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Theoretical study on 3-hydroxykynurenine transaminase by homology modeling and molecular dynamics

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Abstract

The three-dimensional (3D) model of the 3-hydroxykynurenine transaminase (3-HKT) is constructed based on the crystal structure of the alanine-glyoxylate aminotransferase (EC 2.6.1.44, PDB code 1VJO) by using InsightII/Homology module. With the aid of the molecular mechanics and molecular dynamics methods, the last refined model is obtained and further assessed by Profile-3D and ProStat, which confirm that the refined model is reliable. With this model, a flexible docking study is performed and the result indicates that Trp104 and Gln204 are important residues as they have strong hydrogen bonding interactions with 3-HK respectively and they will act as a vital role in catalysis of 3-HKT. The Trp104 is in good agreement with the experimental result by Li et al. From the docking studies, we also suggest that the residues of Lys205 and Pro211 in 3-HKT are two important determinant residues in binding as they have strong van der Waals contacts with the 3-HK. Our results may be helpful for further experimental investigations.

Keywords: 3-Hydroxykynurenine transaminase; Docking; Homology modeling

1. Introduction

3-Hydroxykynurenine (3-HK) is a metabolic intermediate of the kynurenine pathway, which is the major catabolic route of tryptophan. However, it is oxidized easily, stimulating the production of reactive oxygen species [1–5]. Several studies have demonstrated that 3-HK concentration is elevated in the brains of patients with AIDS-related dementia [6], Parkinson's disease [7], Huntington's disease[8,9], and hepatic encephalopathy [10]. Li et al.'s study showed that mosquitoes have an efficient mechanism for controlling the level of 3-HK through the conversion of the chemically reactive and potentially toxic 3-HK to a chemically stable xanthurenic acid (XA) by transaminase-mediated reactions. Therefore, the 3-HK to XA pathway is considered an essential detoxification pathway in mosquitoes [11].

The mechanism of 3-HK-induced pathogenesis has been extensively studied. However, it has not been well elucidated, for the 3D structure of the 3-hydroxykynurenine transaminase (3-HKT) has not been known. To our best knowledge, the homology model is an efficient method for the 3D structure construction of protein [12]. In the present investigation, we construct a 3D model of 3-HKT and search for the binding site of substrate. The 3D features of the model are obtained by a homology modeling procedure based on the crystal structure of alanine-glyoxylate aminotransferase (EC 2.6.1.44, PDB code 1VJO) [13]. The model can be used to explain substrate specificity and relationship of enzyme function and structure. The docking complex by Affinity module would be used to identify the key residues for further revealing the substrate reaction mechanism, in particular identifying the binding residues with 3-HK.

2. Theory and methods

All simulations are performed on the SGI O3800 workstations using InsightII software package developed by Accelrys Inc. [14]. The sequence of 3-HKT is obtained from the databank in the National Center for Biotechnology

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Information (www.ncbi.nlm.nih.gov). The consistent-valence forcefield (CVFF) is used for energy minimization and molecular dynamics (MD) simulations.

2.1. 3D model building

The homology module [15] is used to build the initial model of 3-HKT.

The first step is searching a number of related sequences to find a related protein as a template. BLAST search algorithm [16] is used for the search on line (http://pir.georgetown.edu/). Program Modeler is performed to build the 3D structure of 3-HKT [17]. Modeler is an implementation of an automated approach to comparative modeling by satisfaction of spatial restraints [18–20]. For the remaining side chains, library values of rotamers are adopted. Through the procedure mentioned above, an initial model is thus completed.

The initial model is improved by energy minimization. After 200 steps of conjugate gradient (CG) minimization performed, the MD simulation is carried out to examine the quality of the model structures by checking their stability via performing 150 picoseconds (ps) simulations at a constant temperature 298 K. An explicit solvent model TIP3P water is used, and the homology solvent model is constructed with a 20 Å water cap from the center of mass of 3-HKT. Finally, a conjugate gradient energy minimization of full protein is performed until the root mean-square (rms) gradient energy is lower than 0.001 kcal mol^{-1} $Å^{-1}$. All calculations mentioned above are accomplished by using Discover3 software package [21]. In this step, the quality of the initial model is improved.

After the optimization procedure, the structure is checked using Profile-3D [22,23] and ProStat. The Profile-3D method measures the compatibility of an amino acid sequence with a known three-dimensional protein structure. This is especially useful in the final phase of the protein structure modeling. The ProStat module of InsightII identifies and lists the number of instances where structural features differ significantly from the average values calculated from known proteins.

2.2. Binding site analysis

The binding-site module [24] 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 is used to identify protein active sites and binding sites by locating cavities in the 3-HKT structures. When the search is completed, the largest site is automatically displayed on the structure. And then, by using Asite-Display, other two sites are also obtained. The results can be used to guide the protein–ligand docking experiment.

Molecular electrostatic potential (MEP) is one of the most important factors, which influence the structure and biological activity of macromolecules. To understand the surface property of the 3-HKT and the 3-HK better, the electrostatic potential is calculated by solving the Poisson-Boltzmann equation using the finite difference method (Delphi module in InsightII).

2.3. Docking ligand to 3-HKT

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) [25]. By means of the 3D structure of 3-HK which is built through the InsightII/Builder program, the automated molecular docking is 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 (active) 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 is assigned by using the CVFF and the cell multipole approach is used for non-bonding interactions. To account the solvent effect, the centered enzyme–ligand complex is solvated in a sphere of TIP3P water molecules with radius 20 Å. Finally, the docked complex of 3-HKT with 3-HK is selected by the criteria of interacting energy combined with the geometrical matching quality. The complex is used as the starting conformation for further energetic minimization and geometrical optimization before the final model is achieved.

3. Results and discussion

3.1. Homology modeling of 3-HKT

BLAST search algorithm [16] is used for the search on line (http://pir.georgetown.edu/). The high sequence identity between the 3-HKT and the reference protein 1VJO is 41% which allows for rather straightforward sequence alignment (Fig. 1). In our study, automated homology model building is performed using protein structure modeling program Modeler. All the side chains of model protein are set by rotamers. With this procedure, the initial model is completed. This model is refined by MM optimization and MD simulations, and then the final stable structure of 3-HKT is obtained as displayed in Fig. 2. From Fig. 2 we can see that this enzyme has thirteen helices and thirteen sheets. The analysis by ProStat shows that there is no significantly different appeared between the calculated values of the bond lengths and bond angles and that of the known proteins for the total residues. The final structure is further checked by profile-3D and the results are presented in Fig. 3. Checking by profile-3D, the self-compatibility score for this protein is 159.13 which is higher than the low score 79.82 and close to the top score 177.38. Note that compatibility scores above zero correspond to 'acceptable' side chain environment. From Fig. 3, we can see that most

Fig. 1. Sequence alignment of 3-HKT with the alanine–glyoxylate aminotransferase (EC 2.6.1.44, PDB code 1VJO).

residues are reasonable, but only the variable regions Asn39-Leu42, Asn44-Phe50 and Phe306 are built poorly and should be considered as unreliable. Fortunately, these twelve unreliable residues locate far from the active site of 3-HKT, and these twelve unreliable residues would have not influence much on the following study. Fig. 4 shows the structure alignment of Ca trace between 3-HKT and 1VJO. The root mean square deviation of the Ca atoms (Ca RMSD) between 3-HKT and 1VJO is 1.45 Å. The above results indicate that the homology model is reliable.

3.2. Identification of substrate-binding region in 3-HKT

The 3-HKT and 1VJO are well conserved in both sequence and structure, their biological functions should be identical. And thus we can presume that the **3**-HK binds in a manner similar to both 3-HKT and 1VJO. In order to investigate the interaction between 3-HKT and 3-HK, the binding pocket is defined as a subset that contains residues in which any atoms are within 5.0 Å from 3-HK. The binding-site is searched by InsightII/Binding-Site module, which can be used to guide the protein–ligand docking experiment. The binding pocket is composed of 15 residues (Pro24, Gly25, Pro26, Ser27, Gly76, Ser77, Ala78, Trp104, Ser203, Gln204, Lys205, Ala209, Pro210, Pro211 and Gly212), in which, Pro24, Gly25, Pro26, Ser27, Gly76, Ser203, Gln204, Pro211 and Gly212 are nine conserved residues. Fig. 5 shows the Connolly surface of 3-HK and the binding pocket. From Fig. 5, which depicts the size, the shape and the electrostatic surface potential of the binding pocket are matching for that of the 3-HK, we can see that there is a pocket obviously in the protein surface including

Fig. 2. The final 3D-structure of 3-HKT. The α-helix is represented by red color and the β-sheet is represented by yellow color.

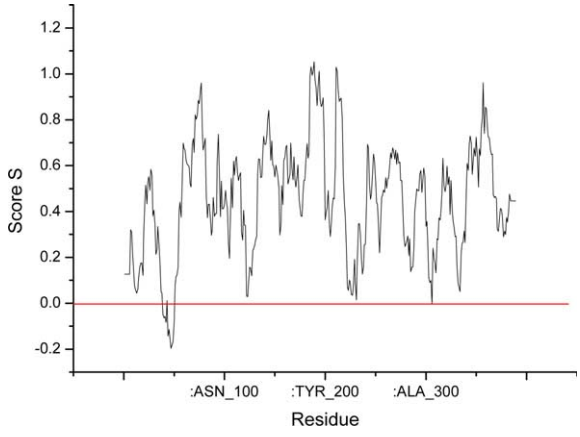

Fig. 3. The 3D profiles verified results of 3-HKT model, residues with positive compatibility score are reasonably folded.

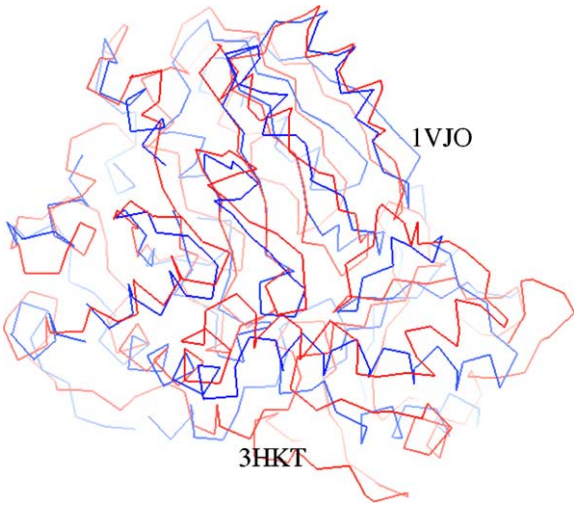

Fig. 4. Ca trace of 3-HKT (represented by red color) and 1VJO (represented by blue color).

the 15 residues mentioned above. Thus, in this study this site is chosen as the more favorable binding site to dock the ligand.

3.3. Docking study

3-Hydroxykynurenine (3-HK) is an endogenous metabolite in the tryptophan oxidation pathway. We are interested in investigating the mechanisms of ligand binding and the interaction between the 3-HKT and the 3-HK.

The 3D structure of 3-HK is built with the InsightII/Builder program and the geometry of 3-HK is further optimized using AM1 method. The 3D conformation of 3-HK is shown in Fig. 6.

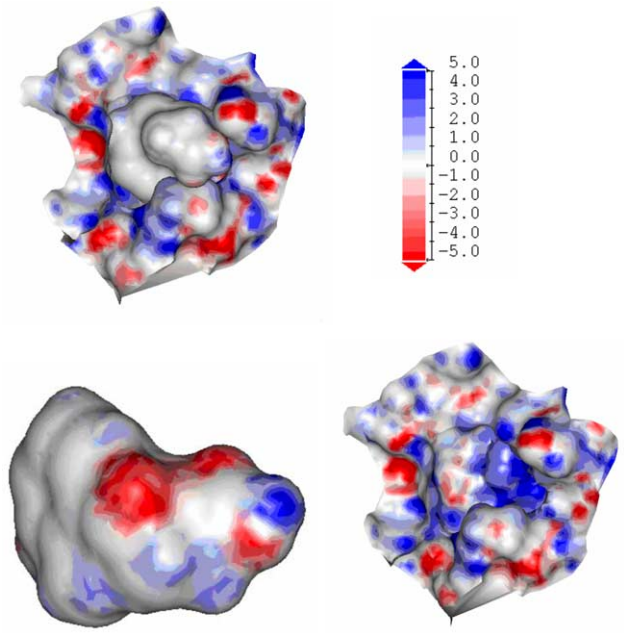

Fig. 5. The electrostatic surface potential of the binding pocket of the 3-HKT and the 3-HK. In the figure, the red denoted negative electrostatic potential and the blue denoted positive electrostatic potential.

3.4. Docking of the substrate into the active site

To understand the interaction between 3-HKT and 3-HK, the complex of 3-HKT with 3-HK (T-h) is generated by InsightII/Affinity module and the binding 3D conformation of the T-h complex is described in Fig. 7. This figure shows that the 3-HK locates in the center of the binding pocket. As is well known, hydrogen bonds play an important role for structure and function of biological molecules, especially for the enzyme catalysis. The hydrogen bonds presented in the T-h complex are listed in Table 1 and Fig. 8, respectively. One hydrogen bond forms between the carbonyl O of Gln204 and Hydroxyl of 3-HK. It should be pointed out that with the aid of H_2O, one pair of hydrogen bonds is also formed between 3-HKT and 3-HK. This kind of hydrogen bond forms between indole H of Trp104 and amino N of 3-HK by taking H_2O as a bridge. These hydrogen bonding interactions enhance the stability of the T-h complex.

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 of 3-HKT are calculated. Significant binding-site residues in the models are identified by the total interaction energy between the substrate and each amino acid residues in the enzyme. This identification, compared with a definition based on the distance from the substrate, can clearly show the relative significance for every residue. Table 2 gives the interaction energies including the total, van der Waals and electrostatic energies with the total energies lower than -0.40 kcal $\cdot$ mol^{-1} for all residues in T-h complex. From Table 2 we can also see that the T-h complex has a large favorable total interaction energy and the total interaction energy is $-$

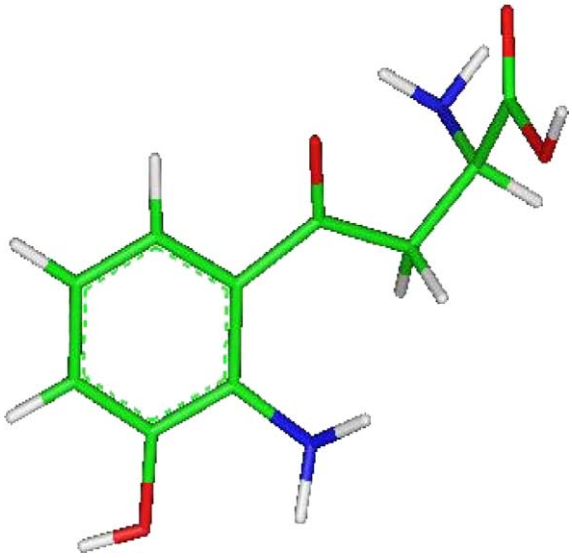

Fig. 6. A stereo picture of 3-HK.

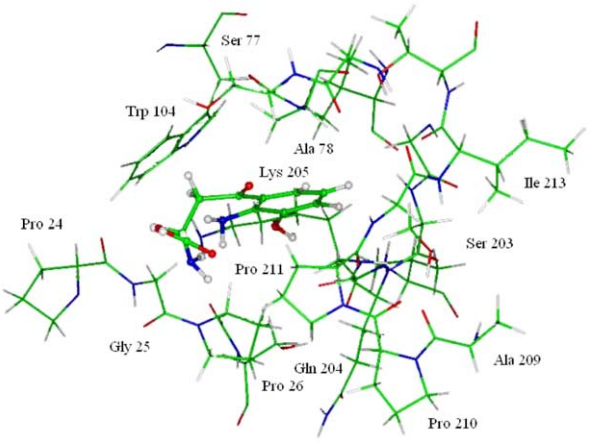

Fig. 7. A stereo picture of the active site's residues and the 3-HK.

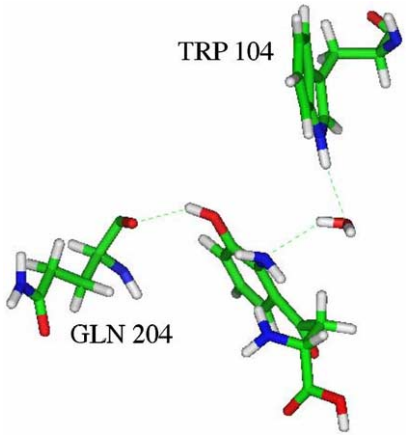

Fig. 8. The hydrogen bonding interaction of the complex T-h.

37.35 kcal mol^{-1}, the van der Waals and electrostatic energies are −34.41 and −2.94 kcal mol^{-1}, respectively. These results indicate that the attractive interaction is important. Through interaction analysis, we know that Pro211, Gln204, Ser77, Lys205, Ala78, Pro26, Ser203, Gly25, Trp104, Ile213, Pro210, Ala209 and Pro24 are important anchoring residues for 3-HK and have main contribution to the substrate interaction. For the hydrophobic residues of Pro211, Ala78, Pro26, Trp104 and Pro24, the interaction energies with 3-HK are mainly contributed by van der Waals interaction. Although the interaction energies of Trp104 and Gln204 are not prominent compared with the other residues in Table 2, the residues of Trp104 and Gln204 can form strong hydrogen bonds with 3-HK and play a major role in catalysis of 3-HKT. Thus, the Trp104 and Gln204 may be important residues, and the Trp104 is in good agreement with the experimental result by Li et al. [11]. On the other hand, we can conjecture that the residues of Lys205 and Pro211 in 3-HKT are two important determinant residues in binding as they have strong van der Waals contacts with 3-HK. The energy information in Table 2 may guide the selection of candidate sites for further experimental studies of site-directed mutagenesis.

4. Conclusion

We have developed a three-dimensional model of the 3-HKT by InsightII/Homology module. After energy minimization and molecular dynamics simulations, the refined model structure is obtained. The last refined model is further assessed by Profile-3D and ProStat, and the results show that this model is reliable. The stable structure is further used to perform the docking of 3-HK. Through the docking study, the model structure of the ligand–receptor complex is obtained. The docking results indicate that the conserved amino acid residues in 3-HKT play an important role in maintaining a functional conformation and are directly involved in binding to donor and acceptor substrates. The interactions of the 3-HKT and 3-HK proposed in this study are useful to understand the potential mechanisms of the 3-HKT and 3-HK. In particular, with the aid of H_2O, some hydrogen bonds are formed in the docked complex by taking H_2O as a bridge. The residues of Trp104 and Gln204 are important as they have strong hydrogen bonding interactions with 3-HK and they will play a major role in catalysis of 3-HKT. The Trp104 is in good agreement with the experimental result by Li et al. [11]. On the other hand, the results reported here lead to the proposal of Lys205 and Pro211 as the key residues, for they have strong van der Waals contacts with the 3-HK. Furthermore, these residues, as well as the others in Table 2, are suggested as candidates for further experimental studies of structure–function relationships.

Acknowledgements

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Table 1
The hydrogen bonds between the 3-HK and active site residues of 3-HKT

3-HKT		3-HK (Atom)	H_2O (Atom)	Distance (Å)	Angle (degree)
Residue	Atom				
GLN 204	O	Hydroxyl H		2.16	140.41
TRP 104	H		O	2.30	167.36
		Amino N	H	2.45	141.89

Table 2
The total energy (E_{total}), Van Der Waals energy (E_{vdw}) and electrostatic energy (E_{ele}) between 3HK and individual residues ($E_{total} < -0.40$ kcal mol^{-1} listed in energy rank order)

Residue	E_{vdw} (kcal mol^{-1})	E_{ele} (kcal mol^{-1})	E_{total} (kcal mol^{-1})
Total	−34.41	−2.94	−37.35
PRO 211	−5.16	−0.83	−5.99
GLN 204	−2.92	−1.90	−4.82
SER 77	−4.21	0.04	−4.17
LYS 205	−2.29	−1.27	−3.56
ALA 78	−3.19	−0.11	−3.30
PRO 26	−3.39	0.51	−2.87
SER 203	−2.26	0.12	−2.14
GLY 25	−1.84	0.05	−1.79
TRP 104	−1.14	−0.01	−1.15
ILE 213	−0.66	−0.41	−1.07
PRO 210	−0.43	−0.43	−0.86
ALA 209	−0.18	−0.30	−0.48
PRO 24	−0.39	−0.02	−0.41

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References

[1] Hiraku Y, Inoue S, Oikawa S, Yamamoto K, Tada S, Nishino K, Kawanishi S. Carcinogenesis 1995;16:349.
[2] Nakagami Y, Saito H, Matsuki N. Neurosci Lett 1996;216:211.
[3] Okuda S, Nishiyama N, Saito H, Katsuki H. J Neurochem 1998;70:299.
[4] Okuda S, Nishiyama N, Saito H, Katsuki H. Proc Natl Acad Sci USA 1996;93:12553.
[5] Sardar AM, Reynolds GP. Neurosci Lett 1995;187:9.
[6] Sardar AM, Bell JE, Reynolds GP. J Neurochem 1995;64:932.
[7] Ogawa T, Matson WR, Beal MF, Myers RH, Bird ED, Milbury P, Saso S. Neurology 1992;42:1702.
[8] Pearson SJ, Reynolds GP. Neurosci Lett 1992;144:199.
[9] Guilarte TR, Eastman CL. J Neurol Sci 1993;116:227.
[10] Pearson SJ, Reynolds GP. J Chromatogr 1991;565:436.
[11] Li J, Li G. Insect Biochem Mol Biol 1997;27:859.
[12] Eisenhaber F, Persson B, Argos P. Crit Rev Biochem Mol Biol 1995;30:1.
[13] Joint Center for Structural Genomics: Crystal Structure of Alanine–Glyoxylate Aminotransferase (Alr1004) from Nostoc Sp. At 1.70 A Resolution To be Published.
[14] InsightII, version 98.0.San Diego: Accelrys Inc. 1998.
[15] Homology User Guide, Accelrys Inc., San Diego, USA 1999.
[16] Altschul SF, Gisli W, Miller W, Mayer EW, Lipman DJ. J Mol Biol 1990;25:403.
[17] Sali A, Blundell TL. J Mol Biol 1993;234:779.
[18] Sali A, Overington JP. Protein Sci 1994;3:1582.
[19] Sali A, Potterton L, Yuan F, Vlijmen H, Karplus M. Proteins 1995;23:318.
[20] Sali A. Curr Opin Biotechnol 1995;6:437.
[21] Discover3 User Guide, Accelrys Inc., San Diego, USA 1999.
[22] Luthy R, Bowie JU, Eisenberg D. Nature 1992;356:82.
[23] Profile-3D User Guide, Accelrys Inc., San Diego, USA 1999.
[24] Binding Site Analysis User Guide, Accelrys Inc., San Diego, USA 1999.
[25] Affinity User Guide, Accelrys Inc., San Diego, USA 1999.