49 (−21.24 kcal mol^{-1}), Trp339 (−7.22 kcal mol^{-1}), Trp10 (−11.81 kcal mol^{-1}), Tyr13 (−5.45 kcal mol^{-1}), Trp78 (−5.83 kcal mol^{-1}), Glu119 (−4.88 kcal mol^{-1}) are important anchoring residues for allosamidin, and have a main contribution to the substrate interaction. All the residues mentioned above are all conserved from the alignment result in this subfamily except Glu49 and Glu276. Gln12 is not listed in Table 1 as having a total calculated bonding energy more than −1 kcal mol^{-1}. We think that Gln12 may be an important residue because it forms a hydrogen bond with allosamidin. Thus, the residue of Glu119 can form hydrogen bond with hAMCase and play a major role in catalysis of chitinase substrates, which is in good agreement with the experimental result by Yi-Te et al. [35]. On the other hand, we can conjecture that the residue Trp10, Glu49, Glu192 and Glu276 are important determinant residues in binding as they have strong van-der-Waals and electrostatic contacts with allosamidin, respectively.

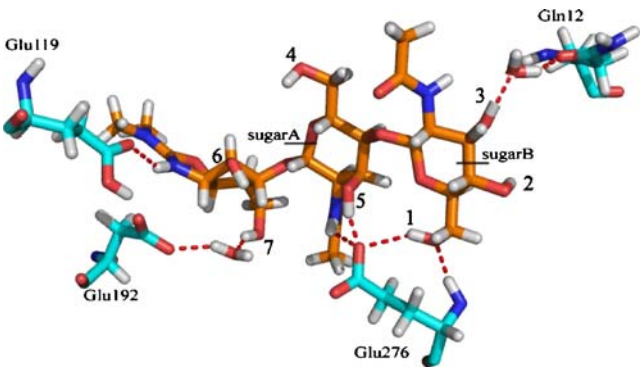

Fig. 7 The hydrogen bonding interaction in complex of hAMCase with allosamidin

Docking of the ligand into the active site

To understand the interaction between hAMCase and NAG_2, the NAG_2- hAMCase (N-h) complex was generated using the InsightII/Affinity module. The results show that the orientation within the active site pocket of NAG_2 is similar to those of the hAMCase and allosamidin, but there are only four hydrogen bonds in the N-h complex. The amino acids that recognize and interact with NAG_2 are Glu49 and Glu276. As seen from Fig. 8, we can see that there are two hydrogen bonds between carboxyl O of Glu49 and OH1 of sugar A (1.72Å, 2.43 Å), and the carboxyl O of Glu276 forms another two hydrogen bonds with OH3 and amino H of sugar A (1.78Å, 2.04Å). The absence of critical hydrogen bond interactions with the catalytic residue Glu119, and weak hydrogen bond interaction of NAG_2 with Glu276 and Glu49, presumably lead to the interaction energy of NAG_2 with the active-site residues of hAMCase (−74.51 kcal mol^{-1}) that is lower than that in the complex of hAMCase with allosamidin (−148.31 kcal mol^{-1}). Through the interaction analysis (in Table 1), we know that Glu276 (−25.08 kcal mol^{-1}), Trp10 (−12.73 kcal mol^{-1}), Glu49 (−11.24 kcal mol^{-1}), Trp78 (−10.91 kcal mol^{-1}), Trp339 (−7.65 kcal mol^{-1}), Asp192

Table 1 The total energy (E_{total}), Van Der Waals (E_{vdw}) and electrostatic energy (E_{ele}) between individual residues of hAMCase and allosamidin or NAG_2 (kcal mol^{-1})

Residue	Allosamidin			NAG_2		
	E_{vdw}	E_{ele}	E_{total}	E_{vdw}	E_{ele}	E_{total}
Total	−35.54	−112.77	−148.31	−39.49	−35.02	−74.51
Trp10	−11.52	−0.29	−11.81	−11.58	−1.15	−12.73
Tyr13	−4.42	−1.03	−5.45	−2.07	0.21	−1.85
Glu49	−1.03	−20.21	−21.24	−0.89	−10.35	−11.24
Trp78	−5.51	−0.32	−5.83	−8.97	−1.94	−10.91
Glu119	−1.55	−3.33	−4.88	−4.20	−1.07	−5.27
Asp192	−3.28	−56.13	−59.41	−1.22	−4.77	−5.99
Glu276	−0.60	−51.40	−52.00	−0.53	−24.55	−25.08
Ile279	−1.78	0.07	−1.70	−3.67	−1.35	−5.02
Trp339	−6.88	−0.34	−7.22	−7.25	−0.40	−7.65

(−5.991 kcal mol^{-1}), Glu119 (−5.27 kcal mol^{-1}) and Tyr13 (−1.85 kcal mol^{-1}) are important anchoring residues for NAG_2, and have main contribution to the substrate interaction. As seen from the alignment result that all the residues mentioned above are all conserved in this subfamily except Glu49 and Glu276.

For the docked structures, the interaction energies order are allosamidin > NAG_2. For the Ludi scores, the interaction energies order are allosamidin > NAG_2 (In Table 2). These results qualitatively validate the modeling and docking studies described for this subfamily.

In summary, the above results show that total interaction energy between hAMCase with allosamidin is higher than that between NAG_2 and hAMCase. The number of hydrogen bonds in the A-h complex is more than that in the N-h complex. This means that the A-h complex is more stable than that of the N-h complex. Furthermore, there are many common important residues in the hAMCase binding to allosamidin and NAG_2. This indicates that there is a simple competitive inhibition between allosamidin and NAG_2, and allosamidin is the more preferred ligand. Compared with allosamidin and NAG_2, they have the same main moiety, but the only difference is that there exists an addosamizoline moiety in allosamidin. They can both have strong interactions with hAMCase, whereas the addosamizoline moiety in the allosamidin is a strong attractive group and can induce the conformational changes in the active site residues of hAMCase and make the binding of allosamidin with hAMCase more energy favorable. In the case of NAG_2, it essentially has poor interactions with hAMCase, mainly because it lacks addosamizoline moiety and loosely binds to the enzyme, not well fixed in the substrate-binding pocket of the active site. Thus, this explains why the complex of NAG_2 and hAMCase have lower interaction. The results are in good agreement with the experiment reported by Yi-Te et al. [35].

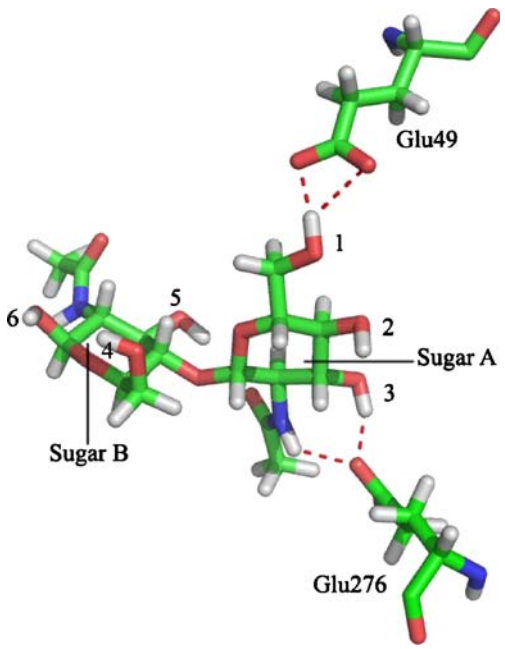

Fig. 8 The hydrogen bonding interaction in the complex of hAMCase with NAG2

Conclusions

The 3D structure of human acidic mammalian chitinase (hAMCase) has not been known. In this investigation, the

Table 2 The interaction energies (kcal mol^{-1}) between the ligands and hAMCase

Substrate	Evdw (kcal mol^{-1})	Eele ($kcalmol^{-1}$)	Etotal ($kcalmol^{-1}$)	Ludi Score	No. of H −bonds
allosamidin	−35.54	−112.77	−148.31	533	7
NAG_2	−39.49	−35.02	−74.51	481	4

3D structure of hAMCase was built by homology modeling, which was based on the known crystal structure of human chitotriosidase (PDB code 1HKK). Then energy minimization and molecular dynamics were used to refine the structure. With this model, a flexible docking study was performed and the docking results indicate that the addosamizoline moiety in allosamidin can stabilize the position and orientation in the active site of hAMCase. Glu49 and Glu276 may be the key amino acids residues interacting with the substrates, and Asp192 and Trp10 may help allosamidin steadily interact with hAMCase. These results will offer further experimental studies of structure-function relationships.

Acknowledgement This work is supported by the Natural Science Foundation of China, Key Projects in the National Science & Technology Pillar Program, and Specialized Research Fund for the Doctoral Program of Higher Education(Grant Nos. 20573042, 2006BAE03B01, and 20070183046)

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