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Interactions of curcumin with the PfATP6 model and the implications for its antimalarial mechanism

Hong-Fang Ji, Liang Shen *

Shandong Provincial Research Center for Bioinformatic Engineering and Technique, Center for Advanced Study, Shandong University of Technology, Zibo 255049, PR China

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ABSTRACT

Despite curcumin has been proved to possess antimalarial effects, the underlying mechanism remains to be elucidated. In this letter, the active site binding modes of curcumin in PfATP6, an important antimalarial target, were investigated using computational docking. It was revealed that curcumin interacts with PfATP6 mainly through hydrophobic interactions and hydrogen bonds. Moreover, the theoretically predicted binding affinity implies that curcumin can efficiently inhibit PfATP6, which gains some deeper insights into the antimalarial mechanism of curcumin.

As an important natural phytochemical found in the rhizomes of *Curcuma longa* or turmeric, curcumin (Fig. 1) has attracted considerable attention in recent years due to its remarkable pharmacological effects, including antioxidant, antitumor, and antiinflammatory activities.[1–5] As we know, despite much effort has been devoted to combat malaria in the past few decades, it remains a major public health concern worldwide. So, it is interesting to find that curcumin possesses antimalarial effects in *Plasmodium falciparum* culture and *Plasmodium berghei*-infected mice.[6,7] Recently, PfATP6, the parasite orthologue of mammalian sarcoplasmic–endoplasmic reticulum Ca^{2+}–ATPase (SERCA), has been proved to be the molecular target of artemisinins, which are the most potent antimalarials available.[8] However, the curcumin's antimalarial mechanism underlying needs to be elucidated. In the present study, through docking simulation study, the active site binding modes of curcumin with PfATP6 were investigated and the theoretically predicted binding affinity implies that curcumin can inhibit PfATP6, which may be involved in the curcumin's antimalarial mechanism.

As shown in Figure 1, curcumin is unique for possessing two isomers, the keto and enol forms, both are considered during the docking calculations.

A BLAST search in the protein data bank (PDB)[9] with PfATP6 sequence (the amino acid sequence of PfATP6 was obtained from the official database of the malaria parasite genome project (PlasmoDB)) revealed 43.5% identity with a sarco/endoplasmic reticulum Ca^{2+}–ATPase(SERCA) [Swissprot sequence: AT2A1_RABIT (P04191)]. As there is no structure of PfATP6 in PDB, structure coordinates for SERCA in PDB file 1IWO[8] was used to construct the PfATP6 model by employing the homology modelling module of Insight II software.[10] Firstly, hydrogens were added to the constructed model at pH 7.0 by employing the biopolymer module of Insight II software. Then, molecular dynamics (MD) equilibration was performed with the consistent-valence force field (CVFF)[11–13] on a SGI origin 350 server. The model was minimized by 1000 conjugate gradient steps for equilibration, heated from 2 K to 300 K during 35 ps at temperature increment of 50 K per 5 ps, then the constant temperature and pressure algorithm was applied at 300 K for 200 ps. The velocity verlet integrator was used with an integration step of 2 fs. Moreover, the feasibility of modelled structure (Fig. 2) of PfATP6 was evaluated by Verify3D, which calculated structural compatibility scores based on 3D–1D profiles. The predicted structure of PfATP6 had an acceptable 3D–1D self-compatibility score, beyond the incorrect fold score threshold.[14] Moreover, the root–mean–square deviation (RMSD) of the Cα coordinates between 1IWO and the PfATP6 model is 1.9 Å as calculated by the corresponding aligned residues, which also verifies the accuracy of the modelled structure.

Considering the fact that the structure of 1IWO contains the highly specific inhibitor thapsigargin, which maintains the same spatial coordinates in PfATP6 and SERCA,[8] we constructed the binding site of PfATP6 by using thapsigargin as reference ligand. Standard parameters of the program FlexX,[15] as implemented in the molecular modelling software SYBYL 7.0,[16] were used to explore the binding modes of curcumin with PfATP6. The Ludi module of Insight II was used to estimate the binding affinities. The Ludi score

* Corresponding author. Tel./fax: +86 533 278 0271.
E-mail address: shen@sdut.edu.cn (L. Shen).

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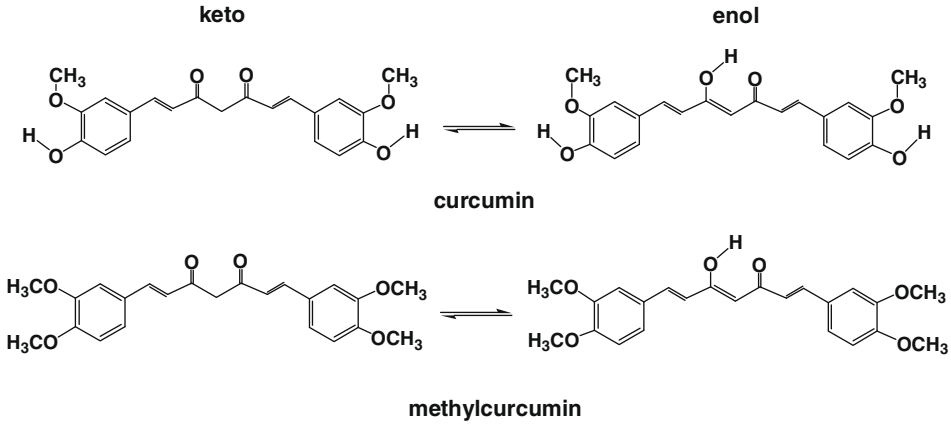

Figure 1. Chemical structures of the keto and enol forms of curcumin and methylcurcumin.

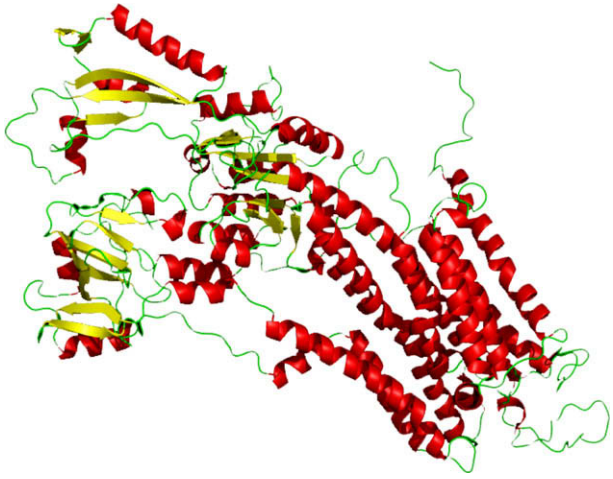

Figure 2. Cartoon structure of PfATP6 generated using PyMol (http://www.pymol.org). Red: α-helix; yellow: β-sheet; green: random coil.

derived by the program is empirically related to the dissociation constant K_d: Ludi score = $-100 \log K_d$.

The docking conformations of the keto and enol forms of curcumin in the active site of PfATP6 with all interacting residues are depicted in Figure 3. It can be seen that the feruloyl moieties are twisted relative to each other for both the keto and enol forms of curcumin. The binding pocket is defined by those residues that have at least one atom with a distance 5 Å from a heavy atom of curcumin. According to Figure 3a, the binding pocket for the keto form of curcumin to PfATP6 consists of eleven residues, that is, Leu263, Phe264, Gln267, Ile271, Ile977, Ile981, Val984, Ala985, Asn1039, Leu1040 and Ile1041. In comparison, nine residues, that is, Leu263, Phe264, Gln267, Leu268, Ile271, Ile977, Ile981, Leu1040 and Ile1041 are involved in the interactions of the enol form of curcumin with the protein (Fig. 3b). Among the two groups of binding residues eight are same, which suggests that the keto and enol forms of curcumin share a common binding region. Moreover, it can be seen that most of the residues in the two binding cavities are hydrophobic (Fig. 3). In view of the hydrophobic feature of curcumin, it can be inferred that the hydrophobic interactions should play important roles in the binding of curcumin with PfATP6. Hydrophobic interactions have also been proposed as the main driving forces for the binding of artemisinin derivatives to PfATP6.[17]

In addition, curcumin possesses the phenolic hydroxyl and keto-enol moiety, which tend to form hydrogen bond with surrounding residues. Therefore, the hydrogen bonds also contribute to the binding of curcumin to PfATP6. Shown in Figure 4 is a close view of the residues involved in forming hydrogen bonds between PfATP6 and the keto and enol forms of curcumin. In the complex of PfATP6 with the keto form of curcumin, four residues are involved in the hydrogen bond formation between the ligand and protein, Gln267 is with the keto oxygen, Leu1040 and Ile1041 with one of the phenolic oxygens and Ala985 with the other phenolic oxygen (Fig. 4a). In comparison, because of the rotation of the phenyl ring, the hydrogen bond between one of the phenolic oxygen and Ala985 is absent in the complex of PfATP6 with the enol form of curcumin (Fig. 4b).

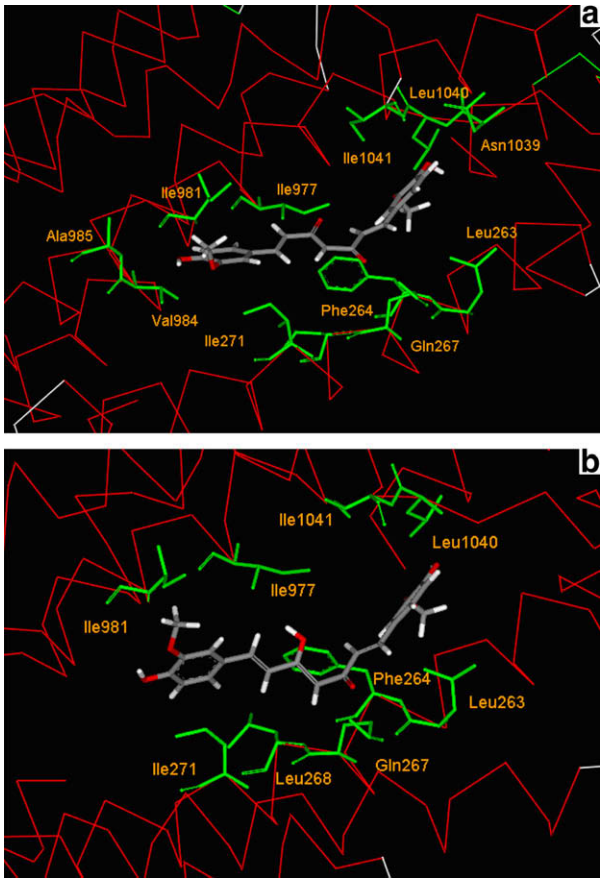

Figure 3. The bound conformation of curcumin in the active site of PfATP6 with all interacting residues shown as stick models. (a) keto form; (b) enol form.

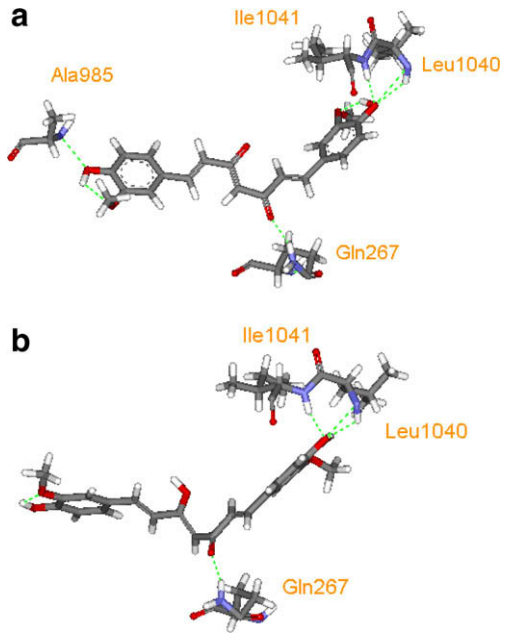

Figure 4. Close-up views of hydrogen bonds (marked in green dotted lines) formed between PfATP6 and the keto form (a) and enol form (b) of curcumin.

To estimate the binding affinity of curcumin to PfATP6, the Ludi scores for the PfATP6/curcumin complexes were calculated, which is 594 for the keto form and 561 for the enol form of curcumin. Then, according to the equation: Ludi score = $-100 \log K_d$, the binding affinity is estimated to be 1.15 μM and 2.45 μM for the keto and enol forms of curcumin with PfATP6, respectively. The theoretical binding affinity suggests that curcumin can be considered as an effective inhibitor of PfATP6. It has been reported that artemisinin inhibits PfATP6 with a K_d value of ~150 nM.[8] Therefore, the activity of curcumin in inhibiting PfATP6 should be relatively weaker relative to artemisinin. This is in good agreement with the experimental results that the IC_{50}s for artemisinin and curcumin are 45–50 nM and 15–18 μM in *P. falciparum* culture, respectively.[7] Moreover, previous study revealed that curcumin can inhibit mammalian SERCA.[18] Thus, it is rational to infer that curcumin and artemisinin possess similar antimalarial mechanism.

In addition, the phenolic hydroxyls are important for the binding of curcumin to PfATP6 as shown in Figs. 3 and 4 and their chemical modifications may affect the binding ability of curcumin. Thus, we also performed parallel calculations on the methylcurcumin, in which, the hydroxyl groups are replaced by $-OCH_3$ groups (Fig. 1). As is expected, the Ludi scores decreased to 528 and 504 for complexes of PfATP6 with the keto and enol forms of methylcurcumin.

In summary, the interactions of curcumin in PfATP6 were investigated by molecular docking. The effective inhibition of curcumin to PfATP6 as characterized by the theoretical binding ability provides some new clues to the antimalarial mechanisms of curcumin.

Acknowledgments

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