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Three-dimensional structure of *Plasmodium falciparum* Ca^{2+}-ATPase(PfATP6) and docking of artemisinin derivatives to PfATP6

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Dedicated to Professor Sir Jack E. Baldwin on the occasion of his 66th birthday

Abstract—Construction of the 3D structure of PfATP6 by homology modeling and docking simulation of artemisinin derivatives to this protein model are reported. Docking and consequent LUDI scores show good relation with in vitro antimalarial activities. The main binding source of artemisinins to the PfATP6 is hydrophobic interaction and biologically important peroxide bonds were exposed to outside of the binding pocket. This study suggests binding of artemisinin to PfATP6 precedes activation of peroxide bond by Fe^{2+} species.

Artemisinin, a sesquiterpene endoperoxide isolated from *Artemisia annua*, and its derivatives have been clinically used to treat drug-resistant malaria.[1–3] It is acting rapidly to the asexual stages of *Plasmodium falciparum*, the most malignant form of malaria, that kills more than 2 million people a year today. Though the chemistry and biology of artemisinins have been extensively studied for about twenty years,[4–8] their molecular target has been discovered only recently.[10] Carbon-centered radical generation via reaction between the peroxide bond of artemisinin derivatives and Fe ion species was thought to be the biological action mechanism of artemisinins.[9] It is recently known that artemisinins inhibit the sarco/endoplasmic reticulum Ca^{2+}-ATPase(SERCA) orthologue (PfATP6) of *P. falciparum* in *Xenopus* oocytes.[10] PfATP6 is thought to be the real molecular target of artemisinins in spite of some disagreements to be resolved.[11] PfATP6 is the only SERCA-type Ca^{2+}-ATPase sequence in the parasite's genome. The amino acid sequence of PfATP6 is known[12] but the three-dimensional structure is not available.

In this letter, we report the construction of the three-dimensional structure of PfATP6 by using homology modeling and docking simulation of artemisinins to this target protein, PfATP6, and subsequent good correlation of docking scores with antimalarial activities. The first binding interaction and mode of artemisinins to PfATP6 are also proposed.

We obtained the amino acid sequence of PfATP6 from PlasmoDB, the official database of the malaria parasite genome project.[13,14] Gene PFA0310c located in *P. falciparum* chromosome 1 and annotated by Sanger encoded the only SERCA-type calcium transporting ATPase protein. This protein comprises 1228 amino acids. The predicted amino acid sequence can be downloaded via internet.[15]

Sequence similarity search with BLAST in Protein Data Bank (pdb) database gives only one similar protein (43.5% identical), SERCA (pdb code: 1iwo). This structure is determined at 3.1 Å resolution and contains the highly specific inhibitor thapsigargin (TG).[10] It has three functional domains, ATP-binding domain, calcium ion binding domain, and α-helix ion channel domain, where TG is located. The binding of TG to the ion channel domain is derived almost only through hydrophobic

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interaction with proposed hydrogen bonding of TG O8 and I819 backbone amide hydrogen.

We performed the pairwise alignment of PfATP6 with 1iwo as reference using the homology module of Insight II software package.[16] Homology-modeled structure was minimized with CFF force field, first with heavy atoms fixed and later without any constraint. The structure of the PfATP6 α-helix domain is very similar to the corresponding TG-binding site of SERCA. But the ATP-binding domain and calcium ion binding domain showed relatively low similarity to SERCA or other proteins. Therefore, we removed the mismatched sequence part (375–707) from the whole sequence, and then constructed the three-dimensional structure of PfATP6 (Fig. 1). We validated it by docking simulation, confirming that thapsigargin was placed in the same binding site as in 1iwo. Figure 2 compares the structures of SERCA and homology-modeled PfATP6.

Docking simulation of artemisinin derivatives to the homology-modeled PfATP6 was performed using the LigandFit[17] module in Cerius2[18], which is known to outperform FlexX in docking of hydrophobic ligands such as artemisinin. First, overall 74 artemisinin derivatives with known antimalarial activities (W2 clone)[19–21] and thapsigargin were energy minimized with CFF 1.01 force-field method.[22] Thapsigargin in PfATP6 maintained the same spatial coordinates as in SERCA. The binding site of PfATP6 was constructed with thapsigargin as reference ligand. Docking of artemisinin derivatives to this binding site was performed, and the LUDI scores[23] were recorded simultaneously (Table 1). These LUDI scores well explained the activities of artemisinin derivatives. Chart 1 shows good correlation of LUDI scores and relative antimalarial activities compared with artemisinin.

The binding modes of artemisinin and its derivatives (Fig. 3) showed hydrophobic interactions with PfATP6 and some consistent pattern; that is, biologically important peroxide bonds were exposed to the outside of the binding pocket. Side chains at C-12 β-position can further contribute to the binding through hydrophobic interaction with hydrophobic residues of PfATP6 such as LEU263, ILE272, and PHE273.

On the other hand, substituents at C-12 α-position exposed to the outside of the binding site destabilize the binding. The ethyl ester moiety of C-12 β-position in 12-(1′-ethylacetic)-*O*-benzyldeoxoartemisinin interacts with the amide nitrogen proton of Ile1041 through hydrogen bond (green dotted line in Fig. 3). The length of this hydrogen bonding is 2.26 Å and it could afford an extra stabilization. This potential hydrogen bond of carbonyl in TG with backbone of Ile1041 can be observed in X-ray crystal structure of SERCA–TG complexes.[10] These observations enable the hypothesis that artemisinin derivatives bind to PfATP6 with almost hydrophobic interactions and it should be the preorganized shape binding[24,25] between the rigid structure of artemisinin analogues and the binding pocket of PfATP6.

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1iwo  :       MEAAHSKSTEECLAYFGVSETTGLTPDQVKRHLEK
PfATP6:  MEEVIKNAHTYDVEDVLKFLDVNKDNGLKNEELDDRRLK

1iwo  :  YGHNELPAEEGKSLWELVIEQFEDLLVRILLLAACISFV
PfATP6:  YGLNELEVEKKKSIFELILNQFDDLLVKILLLAAFISFV

1iwo  :  LAWFEEGEETITAFVEPFVILLILIANAIVGVWQERNAE
PfATP6:  LTLLDMKHKKIEICDFIEPLVIVLILILNAAVGVWQECN

1iwo  :  NAIEALKEYEPEMGKVYRADRKSVQRIKARDIVPGDIVE
PfATP6:  AEKSLEALKELQPTKAKVLRDGKWEIIDSKYLYVGDIIE

1iwo  :  VAVGDKVPADIRILSIKSTTLRVDQSILTGESVSVIKHT
PfATP6:  LSVGNKTPADARIIKIYSTSLKVEQSMLTGESCSVDKYA

1iwo  :  EPVPDP--RAVNQDKKNMLFSGTNIAAGKALGIVATTGV
PfATP6:  EKMEDSYKNCEIQLKKNILFSSTAIVCGRCIAVVINIGM

1iwo  :  STEIGKIRDQM--AATEQDKTPLQQKLDEFGEQLSKVIS
PfATP6:  KTEIGHIQHAVIESNSEDTQTPLQIKIDLFGQQLSKIIF

1iwo  :  LICVAVWLINIGHFNDPVHGGSWIRGAIYYFKIAVALAV
PfATP6:  VICVTVWIINFKHFSDPIH-GSFLYGCLYYFKISVALAV

1iwo  :  AAIPEGLPAVITTCLALGTRRMAKKNAIVRSLPSVETLG
PfATP6:  AAIPEGLPAVITTCLALGTRRMVKKNAIVRKLQSVETLG

1iwo  :  CTSVICSDKTGTLTTNQMS----------VCKMFIIDKV
PfATP6:  CTTVICSDKTGTLTTNQMTTVFH---------------

1iwo  :  DGDFCSLNEFSITGSTYAPEGEVLKNDKPIRSGQFDGLV
PfATP6:  ----------------------------------------

1iwo  :  ELATICALCNDSSLDFNETKGVYEKVGEATETALTTLVE
PfATP6:  ----------------------------------------

1iwo  :  KMNVFNTEVRNLSKVERANACNSVIRQLMKKEFTLEFSR
PfATP6:  ----------------------------------------

1iwo  :  DRKSMSVYCSPAKSSRAAVGNKMFVKGAPEGVIDRCNYV
PfATP6:  ----------------KKEIILYCKGAPENIIKNCKYY

1iwo  :  RVGTTRVPMTGPVKEKILSVIKEWGTGRDTLRCLALATR
PfATP6:  LTKNDIRPLNETLKNEIHNKIQNMG----KRALRTLSFA

1iwo  :  DTPPKREEMVLDDSSRFMEYETDLTFVGVVGMLDPPRKE
PfATP6:  YKKLSSKDLNIKNTDDYYKLEQDLIYLGGLGIIDPPRKY

1iwo  :  VMGSIQLCRDAGIRVIMITGDNKGTAIAICRRIGIFGEN
PfATP6:  VGRAIRLCHMAGIRVFMITGDNINTARAIAKEINILNKN

1iwo  :  EEVADR--------------AYTGREFDDLPLAEQREAC
PfATP6:  EGDDEKDNYTNNKNTQICCYNGREFEDFSLEKQKHILKN

1iwo  :  RRACCFARVEPSHKSKIVEYLQSYDEITAMTGDGVNDAP
PfATP6:  TPRIVFCRTEPKHKKQIVKVLKDLGETVAMTGDGVNDAP

1iwo  :  ALKKAEIGIAMG-SGTAVAKTASEMVLADDNFSTIVAAV
PfATP6:  ALKSADIGIAMGINGTEVAKEASDIVLADDNFNTIVEAI

1iwo  :  EEGRAIYNNMKQFIRYLISSNVGEVVCIFLTAALGLPEA
PfATP6:  KEGRCIYNNMKAFIRYLISSNIGEVASIFITALLGIPDS

1iwo  :  LIPVQLLWVNLVTDGLPATALGFNPPDLDIMDRPPRSPK
PfATP6:  LAPVQLLWVNLVTDGLPATALGFNPPEHDVMKCKPRHKN

1iwo  :  EPLISGWLFFRYMAIGGYVGAATVGAAAWWFMYAEDGPG
PfATP6:  DNLINGLTLLRYIIIGTYVGIATVSIFVYWFLFYPDSDM

1iwo  :  VTYHQLTHFMQCTE----------DHPHFEGLDCEIF
PfATP6:  HTLINFYQLSHYNQCKAWNNFRVNKVYDMSEDHCSYFSA

1iwo  :  EAPEPMTMALSVLVTIEMCNALNSLSENQSLMRMPPWVN
PfATP6:  GKIKASTLSLSVLVLIEMFNALNALSEYNSLFEIPPWRN

1iwo  :  IWLLGSICLSMSLHFLILYVDPLPMIFKLKALDLTQWLM
PfATP6:  MYLVLATIGSLLLHVLILYIPPLARIFGVVPLSAYDWFL

1iwo  :  VLKISLPVIGLDEILKFIARNYLEG
PfATP6:  VFLWSFPVIILDEIIKFYAKRKLKEEQRTKKIKID
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Figure 1. Alignment of PfATP6 sequence with 1iwo as reference protein.

As the Fe^{2+}-dependent activation and antimalarial activity of artemisinin do not depend on the haem binding,[26] we can propose that the production of the carbon-centered free radical[9] should not precede the binding to PfATP6. Therefore, artemisinin should be bound to PfATP6 before activation by Fe^{2+} ion.

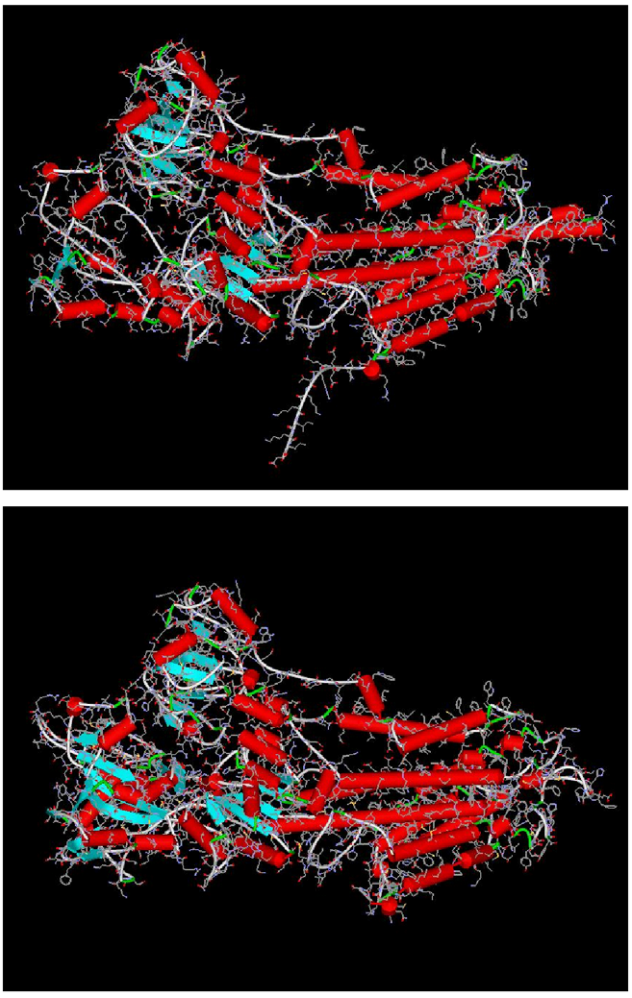

Figure 2. Schematic display of PfATP6 (above) and SERCA (below) generated using DS ViewerPro 5.0 for Windows. Helices and sheets are represented as red cylinders and cyan arrows, respectively.

Table 1. Complete list of actual activity (expressed as Log RA, where Log RA = −log (biological activity of compound/activity of artemisinin))

Compound number	Log RA	LUDI score
1	0.45	285
2	−0.32	164
3	−0.28	243
4	1.36	340
5	−0.48	137
6	0.86	234
7	0.10	221
8	0.37	230
9	1.37	363
10	0.63	298
11	0.78	253
12	−0.07	283
13	0.70	245
14	−0.55	250
15	0.58	279
16	−1.70	152
17	0.16	270
18	.40	353
19	−0.67	219
20	0.65	250
21	1.06	287
22	0.10	333
23	0.37	285
24	0.72	294
25	.20	185
26	−0.87	208
27	0.33	253
28	0.75	273
29	−0.59	258
30	0.27	281
31	−0.81	209
32	0.23	259
33	−0.60	228
34	−0.04	254
35	0.32	220
36	0.38	268
37	0.17	291
38	0.14	273
39	−1.80	145
40	0.90	285
41	0.90	279
42	0.68	358
43	0.21	299
44	0.10	209
45	1.16	290
46	0.49	308
47	1.03	310
48	0.44	336
49	1.22	326
50	1.53	341
51	1.02	323
52	1.01	253
53	1.95	393
54	0.36	288
55	0.44	337
56	0.94	315
57	0.40	245
58	1.82	359
59	0.15	280
60	0.60	230
61	1.05	250
62	1.44	302
63	1.63	352
64	0.87	298
65	1.42	319
66	0.58	324
67	4.10	339
68	1.53	329
69	1.73	342
70	0.54	281
71	1.84	364
72	0.85	354
73	1.05	342
74	2.62	453

In conclusion, we constructed the 3D structure of PfATP6 by homology modeling and performed docking simulation of artemisinin derivatives to this protein model. In spite of the relatively low resolution (3.1 Å) of template protein, homology modeled protein showed the binding sources well. The LigandFit docking and consequent LUDI scores show good relationship with in vitro activities. The main binding source of artemisinins to the PfATP6 is hydrophobic interaction and biologically important peroxide bonds were exposed to the

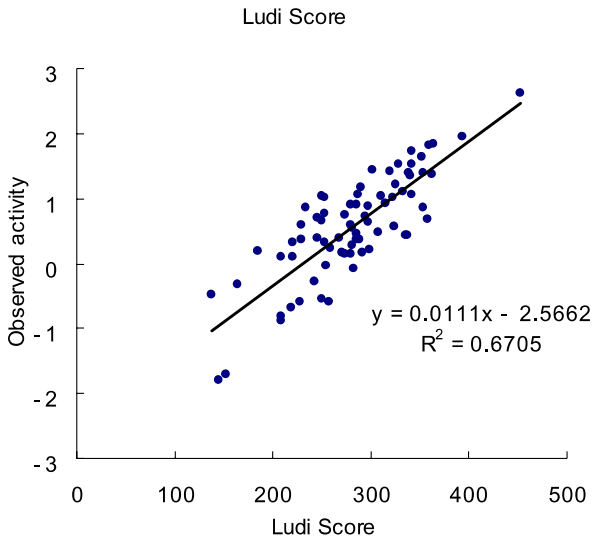

Chart 1. Correlation of Ludi scores versus observed activities of artemisinin derivatives.

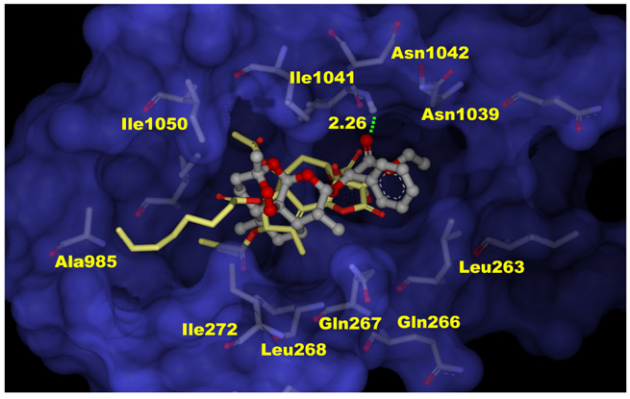

Figure 3. Binding mode of artemisinin derivative (12-(1′-ethylacetic)-*O*-benzyldeoxoartemisinin, 40; gray ball and stick model. TG; yellow cylinder model) in PfATP6. Figure is generated using DS ViewerPro 5.0 for Windows.

outside of the binding pocket. This study strongly suggests binding of artemisinin to PfATP6 precedes the activation of peroxide bond by Fe^{2+} species, thus providing new and invaluable information in biological mechanism studies of artemisinin.

Acknowledgments

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