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ORIGINAL PAPER

Stereoselectivity of chalcone isomerase with chalcone derivatives: a computational study

Yuan Yao · Hui Zhang · Ze-Sheng Li

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Abstract Chalcone isomerase (CHI) catalyzes the intramolecular cyclization of chalcones into flavonoids. The activity of CHI is essential for the biosynthesis of flavonoids precursors of floral pigments and phenylpropanoid plant defense compounds. In the present study, we explored the detailed binding structures and binding free energies for two different active site conformations of CHI with *s*-cis/*s*-trans conformers of three chalcone compounds by performing molecular dynamics (MD) simulations and binding free energy calculations. The computational results indicate that *s*-cis/*s*-trans conformers of chalcone compounds are orientated in the similar binding position in the active site of CHI and stabilized by the different first hydrogen bond network and the same second hydrogen bond network. The first hydrogen bond network results in much lower binding affinity of *s*-trans conformer of chalcone compound with CHI than that of *s*-cis conformer. The conformational change of the active site residue T48 from indirectly interacting with the substrate via the second hydrogen bond network to directly forming the hydrogen bond with the substrates cannot affect the binding mode of both conformers of chalcone compounds, but remarkably improves the binding affinity. These results show that CHI has a strong stereoselectivity. The calculated binding free energies for three chalcone compounds with CHI are consistent with the experimental activity data. In addition, several valuable insights are suggested for future rational design and discovery of high-efficiency mutants of CHI.

Keywords Chalcone isomerase · MM/PBSA · Molecular dynamics simulation · Stereoselectivity

Y. Yao · Z.-S. Li (✉)
Academy of Fundamental and Interdisciplinary Science, Harbin Institute of Technology, Harbin 150080, People's Republic of China
e-mail: zeshengli@bit.edu.cn

H. Zhang (✉)
College of Chemical and Environmental Engineering, Harbin University of Science and Technology, Harbin 150080, People's Republic of China
e-mail: hust_zhanghui11@hotmail.com

Z.-S. Li
Key Laboratory of Cluster Science of Ministry of Education & School of Chemistry, Beijing Institute of Technology, Beijing 100081, People's Republic of China

Introduction

Flavonoids and the related compounds play several physiological roles in plants, e.g., as protection against damaging UV light, as floral pigments for attracting pollinators, as inducers of *Rhizobium* nodulation genes, and as potent anti-microbial phytoalexins against pathogen infestations [1–3]. This kind of compound can also be used as common constituents in human diets, showing medicinal properties [4, 5]. In solution, flavonoid can be produced by spontaneous cyclization of chalcone. In flavonoid biosynthetic pathways, chalcone isomerase (CHI, EC 5.5.1.6) not only catalyzes the cyclization of chalcone into flavanone via a diffusion-controlled reaction with a ~10^7-fold higher rate than spontaneous conversion [6] but also guarantees the formation of the biologically active (*2S*)-flavonoids. Therefore CHI has been considered as an attractive target for metabolic engineering to provide enhanced feed crops, food sources, and medicinal agents [7, 8]. To achieve this goal, the detailed structural and mechanistic information of CHI is required.

The crystal structures of CHI and its complexes with different flavanones have been reported [9–12]. In the active site of CHI, the bound flavanone interacts with the enzyme through two hydrogen bond networks (shown in Fig. 1). The first hydrogen bond network consists of N113 and T190 residues and contacts the 7-hydroxyl group of the bound flavanone. The second set of interactions includes three conserved residues (T48, Y106, and K97) mediating a hydrogen

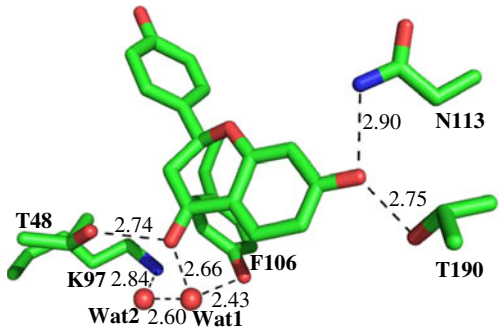


Fig. 1 The active site conformation of CHI with the bound flavonoid

bond network with the involvement of two water molecules (denoted as CHI(**I**)). An alternate conformation (denoted as CHI(**II**)) of the side chain of T48 residue is also observed to point toward the flavanone ketone group. Based on the binding of CHI with flavanones, the reaction mechanism for cyclization of chalcone catalyzed by CHI has been proposed. The deprotonation of the 2′-hydroxyl group of chalcone spontaneously occurs in solution at physiologic pH, resulting in a reactive oxyanion. This has been supported by the pH-dependence result of the reaction [11]. Once bound within the active site of CHI, the 2′-oxyanion of chalcone is positioned for a stereochemically defined Michael addition to the α,β-unsaturated double bond of chalcone. Via an enolate intermediate, the product is generated. Several factors, which remarkably affect the transition state stabilization [13, 14], the free energy barriers [15], and reaction rates of CHI [16] in the different active site conformations, have been revealed through computational studies. However, chalcone has two different conformations in chemistry, including the unproductive *s*-cis conformer and the productive *s*-trans conformer (depicted in Scheme 1). Without any available structure information of CHI-chalcone complex, the binding modes of *s*-cis/*s*-trans conformers of chalcones with CHI remain unresolved and the effect of the different active site conformations on the binding affinity with chalcone compounds remains unclear. This may block the comprehensive understanding of the detailed reaction mechanism of CHI and rational design of high-efficiency mutant of CHI.

In the present study, we have carried out molecular dynamics (MD) simulations and binding free energy calculations to investigate the binding modes of CHI with two different active site conformations. Three chalcone compounds, 4,2′,4′,6′-Tetrahydroxylchalcone (**a**), 4,2′,4′-Trihydroxylchalcone (**b**), and 2′,4′-Dihydroxylchalcone (**c**) were chosen as the substrates. The computational results clearly reveal the binding mode and the stereoselectivity of CHI for *s*-cis/*s*-trans conformers of chalcone. Several factors affecting the binding affinity are also identified. A comparative analysis of the obtained results is helpful to understand not only the origin of catalytic reaction of CHI but also the relationship between the structure and the catalysis.

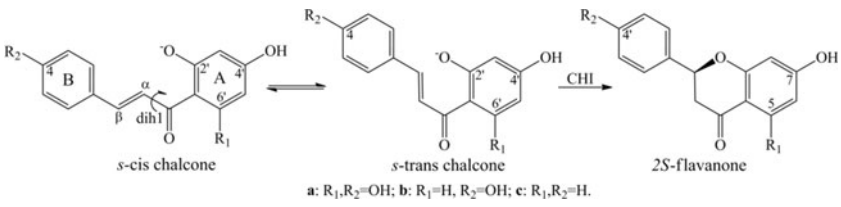
Computational details

Molecular dynamics simulation As chalcone compounds have similar structures as their related products, flavanones, the initial coordinates of the enzyme-substrate complexes were generated on the basis of the X-ray crystal structure 1FM7 of CHI with 7,4′-Dihydroxyflavanone [11] through replacing the product by the related substrates. The partial atomic charges for the atoms in the substrate were calculated by using the RESP protocol implemented in the Antechamber module in Amber9 package [17, 18] after electrostatic potential (ESP) calculation at HF/6-31G* level using Gaussian03 program [19]. All missing hydrogen atoms were added by LEaP module in Amber 9 package. The systems were then solvated in a rectangular box of TIP3P water molecules [20] with a minimum solute wall distance of 10 Å. The prepared systems were fully energy minimized followed by the equilibration through gradually increasing the temperature from 10 to 298.15 K. Then the production MD simulations were kept running for ~10 ns. During MD simulation, the time step was set as 2 f. with a cutoff of 10 Å for nonbond interactions. The shake procedure [21, 22] was employed to constrain all bonds involving hydrogen atoms. The MD simulations were performed by the Sander module in Amber9 package.

Binding free energies calculations The binding free energies were calculated by using the molecular mechanics–Poisson–Boltzmann surface area (MM–PBSA) method [23]. In the MM–PBSA method, the free energy of the protein-substrate binding, ΔG_{bind}, is obtained from the difference between the free energies of protein-substrate complex (G_{cpx}) and the unbound receptor/protein (G_{rec}) and ligand (G_{lig}) as follows:

$$\Delta G_{\text{bind}} = G_{\text{cpx}} - G_{\text{rec}} - G_{\text{lig}}. \quad (1)$$

Scheme 1 The overall reaction catalyzed by CHI



The binding free energy (ΔG_{bind}) is evaluated as a sum of the changes in the molecular mechanical (MM) gas-phase binding energy (ΔE_{MM}), solvation free energy (ΔG_{solv}), and entropic contribution ($-T\Delta S$):

$$\Delta G_{bind} = \Delta E_{MM} + \Delta G_{solv} - T\Delta S. \quad (2)$$

The molecular mechanical energy ΔE_{MM} is further divided into the internal energy (ΔE_{int}), the Coulomb energy (ΔE_{ele}), the van der Waals energy (ΔE_{vdW}) in gas phase:

$$\Delta E_{MM} = \Delta E_{int} + \Delta E_{ele} + \Delta E_{vdW}. \quad (3)$$

The salvation free energy is divided into a polar part (ΔG_{PB}) and a nonpolar part (ΔG_{np})

$$\Delta G_{solv} = \Delta G_{PB} + \Delta G_{np}. \quad (4)$$

Here, the polar contribution (ΔG_{PB}) is calculated by solving the Poisson–Boltzmann (PB) equation [24] as implemented in Amber9 package. The value of the interior dielectric constant and exterior dielectric constant were set to 1 and 80, respectively. The nonpolar solvation energy (ΔG_{np}) was calculated from the solvent–accessible surface area (SASA) using the hard-sphere atomic model. The probe radius of the solvent was set to 1.4 Å. ΔG_{np} is calculated using

$$\Delta G_{np} = \gamma \cdot \Delta SASA + \beta. \quad (5)$$

where the surface tension γ and the offset β were set to the standard values of 0.00542 kcal $mol^{-1} \cdot Å^2$ and 0.92 kcal mol^{-1}, respectively.

Normal-mode analysis (NMA) is useful to estimate the change in solute entropy during ligand binding. However, the NMA calculation is considered to be problematic and time-consuming and the NMA approach also does not take into consideration the solvent entropy. In addition, the three substrates used in the present study are very similar. According to the previous studies [25–28], the entropy differences should be very small so that the correlation between the experimental K_m value and the calculated binding free energy may not be greatly improved. Therefore, the solute entropy term was neglected in the present study. For each MD-simulated CHI-substrate complex, we calculated the ΔG_{bind} values for the 100 snapshots of the MD trajectory (one snapshot for each 2 ps during the last 200 ps of the stable trajectory) and the final ΔG_{bind} value was the average of the calculated ΔG_{bind} values for these snapshots.

Results and discussion

Binding structure The binding modes of CHI with chalcone compounds is shown in Fig. 2 and plots of MD-simulated internuclear distances and RMSD for Cα atoms versus simulation time for each of the CHI-chalcone complexes are provided in Figs. 3 and 4. It was shown that each of the CHI-chalcone complexes was dynamically stable and both *s*-cis/*s*-trans chalcone compounds (**a**, **b**, and **c**) were stabilized in the active site of CHI through two hydrogen bond networks. The first hydrogen bond network consists of the side chains of N113 and T190 residues when *s*-trans chalcone compounds are the substrates. In comparison, only the main chain of T190 residue is involved in the first hydrogen bond network when binding *s*-cis chalcone compounds. This hydrogen bond network directly forms strong hydrogen bonds with the 4′-hydroxyl group of chalcone compounds. The second hydrogen bond network consists of three conserved residues (T48, Y106, and K97) and interacts with the ketone group of chalcone compounds through two water molecules in both *s*-cis/*s*-trans chalcone compounds. In CHI(**I**), the side chain hydroxyl group in T48 residue forms a strong hydrogen bond with the second water molecule involved in the second hydrogen bond network. By contrast, it does not join the second hydrogen bond network but directly forms the hydrogen bond with the ketone group of chalcones in CHI(**II**). Overall, this binding mode is similar to that of CHI with flavanones as shown in Fig. 1.

Clearly, CHI(**I**) shares the binding mode with CHI(**II**), no matter which conformer of chalcone compound is bound. It is interesting to know why this happens. The main difference between *s*-cis/*s*-trans chalcone compounds is caused by the change of dihedral angle (dih1) as shown in Scheme 1. In *s*-cis conformer of chalcone compounds, dih1 almost equals to 180° and C_β atom locates away from O2′ atom, whereas *s*-trans conformer of chalcone compound has C_β atom and O2′ atom in proximity with dih1 of 0°. So, *s*-cis conformer of chalcone compounds has a linear conformation, but a bent conformation for *s*-trans conformer of chalcone compounds. In Fig. 5, one can see that rings B of *s*-cis/*s*-trans conformers of chalcone compounds point toward the entrance of the binding pocket and they are surrounded by several hydrophobic residues (L38, F47, and L101). The ketone groups are stabilized and anchored by the second hydrogen bond network, resulting in sharing the same location within the active site of CHI. Therefore, 4′-hydroxyl groups in rings A in *s*-cis/*s*-trans conformers of chalcone compounds are forced to point toward the different directions and form the different hydrogen bonds with the first hydrogen bond networks as mentioned above. Note that rings A are surrounded by several hydrophobic residues (I51, I93, V197, and L201). Thus, the orientations of rings A of *s*-cis/*s*-trans conformers of chalcone compounds are slightly different. In comparison with **a**, one can see that

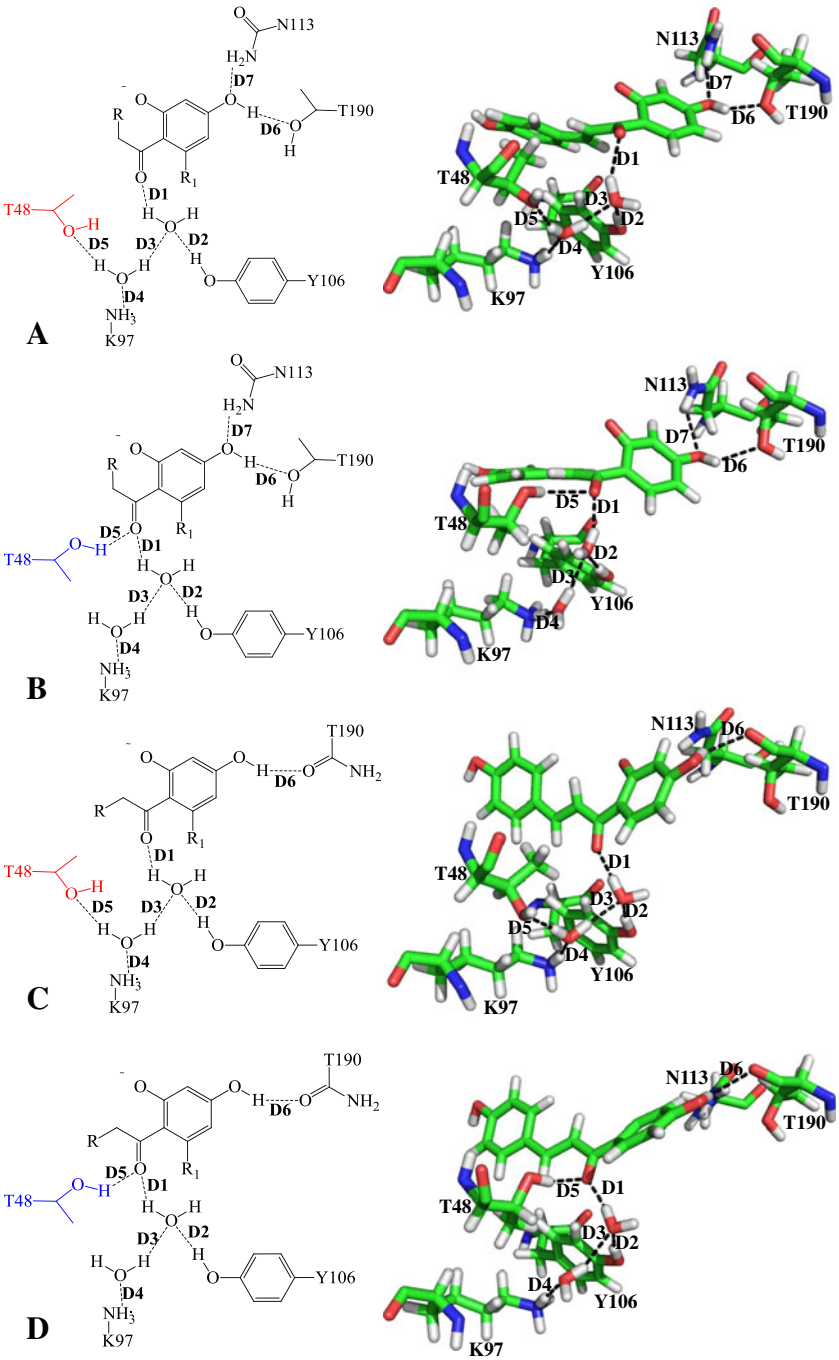


Fig. 2 Two conformations of the CHI active site with chalcone compounds bound within them. **a** *s*-trans conformer of chalcone compounds with CHI(I), **b** *s*-trans conformer of chalcone compounds with CHI(II), **c** *s*-cis conformer of chalcone compounds with CHI(I), **d** *s*-cis conformer of chalcone compounds with CHI(II)

the 6′-hydroxyl group is substituted by a hydrogen atom in **b** and both 4- and 6′-hydroxyl groups are substituted by hydrogen atoms in **c**. The 4- and 6′-hydroxyl groups in **a** are not directly involved in anchoring chalcone compounds as they do not form any hydrogen bond with the active site residues, therefore the loss of these hydroxyl groups in **b** or **c** does not affect the binding position of different chalcone compounds in the active site of CHI. These may explain why CHI shares the binding mode of the different conformations of chalcone compounds. The discussion above conclusively indicates that the rotation of the side chain of T48 residue cannot affect the orientation of the substrate within the active site of CHI. Also, neither the *s*-cis/*s*-trans conformer nor the different substituent groups on the 4- and 6′- positions of chalcone compounds change the orientation of the substrate within the active site of CHI.

Binding free energies The calculated binding free energies for three chalcone compounds with CHI are summarized in Table 1. As listed in Table 1, one can see that the calculated

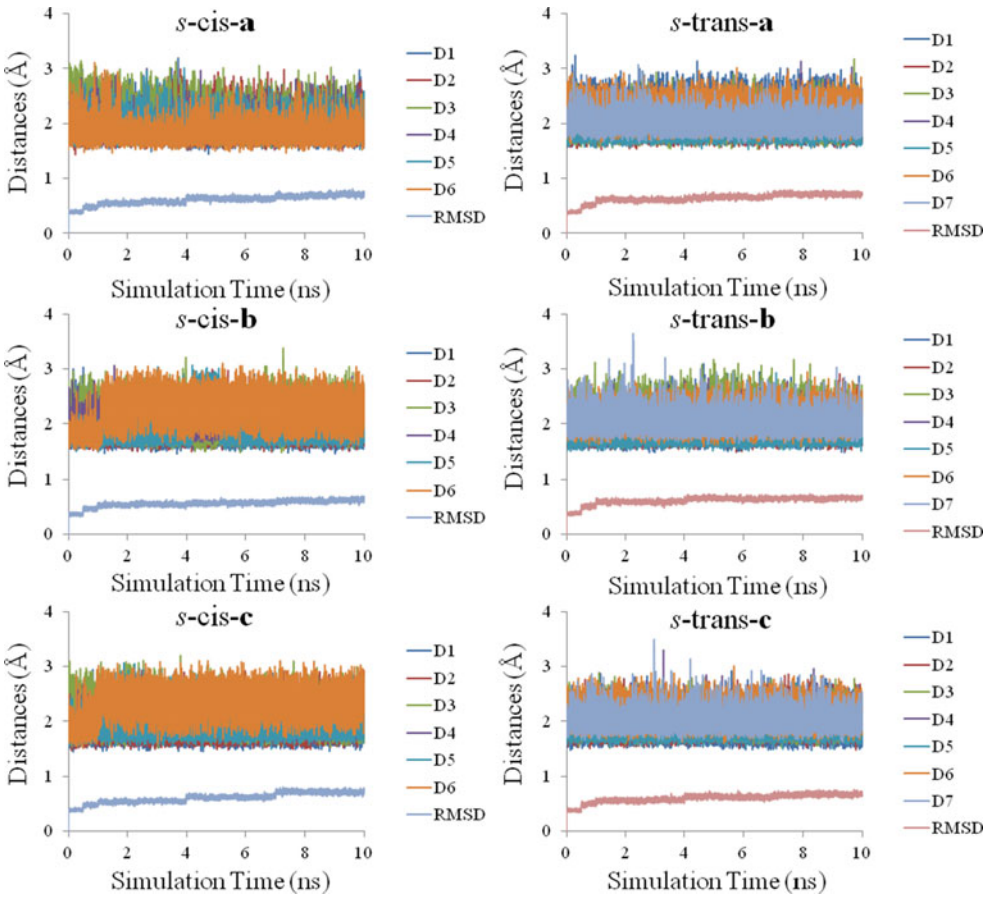


Fig. 3 Plots of MD-simulated internuclear distances and RMSD versus simulation time for CHI(I) with *s*-cis/*s*-trans chalcone compounds

binding free energy for *s*-trans conformers of each chalcone compound is much lower than that for the corresponding *s*-cis conformers with both CHI(**I**) and CHI(**II**), showing that *s*-trans conformer of chalcone compound is a more favorable substrate than *s*-cis conformer. In addition, each chalcone compounds has much lower binding free energy with CHI(**II**) than CHI(**I**). It can be concluded that CHI(**II**)-*s*-trans chalcone compound complex has the lowest binding free energy and the strongest binding affinity. In the case of the binding of CHI(**II**) with *s*-trans conformer, *s*-trans-**b** has the lowest binding free energy with the value of −24.47 kcal mol^{-1}, and *s*-trans-**a** has the highest binding free energy with the value of −20.06 kcal mol^{-1}. Qualitatively, this is in good agreement with the experimental K_m values listed in Table 2. Conclusively, CHI(**II**)-*s*-trans complexes are the most favorable binding mode for CHI with chalcone compounds. In fact, *s*-trans chalcone compounds are the productive substrates of CHI.

Main factors affecting the CHI-chalcone binding To comprehensively understand the binding of chalcone compounds with CHI, it is necessary to reveal the main factors that may affect the stability of the CHI-chalcone complex. First, five conserved residues including T48, K97, Y106, N113 and T190 play the key role in anchoring and orientating chalcone compounds with CHI through two hydrogen bond networks formed through two water molecules. This can reasonably explain the experimental result that the mutations of these five residues cause the remarkable increase of K_m values and the decrease of k_{cat} values of CHI-chalcone complex [10]. Second, in order to analyze the individual contribution to the binding free energies for *s*-cis/*s*-trans conformers of chalcone compounds, the detailed polar and nonpolar contributions are also listed in Table 1. One can see that the primary contribution to the binding free energy comes from the nonpolar interaction, particularly the vdW term. These favorable nonpolar free energy terms are in good agreement with the structural feature of CHI that several hydrophobic residues locate at the binding pocket (Fig. 5). As observed in the CHI-chalcone complex, the substrates are surrounded by the side chains of L38, F47, I51, I93, L101, V197, and L201 residues, causing a high degree of surface complementarities. In contrast, polar contribution is unfavorable for the binding for chalcone compounds with CHI, especially the electrostatic contribution to the solvation free energy.

Comparison between s-cis/s-trans conformers of chalcone compounds As mentioned above, *s*-cis/*s*-trans conformers

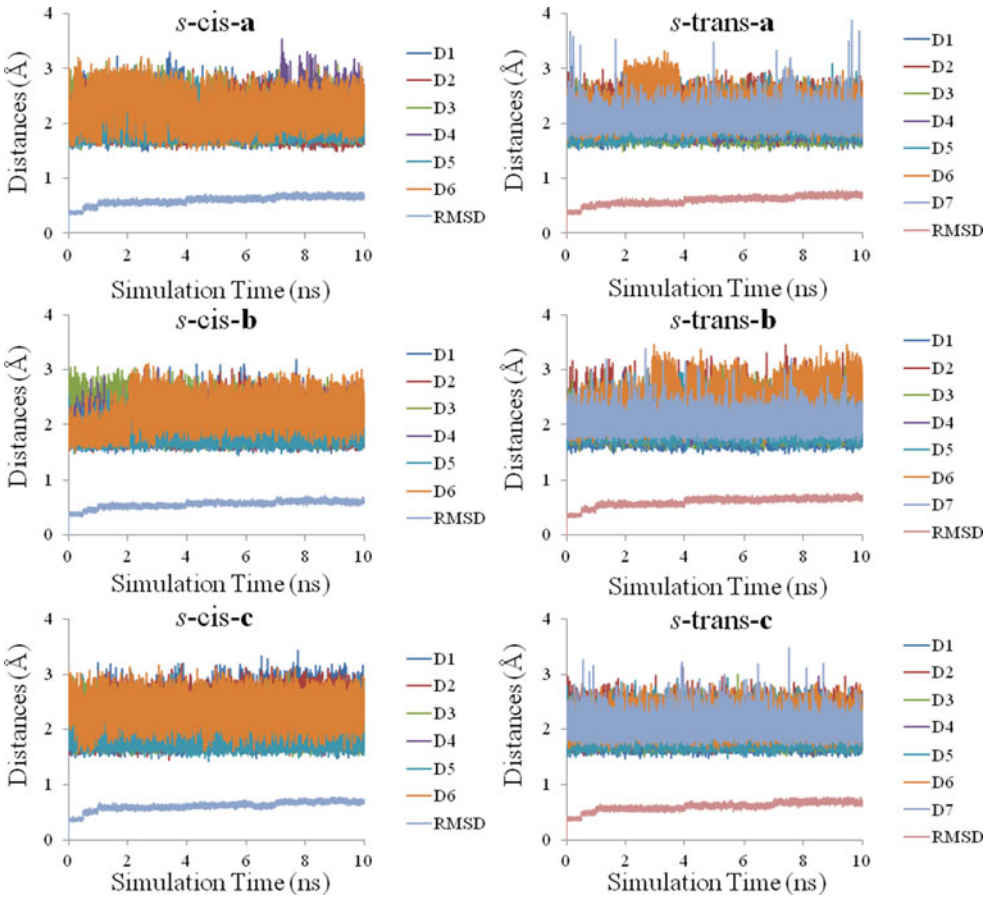


Fig. 4 Plots of MD-simulated internuclear distances and RMSD versus simulation time for CHI(II) with *s*-cis/*s*-trans chalcone compounds

of chalcone compounds share a similar binding mode with CHI. However, as listed in Table 1, it is clear that any *s*-cis conformer of chalcone compounds has much higher binding free energy with both CHI(I) and CHI(II) than the related *s*-trans conformer. Through comparing the binding of two conformers of chalcone compounds, one can see that the main difference between them is the amount of the hydrogen bond between the first hydrogen bond network and the substrate as depicted in Fig. 2. The first hydrogen bond network forms two strong hydrogen bonds with *s*-trans chalcone compounds but only one for *s*-cis chalcone compounds. As we know, the hydrogen bond formed between the enzyme and the substrate plays a very important role in stabilizing the enzyme-substrate complex and improving the binding affinity. Thus, the different first hydrogen bond network should be the main factor to cause the difference in the binding free energies between two conformers of chalcone compounds.

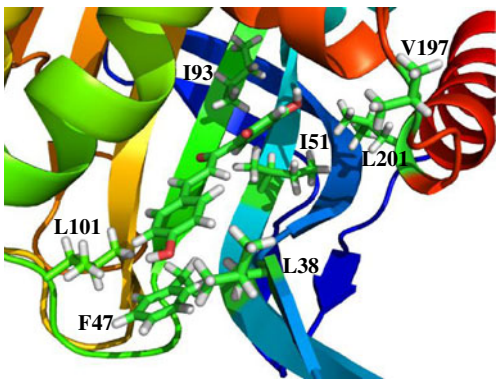


Fig. 5 The hydrophobic residues in the active site

Comparion between CHI(I) and CHI(II) As listed in Table 1, one can see that CHI(II) has much lower binding free energy with any chalcone compound than CHI(I). It is interesting to reveal the remarkable binding affinity difference between CHI(I) and CHI(II) with chalcone compounds. The main difference between CHI(I) and CHI(II) is the conformation of the side chain of T48 residue. As described above, the hydroxyl group in the side chain of T48 residue in CHI(I) forms a hydrogen bond with the second crystal water molecule involving in the second hydrogen bond network, resulting in the interaction with the the ketone group of chalcone, whereas it points toward the ketone group of chalcone and therefore a strong hydrogen bond forms between them in CHI(II). Obviously, this strong hydrogen bond between T48 residue and chalcone compounds in CHI(II) directly enhances the interaction between the ligand and CHI. During the chemical reaction, this hydrogen bond may play an important role in stabilizing the developing negative

Table 1 Calculated binding free energies (all in kcal mol^{-1})

Enzymes	Substrates	ΔE_{ele}	ΔE_{vdW}	ΔG_{PB}	ΔG_{np}	Nonpolar	Polar	ΔG_{bind}
CHI(I)	cis-a	−56.22	−32.58	76.82	−4.60	−37.18	20.60	−16.58
	trans-a	−69.32	−34.61	90.84	−4.48	−39.09	21.52	−17.57
	cis-b	−67.78	−31.38	87.18	−4.51	−35.89	19.40	−16.49
	trans-b	−55.06	−33.34	71.99	−4.34	−37.68	16.93	−20.75
	cis-c	−47.90	−29.41	67.12	−4.45	−33.86	19.22	−14.64
	trans-c	−60.79	−33.10	78.45	−4.35	−37.45	17.66	−19.79
CHI(II)	cis-a	−66.94	−34.61	88.73	−4.41	−39.02	21.79	−17.23
	trans-a	−83.58	−34.59	102.53	−4.42	−39.01	18.95	−20.06
	cis-b	−72.25	−31.60	91.32	−4.48	−36.08	19.07	−17.01
	trans-b	−71.54	−33.69	85.17	−4.41	−38.10	13.63	−24.47
	cis-c	−57.94	−33.56	79.50	−4.41	−37.97	21.56	−16.41
	trans-c	−70.02	−32.86	84.66	−4.34	−37.20	14.64	−22.56

charge on ketone oxygen atom. This may explain the experimental result listed in Table 2 that T48S mutant of CHI has much higher k_{cat} value than that of T48A mutant, as serine residue has a similar hydroxyl group in the side chain as threonine residue. On the other hand, the rotation of the hydroxyl group in the side chain of T48 residue may change the microenvironment of the interface between the ligand and CHI. The evidence to support this proposal is the difference of the calculated polar contributions for CHI listed in Table 1. The polar contribution to the binding for any chalcone compounds with CHI(II) is much lower than that of CHI(I), resulting in the higher binding free energy for CHI(II). Thus, T48 residue may play an important role in enhancing the binding affinity of chalcone compounds with CHI by forming a hydrogen bond with the ketone group.

Insights for future design of high-efficiency CHI mutant The computational results above have demonstrated some important, favorable interaction between CHI and chalcone compounds: (1) the hydrogen bond networks involving five conserved residues T48, K97, Y106, N113 and T190; (2) the conformation of the side chain of T48 residue; (3) the hydrophobic interactions involving several hydrophobic residues in the active site (L38, F47, I51, I93, L101, V197, and L201). It is essential for rational design of CHI mutant with higher efficiency to carefully account for all of these favorable intermolecular interactions. For example, as *s*-trans conformer of chalcone compound is a more favorable substrate for CHI than the unproductive *s*-cis conformer, then a mutation should be designed to increase the binding affinity of CHI with chalcone compounds by enhancing the substrate stereoselectivity of CHI. In CHI-chalcone complexes, the orientations of ring B are different in *s*-cis/*s*-trans conformers of chalcone compounds. Thus, it is promising to introduce a hydrogen bond interaction with 4-hydroxyl group or π-π interaction with ring B of only *s*-trans conformers, rather than *s*-cis conformers of chalcone compounds, resulting that the mutant of CHI only binds *s*-trans conformers of chalcone compounds. In addition, T48 play an important role in enhancing the binding affinity by direct hydrogen bond interaction with the ketone group of chalcone compounds. This hydrogen bond may play an important role in increasing the stabilization of the transition state and improving the catalytic efficiency through stabilizing the developing negative charge on ketone oxygen atom. Therefore a mutation should be designed to keep this special conformation of T48 residue or directly forms a strong hydrogen bond with the ketone group of chalcone compounds.

Conclusions

The combined MD simulations and binding free energy calculations provide valuable insights into the detailed binding of CHI with *s*-cis/*s*-trans conformers of three chalcone

Table 2 Steady-state kinetic constants for wild-type and mutant CHI

	a		b		c	
	K_m (μM)	k_{cat}(min^{-1})	K_m (μM)	k_{cat}(min^{-1})	K_m (μM)	k_{cat}(min^{-1})
CHI	112±28	11180±1380	8.4±2.0	2250±165	22.7±4.2	755±33
T48S	516±341	12900±6500	21.3±4.1	830±86	41.0±4.2	519±63
T48A	182±91	88.6±13	18.9±3.2	1.45±0.11	15.0±2.2	1.07±0.06

All data are from ref [10] and [11]

compounds. The results indicate that *s*-cis/*s*-trans conformers of three chalcone compounds are orientated and stabilized by the different first hydrogen bond network and the same second hydrogen bond network and this binding mode cannot be affected by the conformation of the side chain of T48 residue. The different first hydrogen bond network results in the different binding free energies of *s*-cis/*s*-trans conformers of three chalcone compounds with CHI. The binding affinity between the enzyme and the substrate can also be improved by the direct hydrogen bond interaction between T48 residue in CHI(II) and the substrate. It can be concluded that the binding affinity of CHI(II) with *s*-trans conformer of chalcone compounds are the strongest, showing the remarkable stereoselectivity. The MD-simulated enzyme-substrate binding structures have also revealed important, favorable factors affecting the binding between CHI and chalcone compounds, including (1) the hydrogen bond networks; (2) the variable conformation of the side chain of T48 residue; (3) the hydrophobic interactions involving several hydrophobic residues in the active site (L38, F47, I51, I93, L101, V197, and L201). The results presented here provide a solid base for future rational design of CHI mutant with higher catalytic efficiency.

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