Arch Pharm Res Vol 29, No 1, 50-58, 2006

Archives of Pharmacal Research
http://apr.psk.or.kr

Homology Modeling and Molecular Docking Study of Translationally Controlled Tumor Protein and Artemisinin

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(Received October 4, 2005)

Translationally controlled tumor protein (TCTP), also known as histamine releasing factor (HRF), is found abundantly in different eukaryotic cell types. The sequence homology of TCTP between different species is very high, belonging to the MSS4/DSS4 superfamily of proteins. TCTP is involved in both cell growth and human late allergy reaction, as well as having a calcium binding property; however, its primary biological functions remain to be clearly elucidated. In regard to many possible functions, the TCTP of *Plasmodium falciparum (Pf)* is known to bind with an antimalarial agent, artemisinin, which is activated by heme. It is assumed that the endoperoxide-bridge of artemisinin is opened up by heme to form a free radical, which then eventually alkylates, probably to the Cys14 of *Pf*TCTP. Study of the docking of artemisinin with heme, and subsequently with *Pf*TCTP, was carried out to verify the above hypothesis on the basis of structural interactions. The three dimensional (3D) structure of *Pf*TCTP was built by homology modeling, using the NMR structure of the TCTP of *Schizosaccharomyces pombe* as a template. The quality of the model was examined based on its secondary structure and biological function, as well as with the use of structure evaluating programs. The interactions between artemisinin, heme and *Pf*TCTP were then studied using the docking program, FlexiDock. The center of the peroxide bond of artemisinin and the Fe of heme were docked within a short distance of 2.6Å, implying the strong possibility of an interaction between the two molecules, as proposed. When the activated form of artemisinin was docked on the *Pf*TCTP, the C4-radical of the drug faced towards the sulfur of Cys14 within a distance of 2.48Å, again suggesting the possibility of alkylation having occurred. These results confirm the proposed mechanism of the antimalarial effect of artemisinin, which will provide a reliable method for establishing the mechanism of its biological activity using a molecular modeling study.

Key words: *P. falciparum* TCTP, artemisinin, heme, homology modeling, docking

INTRODUCTION

In 1998, a report accounted for over a million deaths each year worldwide due to malaria, with 273 million cases. The most dangerous malarial parasite, *Plasmodium falciparum,* causes cerebral malaria (El Sayed *et al.*, 2001). A promising class of compounds for the treatment of multidrug resistant *P. falciparum* is artemisinin and its derivatives. Artemisinin is a natural sesquiterpene endoperoxide isolated from *Artemisia annua*, which appears to be selectively toxic to malaria parasites by interacting with heme, a byproduct of hemoglobin digestion, which is present at high levels in the parasite (Bhisutthibhan *et al.*, 1998). Artemisinin derivatives appear to act by a two-step mechanism; first, intraparasitic heme and/or free iron catalyze the decomposition of the endoperoxide-bridge to form the O-radical of artemisinin, which then eventually forms carbon-centered free radicals due to hydrogen deletion. Second, these free radicals act as alkylating agents, reacting with both intraparasitic heme and proteins (Bhisutthibhan and Meshnick, 2001).

TCTP, also known as histamine releasing factor (HRF), is a highly conserved, abundantly expressed family of eukaryotic protein, which has been implicated in both cell growth and human acute allergic responses, but whose intracellular biochemical function remains elusive (Bhisutthibhan *et al.*, 1998; Bommer and Thiele, 2004). Since the discovery of TCTP, it has been reported as being implicated with extracellular histamine releasing (MacDonald *et al.*, 1995), as having a relationship with

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cytoplasmic microtubule (Bommer and Thiele, 2004), upregulation by the earthworm in a heavy metal pollution state (Sturzenbaum *et al.*, 1998), the possibility of calcium binding (Bommer and Thiele, 2004) and as having a relationship with neurodegenerative disorders, such as Alzheimer's disease and Down syndrome (Kim *et al.*, 2001). Meshnick's group identified a target protein of artemisinin, a translationally controlled tumor protein (TCTP) homolog, from the most heavily labeled proteins from *P. falciparum* homogenates. They found, *in vitro*, that hemin binds to the recombinant *Pf*TCTP, and that in the presence of hemin, [^{3}H]dihydroartemisinin, reacts covalently with recombinant TCTP. The group also suggested that cysteine may play an important role in the binding of artemisinin with the protein, as artemisinin binding was decreased when cysteine residue of *Pf*TCTP was blocked by iodoacetamide (Bhisutthibhan *et al.*, 1998).

The interaction with heme should be preceded in order for artemisinin to bind with TCTP. In infected erythrocytes, 70% of the hemoglobin is digested by parasites, with the release of the heme. As heme shows toxicity against parasite, it forms a hemazoin as a detoxification agent as its defensive mechanism. Artemisinin reacts with the released heme; thereby, interfering with the production of hemazoin, allowing maintenance of the toxic heme to kill the parasites (Pandey *et al.*, 1999). Nevertheless, the more important antimalarial mechanism of artemisinin is the opening of the internal peroxide ring of artemisinin to induce the production of a free radical for the alkylation of biomolecules, such as heme or TCTP (Olliaro *et al.*, 2001) (Fig. 1). The activated artemisinin can bind to heme, but when a protein is present, it will bind primarily to the protein (Yang *et al.*, 1994).

The importance of a free radical was proved experimentally by the discovery that a free radical scavenger acts as an antagonist of artemisinin, and the inhibitory activity increases due to a different free radical generator. Therefore, a radical formed by the reaction with heme is reported to be closely related with the antimalarial activity (Paitayatat *et al.*, 1997). When the production of C4-radical is blocked, the antimalarial activity is greatly decreased, supporting the important role of C4-radical in the activity. However, there is no direct evidence that the alkylation of TCTP by artemisinin occurs for the antimalarial activity of the drug (Bhisutthibhan and Meshnick, 2001).

How does artemisinin react with heme and bind to *Pf*TCTP? In this study, to gain a better understanding of the interactions between these molecules, a homology modeling technique was used to generate the unknown 3D structure of *Pf*TCTP using the MODELLER program (Sali and Blundell, 1993). A docking experiment was then carried out with FlexiDock to demonstrate the activation of artemisinin by heme and due to the interaction of *Pf*TCTP with the C4-radical form of artemisinin induced by heme. The results of this work will provide important information on the mechanism of the antimalarial activity of artemisinin.

METHODS

Software and computer system

All programs were executed on a Silicon Graphics Octane Workstation, using the following programs: 1)

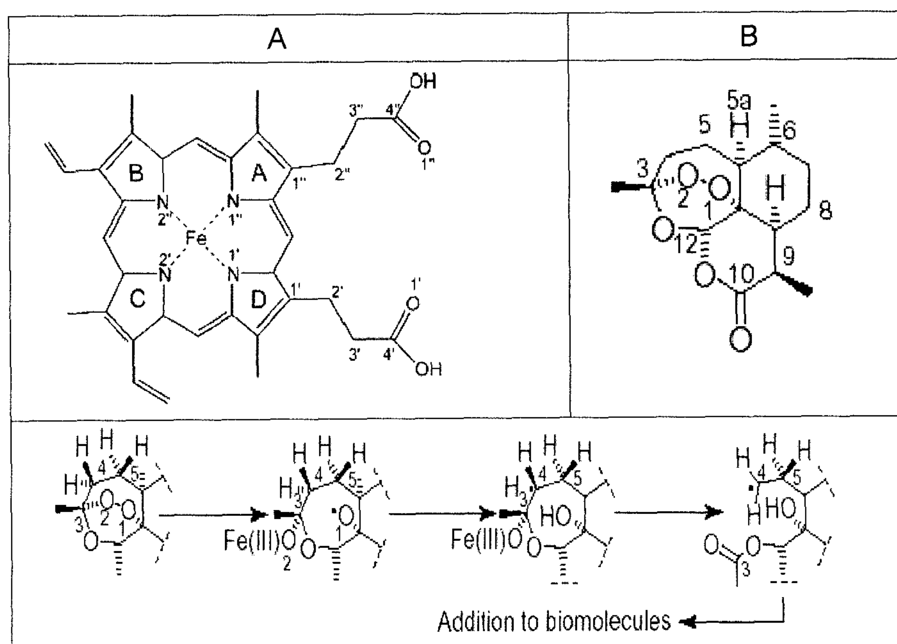

Fig. 1. Proposed reductive scission of the peroxide bond and formation of C-centered radicals. A: heme, B: artemisinin

protein structure modeling program, MODELLER 7v7 (Sali and Blundell, 1993), 2) genetic algorithm-based flexible docking program, FlexiDock in SYBYL 6.9. (Tripos Inc., St. Louis, MO), and 3) structure evaluation programs, PROCHECK (Laskowski *et al.*, 1993) and PROSAII (Sippl, 1993). Other programs for searching the template, sequence analysis or the secondary structure information were used from the Internet.

Sequence analysis and prediction of secondary structures

The sequence of *Pf*TCTP was obtained from Entrez_ Protein of NCBI (Devlin *et al.*, 2002; accession number NP_703454). PSI_BLAST was used to identify homologous proteins against the non-redundant sequence database at NCBI (Altschul *et al.*, 1997). In order to find a template for the homology modeling using the MODELLER program, in addition to PSI_BLAST, the web-available threading method, 3D-PSSM, was also used. It is a method based on matching the predicted secondary structure and solvent accessibility or solvation potentials of the query sequence with those of proteins of known structures (Kelley *et al.*, 2000).

Several secondary structure prediction methods, such as PHD (Rost, 1996), PROSITE (Falquet *et al.*, 2002), and Multicoil network server programs (Wolf *et al.*, 1997), were also employed. PHD predicts one-dimensional protein structures using profile-based neural networks. PROSITE is a database that contains protein families and domains with more than 1,000 proteins. With this program, the important profiles and patterns can be searched to identify to which known protein family (if any) the *Pf*TCTP sequence belongs. The Multicoil server program was used to detect the coil-coil region based on the recent report of the possibility of TCTP protein being a dimer or oligomer. Also, Discrimination of the protein Secondary structure Class (DSC) was used, which predicts the secondary structure by representing each amino acid of a homologous sequence multiple alignment, with more than 70%, as having either a helix, strand or loop form (King and Sternberg, 1996).

Homology modeling and structure validation

A putative 3D structure of *Pf*TCTP was built using the protein structure-modeling program, MODELLER 7v7 (Sali and Blundell, 1993). The sequence alignment between *Pf*TCTP and the template, *Schizosaccharomyces pombe (Sp)* TCTP, was derived using MODELLER after the alignment input file had been manually modified based on the multiple alignment result of 3D-PSSM. A total of five models were built, and their qualities examined with PROSAII and PROCHECK. The PROSAII test was applied to check the energy criteria in comparison with the potential of mean force derived from a large set of known protein structures (Sippl, 1993). The model was stereochemically evaluated using the program, PROCHECK (Laskowski *et al.*, 1993). Through the inspection of the Psi/Phi angles of a Ramachandran plot obtained from this analysis, the backbone conformation of the model was evaluated.

Docking of heme and artemisinin

In order to study the interactions between TCTP, heme and artemisinin, the hemoglobin structure (PDB ID: 1a00) obtained from PDB was selected, and the heme extracted. Since the parameter for a Fe^{2+} ion has not been completely parameterized in SYBYL, this was replaced with Ca^{2+}, which has similar properties to Fe^{2+} (Cheng *et al.*, 2002). Other atom types were checked, and hydrogen atoms added. The energy of this structure was fully minimized by the Tripos force field, using the Gasteiger-Hückel charge and Powell method, until the conjugate gradient reached 0.1 $kcal \cdot mol^{-1} \cdot Å^{-1}$. The rest of the parameters were default values. The structure of artemisinin was constructed using standard geometric parameters within SYBYL, with the energy minimization performed as for the heme structure. A random conformational search was then performed to obtain a thermally stable conformation. The endoperoxide bond of artemisinin was manually prepositioned above the Ca atom, with the distance set to 3Å, based on the experimental data that a Fe atom interacts with artemisinin within this distance range (Olliaro *et al.*, 2001; Cheng *et al.*, 2002). Flexible docking was performed by setting all the rotational bonds in both artemisinin and heme as variables. The default parameters within FlexiDock were set, and the iteration number to 50,000. FlexiDock scored all the orientations and calculated the binding energy for each orientation; thereby, generating the top 20 conformations. A low energy structure with good binding mode was selected from among these, which was used as the final result. The distance between Ca^{2+} and the center of the endoperoxide bond of artemisinin was then measured.

Docking of artemisinin into *Pf*TCTP

The atom types of the putative 3D structure of *Pf*TCTP were checked and hydrogen atoms added to the model. As Cys14 has been reported to be important in the reaction of artemisinin and the protein (Bhisutthibhan *et al.*, 1998), the area around the residue was minimized, using Kollman All charges and Tripos force field, until the conjugate gradient reached 0.1 $kcal \cdot mol^{-1} \cdot Å^{-1}$. The rest of the parameters used were default values.

In the reaction between artemisinin and heme, the endoperoxide ring of artemisinin opens, eventually becoming a C4-radical, which alkylates biomolecules (Olliaro *et al.*, 2001). Based on this theory, the ligand was constructed

by removing hydrogen from the C4 to produce a radical form. Since the radical representation was incomplete in SYBYL, the MOPAC program (Quantum Chemistry Program Exchange, Indiana University, IN) was used to represent the radical type charge around C4 (Stewart, 2002). The structure of modified artemisinin was energy minimized, which was used as a ligand for docking by positioning the C4 radical part to face towards the sulfur atom of the Cys14. Flexible docking experiments were then carried out, with the binding area and ligand set as fully flexible, using the default parameters and iteration of 50,000. The best docked conformation was selected based on the energy and binding mode.

RESULTS

Sequence analysis of *Pf*TCTP

The amino acid sequence of TCTP is highly conserved among the various species, including that of *P. falciparum*. A total of 171 amino acids in the sequence of *Pf*TCTP had 32% identity and 56% similarity with the sequence of human TCTP, and 36% identity and 56% similarity with the sequence of *Sp*TCTP. The *Pf*TCTP contains a single cysteine at position 14, with this residue appearing to be necessary for the reaction between dihydroartemisinin and TCTP (Bhisutthibhan *et al.*, 1998). The 24 full length TCTP sequences currently available in the NCBI databases (Thaw *et al.*, 2001) present the residue 14 as being hydrophobic, with the exception of *C. elegans* and *P. falciparum*, where the residues 14 were Ser and Cys, respectively.

The pattern and profile of the *Pf*TCTP sequence was examined through the PROSITE database, and appears to have six phosphorylation sites; three sites for Protein kinase C phosphorylation and three for Casein kinase II phosphorylation. Among the various species, the most conserved TCTP signatures are TCTP 1 and TCTP 2 (Bommer and Thiele, 2004). The consensus patterns for TCTP 1 and TCTP 2 are [IFAE]-[GA]-[GAS]-N-[PAK]-S-

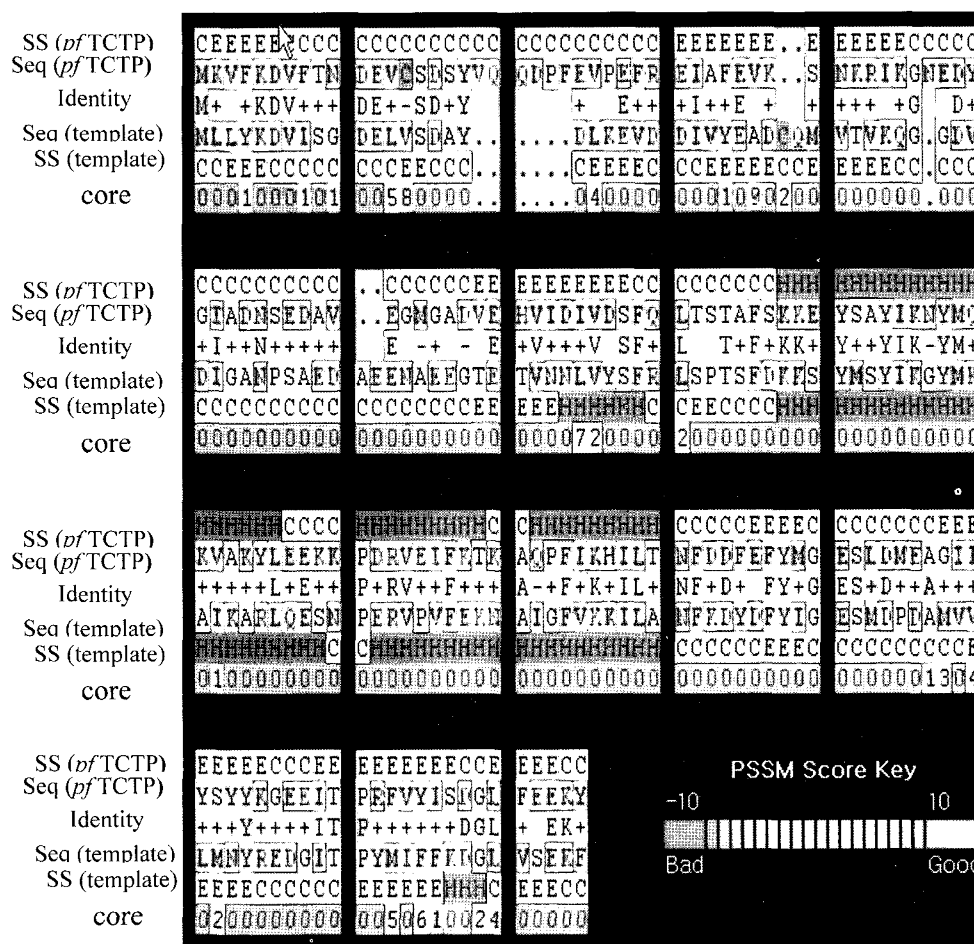

Fig. 2. Sequence alignment of *P. falciparum* and *S. pombe* (template) TCTPs. The secondary structures, and the coding scores, of *P. falciparum* TCTP and the template are displayed. The PSSM Score Key at the bottom right illustrates the spectrum used. The third line, the identity, presents the positive and negative scores for each sequence equivalence, with the sixth line, the core, showing the hydrophobic interaction score. *pf*TCTP: *P. falciparum* TCTP, SS: secondary structure, Seq: sequence, C: coil, E: sheet, H:helix

[GTA]-E-[GDEV]-[PAGEQV]-[DEQGAV] and [FLIV]-x(4)-[FLVH]-[FY]-[MIVCT]-G-E-x(4,7)-[DENP]-[GAST]-x-[LIVM]-[GAVI]-x(3)-[FYWQ], respectively. Most of the eukaryotic TCTPs have TCTP 1 and 2 signatures, with the mobile loop belonging to TCTP 1 (Thaw *et al.*, 2001). However, the residues of TCTP 1 in *Pf*TCTP have a low conservation; hence, was not detected in the PROSITE database. *Pf*TCTP expresses only TCTP 2, corresponding to residues 128-150 of the protein, which represented β-sheets.

The coiled coil region is an important part of a protein, as it constitutes either a dimer or an oligomer by self-interaction (Wolf *et al.*, 1997). The Multicoil server showed that amino acids from 75 to 106 of *Pf*TCTP have the possibility of forming a three-stranded coiled coil. Thus, this presents the tendency for *Pf*TCTP to exist in a multimeric form by self interaction, similarly to the TCTPs of many other species (Gnanasekar *et al.*, 2002).

Three dimensional structure of *Pf*TCTP

The 3D structure of *Pf*TCTP was constructed using the homology modeling program, MODELLER. In order to reduce the errors of the input file of multiple sequence alignment between *Sp*TCTP and *Pf*TCTP, the program was modified according to the data from the 3D-PSSM multiple alignment (Fig. 2). The NMR structure of *Sp*TCTP (PDB ID: 1h6q) was selected as a template, as this sequence homology to that of the target was the highest, not only in PSI-BLAST search, but in the 3D-PSSM search also. Based on the atomic coordination of *Sp*TCTP, five putative structures of *Pf*TCTP were obtained. The degree of restraint violations of a model was determined using a MODELLER objective function (Sali and Blundell, 1993), which combined the spatial restraints and CHARMM energy terms, for which Model 5 showed the lowest value (Table I). Also, the degree of heavy violation of the important residue, Cys14, was 0.34 in model 5, which was the lowest among the five models.

Model 5 was comprised of four β-sheets, designated A to D, with two main α-helices, H2-H3, and a flexible loop (Fig. 3 and Table II). The coil between sheets C and D may be a small helix. Residues Ser83-Lys106 of *Pf*TCTP were composed of α-helix H2, which is exposed outward, and according to the Multicoil server is suggested to be a coiled-coil region. The globular core was formed by the β-sheets of A, B and D, with the coils facing each other. The distal ends of the two-stranded sheet C protruding from the core globular structure anchored the mobile loop of Lys43-Glu66. The helices H2 and H3 faced toward each other, forming a side by side α-helical hairpin structure. Two hydrophobic cores, termed cores 1 and 2, were packed against the two faces of β-sheet A (Thaw *et al.*, 2001). Cys14 was located on β-sheet B, and was part of the hydrophobic core 1.

Table I. The value of objective function of models by MODELLER

Model	1	2	3	4	5
Objective function	1295.7	1248.3	1107.2	1031.3	891.4

Evaluation of the model

To select the best model, the secondary structures of the 5 models were compared with respect to the PHD data. The results from the secondary structure prediction program coincided with those of model 5 in the helical area of Glu86-Asn127 and most of the β-sheets and coils. For further evaluation of the model, the pseudo-potential energies were calculated using PROSAII. In a graph, a good quality model shows a negative energy value, and gives a similar pattern to that of the template (Sippl, 1993), while the non-near native fold shows a high positive energy value. In our results, the plots of model 5 and that of the template presented very similar patterns, with negative energy values (Fig. 4), indicative of a good quality model.

The backbone conformation of model 5 was examined using the PROCHECK program. The Psi/Phi torsion angles of the backbone conformation of each residue were represented by a Ramachandran plot (Fig. 5), which showed 79.5, 18.6 and 1.9% of residues to be in the most favored, allowed and disallowed regions, respectively. The overall conformation of the backbone was in good agreement with the stereochemistry, which was also found to be reliable.

The existence of heme is very important in the binding of artemisinin with the protein. In different species of TCTPs, the fact that aromatic amino acids, such as Phe or Tyr, are well conserved is especially noteworthy, as the pyrrole ring of the heme, as well as the residues with an aromatic ring, could have pi-pi interactions (Bhisutthibhan *et al.*, 1998). The MODELLER model was well constructed with regard to the cavity around Cys14 being surrounded by many aromatic residues: Phe4, Tyr18, Phe34, Phe75, and Tyr161. Summing up the evaluations of the secondary structures and information on biological functions, along with the results from PROSAII and PROCHECK, model 5 obtained from the MODELLER was selected as the most appropriate 3D structure of *Pf*TCTP (Gerczei *et al.*, 2000).

Docking of artemisinin onto heme

To confirm the activation of artemisinin by heme, a docking study was carried out using heme as the receptor and artemisinin as the ligand. The result showed that the two oxygen atoms of the endoperoxide ring of artemisinin were situated directly above the Fe atom, and the drug molecule was docked parallel to the planar ring of the heme (Fig. 6). The distance between the center of the endoperoxide ring and the Ca^{2+} ion was 2.6Å, which was an appropriate distance for the Fe^{2+} ion to react with the

Table II. Secondary structure of Model 5 by MODELLER

Name[a]	Sec[b]	Name	Sec	Name	Sec	Name	Sec	Name	Sec	Name	Sec	Name	Sec
MET1	C	VAL26	S	ALA51	C	GLN76	C	TYR101	H	THR126	C	LYS151	C
LYS2	C	PRO27	S	ASP52	C	LEU77	C	LEU102	H	ASN127	C	GLY152	C
VAL3	S	GLU28	S	ASN53	C	THR78	S	GLU103	H	PHE128	C	GLU153	C
PHE4	S	PHE29	S	SER54	C	SER79	S	GLU104	H	ASP129	C	GLU154	C
LYS5	S	ARG30	C	GLU55	C	THR80	C	LYS105	C	ASP130	C	ILE155	C
ASP6	C	GLU31	C	ASP56	C	ALA81	C	LYS106	C	PHE131	C	THR156	C
VAL7	C	ILE32	C	ALA57	C	PHE82	C	PRO107	C	GLU132	C	PRO157	S
PHE8	C	ALA33	S	VAL58	C	SER83	C	ASP108	C	PHE133	C	ARG158	S
THR9	C	PHE34	S	GLU59	C	LYS84	C	ARG109	C	TYR134	S	PHE159	S
ASN10	C	GLU35	S	GLY60	C	LYS85	H	VAL110	C	MET135	S	VAL160	S
ASP11	C	VAL36	S	MET61	C	GLU86	H	GLU111	H	GLY136	C	TYR161	C
GLU12	C	LYS37	S	GLY62	C	TYR87	H	ILE112	H	GLU137	C	ILE162	C
VAL13	C	SER38	C	ALA63	C	SER88	H	PHE113	H	SER138	C	SER163	C
CYS14	S	ASN39	C	ASP64	C	ALA89	H	LYS114	H	LEU139	C	ASP164	C
SER15	S	LYS40	S	VAL65	C	TYR90	H	THR115	H	ASP140	C	GLY165	C
ASP16	C	ARG41	S	GLU66	C	ILE91	H	LYS116	H	MET141	C	LEU166	C
SER17	C	ILE42	S	HIS67	S	LYS92	H	ALA117	H	GLU142	C	PHE167	S
TYR18	C	LYS43	C	VAL68	S	ASN93	H	GLN118	H	ALA143	C	GLU168	S
VAL19	C	GLY44	C	ILE69	S	TYR94	H	PRO119	H	GLY144	C	GLU169	S
GLN20	C	ASN45	C	ASP70	C	MET95	H	PHE120	C	ILE145	C	LYS170	C
GLN21	C	GLU46	C	ILE71	C	GLN96	H	ILE121	C	ILE146	C	TYR171	C
ASP22	C	ASP47	C	VAL72	C	LYS97	H	LYS122	C	TYR147	S		
PRO23	C	TYR48	C	ASP73	C	VAL98	H	HIS123	C	SER148	S		
PHE24	C	GLY49	C	SER74	C	ALA99	H	ILE124	C	TYR149	C		
GLU25	C	ILE50	C	PHE75	C	LYS100	H	LEU125	C	TYR150	C		

[a]Residue, [b]Secondary structure, C: Coil, H: Helix, S: Sheet

endoperoxide ring. The two carboxyl ethyl groups in heme avoided steric hindrance by being prepositioned perpendicular to and below the porphyrin plane. In addition, the methyl group of the endoperoxide ring interacted with one of imidazole rings of the heme through a cation-π-like interaction (Cheng *et al.*, 2002). The binding modes obtained illustrate how the activation of artemisinin occurs *via* the Fe of the heme.

Docking of activated artemisinin and *Pf*TCTP

Due to the activation, the endoperoxide-bridge of artemisinin is broken, forming an O-centered radical. The C-centered radical is then formed at the C4 position by the removal of hydrogen from within a molecule, which is supposed to alkylate biomolecules, such as TCTP (Olliaro *et al.*, 2001). Docking simulations were performed to verify the alkylation of *Pf*TCTP by the C4-radical form of artemisinin. The radical was docked near the sulfur atom of Cys14, at a distance of 2.48Å (Fig. 7), which again, was within a plausible distance for the reaction. This result strongly supports the hypothesis that alkylation is able to occur between the two molecules.

Artemisinin forms two hydrogen bonds with Tyr18 and Ser15; the carbonyl oxygen atom of δ-lactone with Tyr18 and the oxygen atom in benzodioxepine with Ser15 (Fig. 8). Also, the hydrophobic portion of artemisinin showed interactions with Phe4, Val26, Val36, Ile71, and Phe75; thus, enabling the stability of artemisinin within the cavity. The hydrophobic interactions around Cys14 and the hydrogen bonds with Ser15 and Tyr18 seem to play major contributions to the stable docking of artemisinin near Cys14. These strong interactions pull the drug towards Cys14, close enough for the formation of a covalent bond.

DISCUSSION

The main structural features of TCTP are known to be α-helices, β-sheets and a flexible loop (Bommer and Thiele, 2004). The constructed 3D structure of *Pf*TCTP consisted of all these features, with the overall structure

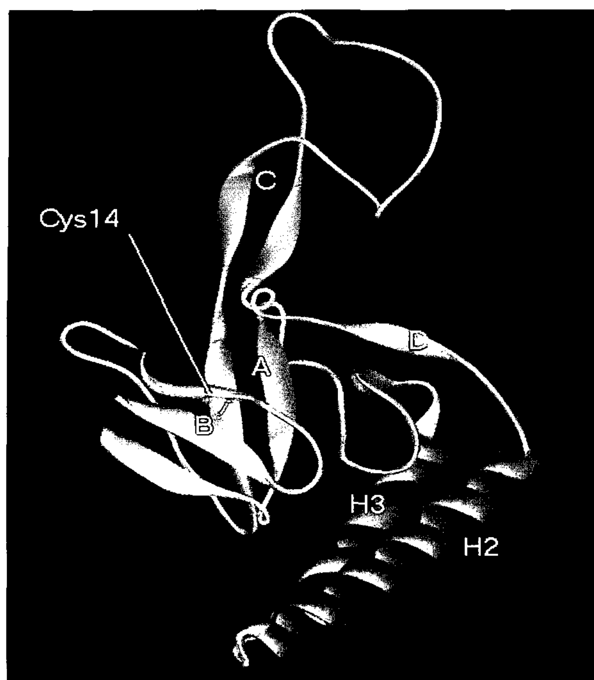

Fig. 3. Three dimensional structure of model 5 by MODELLER. Model 5 has four β-sheets (A, B, C, and D), and two helices (H2 and H3). Cys14 (orange ball and stick) is located in sheet B.

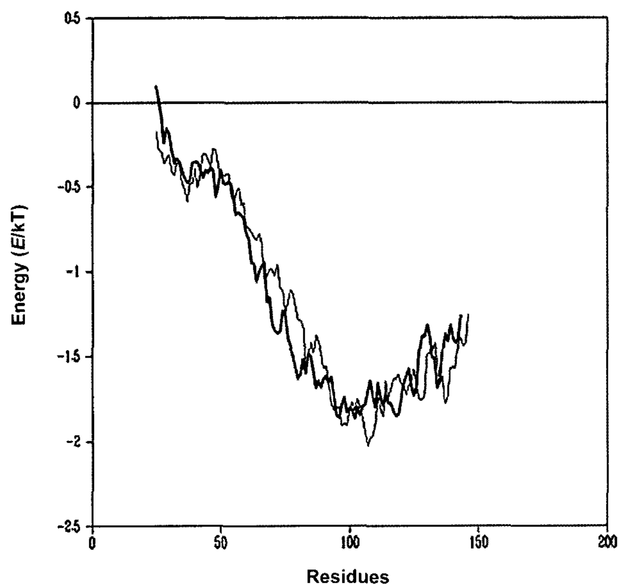

Fig. 4. The pseudo-energy profiles of model 5 and template by PROSAII. Thin line: MODELLER model, Thick line: template.

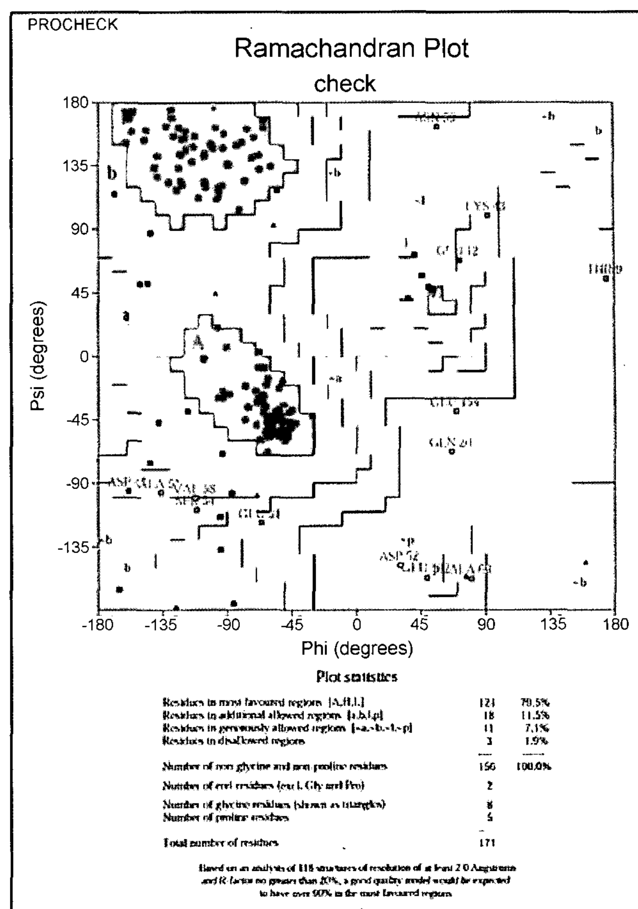

Fig. 5. The Ramachandran plot of model 5 of *P. falciparum* TCTP. The dark grey areas correspond to the "core" regions, which represent the most favorable combinations of phi-psi values. The grey area refers to additionally allowed regions and the light grey to the generously allowed regions. The white areas represent disallowed regions. The regions are labeled as follows: **A**: Core alpha, **a**: Allowed alpha, **~a**: Generous alpha, **B**: Core beta, **b**: Allowed beta, **~b**: Generous beta.

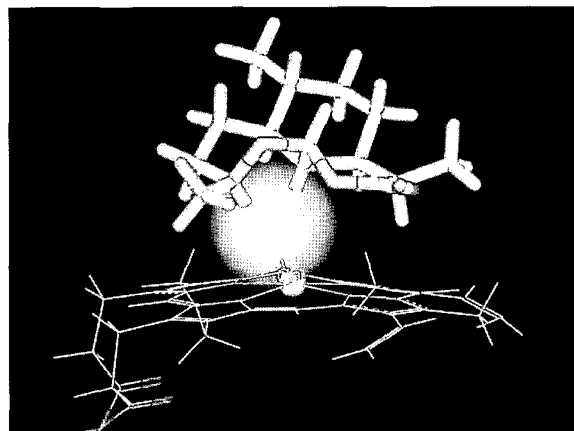

Fig. 6. Docking of Artemisinin into Heme. Endoperoxide bridge of artemisinin, shown in the transparent surface, points towards the Ca^{2+} ion, which is shown as the small ball. The distance between the Ca^{2+} atom and the mid point of the endoperoxide is 2.6Å. Stick: artemisinin, Line: heme.

being similar to that of the template. However, having cysteine instead of a hydrophobic residue at position 14 and representing only TCTP signature 2, were different from that found with other species.

The docking study showed that the endoperoxide-bridge pointed exactly towards the Fe ion in the artemisinin-heme complex, which supports the experimentally derived

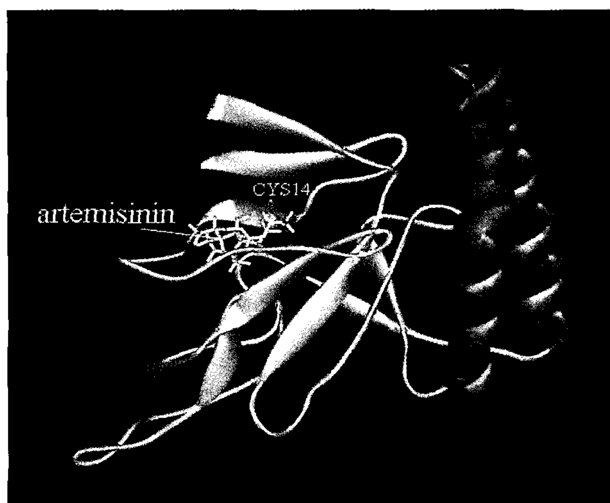

Fig. 7. Binding of Artemisinin onto *P. falciparum* TCTP. Artemisinin (yellow) was docked onto the model 5 of TCTP using Flexidock. C4-radical of artemisinin binds to the sulfur of Cys14 (red), which is the predicted active site residue.

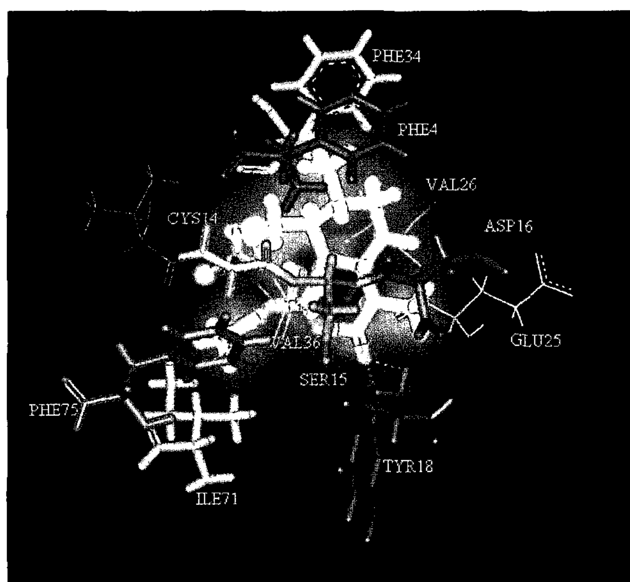

Fig. 8. The final docked configurations of Artemisinin with binding site residues of *P. falciparum* TCTP. Artemisinin (white ball and stick) forms two H-bonds with Ser15 (pink) and Tyr18 (blue). The C4 radical of artemisinin (yellow ball) interacts with the sulfur of Cys14 (orange ball). In addition, artemisinin shows hydrophobic interactions with Phe4 (dark green), Phe75 (dark blue), Val26, Val36 (gray line on the back), and Ile71 (light grey).

hypothesis (Cheng *et al.*, 2002). It is also in agreement with the other docking studies, in that the endoperoxide ring and Fe of the heme were closely located, with distance ranging from 2.6 Å to 2.7Å or 2.8Å, respectively (Cheng *et al.*, 2002; Shukla *et al.*, 1995). The movement of electrons by Fe may break the endoperoxide ring during the activation. The interaction between the methyl group of the endoperoxide ring and the imidazole rings of the heme resembles the docking result of Cheng's group (Cheng *et al.*, 2002); although, the reaction of the two carboxyl ethyl groups of the heme was different. In contrast to our results, the two groups were positioned above the porphyrin plane of the heme. Although the entire reaction process could not be observed through the docking simulations, the results implied the possibility of a reaction occurring between the endoperoxide ring of artemisinin and the Fe ion of heme.

Considering the biological functions of *Pf*TCTP, the existence of heme is very important in the binding of artemisinin and the protein. The recombinant TCTP was found to have two hemin binding sites per molecule, with modest affinity (Bhisutthibhan *et al.*, 1998), which are probably cavities composed of aromatic residues. Based on this assumption, our model seems to be reasonably well constructed, as many hydrophobic as well as aromatic residues were present around the drug binding site.

The specific amino acid residue that is modified by the drug is still unknown; however, the single cysteine appears to be important. It is possible that this cysteine is the actual amino acid modified by the drug. Alternately, it might serve as a source of electrons for the activation of the drug into a free radical (Bhisutthibhan *et al.*, 1998). The C-centered radical at the C4 position of artemisinin seems to alkylate the Cys14 residue of *Pf*TCTP in malaria parasites. The importance of the reaction between artemisinin and cysteine has been studied in several experiments. Artemisinin is known to react with low-molecular-weight thiols (Wu *et al.*, 1999), and in the presence of cysteine, traces of non-heme iron ($FeSO_4$) may rapidly cleave the peroxy bond of artemisinin, where the transient carbon-centered radical, C4, can then attack the sulfur ligand, forming a covalent bond (Wu *et al.*, 1999). Our docking results agree well with these reports, as artemisinin was closely docked to the Cys14 of *Pf*TCTP.

In this study, homology modeling and molecular docking were successfully combined. The 3D structure of *Pf*TCTP initially determined by homology modeling is a reasonable and acceptable structure, and the docking results are consistent with the experimental data. The observed conformations between artemisinin, heme and *Pf*TCTP offer new leads to understanding the biochemical mechanism of action of artemisinin, as well as insights into the physiological role of TCTP.

ACKNOWLEDGEMENT

This work was supported by the Korean Research Foundation Grant funded by the Korean Government (MOEHRD) (R06-2002-011-01003-0)

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