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Bioinformatics analysis and prediction for structure and function of nitric oxide synthase and similar proteins from *Plasmodium berghei*

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ABSTRACT

Objective: To search and analyze nitric oxide synthase (NOS) and similar proteins from *Plasmodium berghei(Pb)*. **Methods:** The structure and function of nitric oxide synthase and similar proteins from *Plasmodium berghei* were analyzed and predicted by bioinformatics. **Results:** *Pb*NOS were not available, but nicotinamide adenine dinucleotide 2′-phosphate reduced tetrasodium (NADPH)-cytochrome p450 reductase(CPR) were gained. *Pb*CPR was in the nucleus of *Plasmodium berghei*, while 134aa-229aa domain was localize in nucleolar organizer. The amino acids sequence of *Pb*CPR had the closest genetic relationship with *Plasmodium vivax* showing a 73% homology. The tertiary structure of PbCPR displayed the forcep-shape with wings, but no wings existed in the tertiary structure of its′ host, *Mus musculus(Mm)*. 137aa-200aa, 201aa-218aa, 220aa-230aa, 232aa-248, 269aa-323aa, 478aa-501aa and 592aa-606aa domains of *Pb*CPR showed no homology with *Mm*CPRs′, and all domains were exposed on the surface of the protein. **Conclusions:** NOS can′t be found in *Plasmodium berghei* and other *Plasmodium* species. *Pb*CPR may be a possible resistance site of antimalarial drug, and the targets of antimalarial drug and vaccine. It may be also one of the mechanisms of immune evasion. This study on *Plasmodium berghei* may be more suitable to *Plasmodium vivax*. And137aa-200aa, 201aa-218aa, 220aa-230aa, 232aa-248, 269aa-323aa, 478aa-501aa and 592aa-606aa domains of *Pb*CPR are more ideal targets of antimalarial drug and vaccine.

1. Introduction

Nitric oxide (NO) is an important gaseous signal molecule in biological tissue. Nitric oxide synthases (NOS) include neuronal nitric oxide synthase (nNOS or NOS1), inducible nitric oxide synthase (iNOS or NOS2) and endothelial nitric oxide synthase (eNOS or NOS3). Recently, NO has been found in or around the food vacuole in *Plasmodium falciparum* parasites[1], but no NOS is found in *Plasmodium falciparum*. Using the bioinformatics database and software, this article is to search NOS and similar proteins from Plasmodium berghei (*Pb*) and analyze their structure and function.

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2. Materials and methods

The gene sequence, mRNA and amino acids (aa) of NOS from *Homo sapiens (Hs)* and *Mus musculus (Mm)* were obtained from NCBI database (http://www.ncbi.nlm.nih.gov), then the amino acids sequences of NOS and similar proteins from *Pb* were blasted. The physical-chemical properties were predicted bases on http://www.expasy.org/tools/protparam.html, and the molecule evolution was analyzed by ClustalX2. The sub-cellular localization of the protein, signal peptide, secondary structures, topological structure and 3D structure were predicted according to http://psort.nibb.ac.jp/ form2.html, http://www.cbs.dtu.dk/services/SignalP/, http://www.predictprotein.org/, and http://geno3d-pbil.ibcp.fr/cgi-bin/geno3d_automat.pl?page=/GENO3D/geno3d_home.html, respectively.

3.Results

3.1. PbNOS and similar proteins

The accession number of the gene, mRNA and aa of *Hs*NOS and *Mm*NOS were listed in Table 1.

No protein with similar mRNA or aa to *Hs*NOS and *Mm*NOS was found in *Pb* and other *Plasmodium* species, but nicotinamide adenine dinucleotide 2′-phosphate reduced tetrasodium (NADPH)NADPH-cytochrome p450 reductase (CYPOR or CPR) of *Pb* was found in part similar to *Hs*NOS and *Mm*NOS. Gene ID of *Pb*CPR was 3426618, and the accession number of mRNA and aa of *Pb*CPR were XM_672962.1 and XP_678054.1. The amino acid sequence of *Pb*CPR was really just like C-terminal amino acid sequence of *Hs*NOS and *Mm*NOS, which includes FNR-like superfamily and FMN-red superfamily. *Pb*CPR 1aa-144aa was located in conserved domains of FMN-red superfamily, while 323aa-684aa in domains of FNR-like superfamily(Table 2 and Figure 1).

Table 1
Accession number of the gene, mRNA and aa of *Hs*NOS and *Mm*NOS.

	NOS1		NOS2		NOS3	
	Hs	*Mm*	*Hs*	*Mm*	*Hs*	*Mm*
Gene	NG_011991.1	–	NG_011470.1	–	NG_011992.1	–
mRNA	NM_000620.2	NM_008712.2	NM_000625.4	NM_010927.3	NM_000603.4	NM_008713.4
Amino acids	NP_000611.1	NP_032738.1	NP_000616.3	NP_035057.1	NP_000594.2	NP_032739.3

Table 2
Similarity of amino acid sequence of *Pb* CPR, *Hs*NOS and *Mm*NOS.

	Sequence			Sequence			Sequence	
Pb CPR	1 – 136	349 – 655	*Pb* CPR	1 – 144	349 – 658	*Pb* CPR	1 – 136	349 – 655
*Hs*NOS1	769 – 941	1092 – 1367	*Hs*NOS2	548 – 686	821 – 1098	*Hs*NOS3	529 – 704	852 – 1127
Pb CPR	1 – 142	349 – 638	*Pb*CPR	1 – 144	342 – 680	*Pb* CPR	1 – 655	–
*Mm*NOS1	764 – 942	1087 – 1362	MmNOS2	349 – 658	815 – 1092	*Mm*NOS3	528 – 1126	–

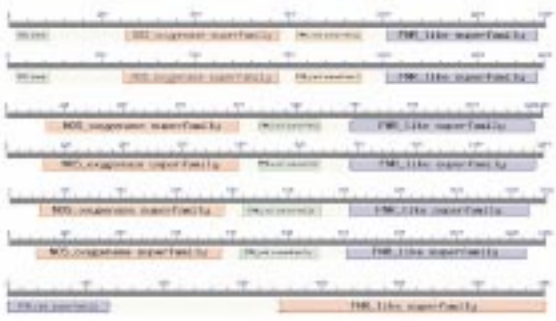

Figure 1. Conserved domains of *Hs*NOS, *Mm*NOS and *Pb*CPR.

3.2. Physical-chemical property of PbCPR

As a non secretory protein, *Pb*CPR is in cell nuclei, 684 aa in length, ans 80237.7 Da in molecular weight. It is unstable and the pI is 8.70.

3.3. Molecular evolution of PbCPR

There were 397 highly conserved amino acids in *Pb*CPR which were homologous to those of *Plasmodium vivax* CPR (*Pv*CPR). The rates of consistency and similarity were 57% and 73%, respectively. While there were 102 highly conserved amino acid which were homologous to those of *Plasmodium falciparum* CPR (*Pf*CPR). And the rates of consistency and similarity were 27% and 48% between *Pb*CPR and *Pf*CPR. *Pb*CPR had close relationship with *Pv*CPR but distant from *Pf*CPR, *Mm*CPR and *Hs*CPR(Figure 2).

3.4. Structure of PbCPR

α-helics accounted for 31.7%, β strands for 14.5%, and loop for 53.9% in the secondary structure of *Pb*CPR. 134aa-229aa was localized in nucleolar organizer regions (NORs) of *Plasmodium berghei*. A total of 50.58% amino acid were exposed on the surface of the protein. *Pb*CPR had 5 N-glycosylation sites, 2 cAMP- and cGMP-dependent protein kinase phosphorylation sites, 14 protein kinase C phosphorylation sites, 6 casein kinase II phosphorylation sites, 3 tyrosine kinase phosphorylation sites, and 1 N-myristoylation site (Figure 3 and 4).

The tertiary structure of *Pb*CPR showed forceps with wings. Conserved domaind of FMN-red superfamily and FNR-like superfamily formed a huge and deep fracture. 134aa-229aa was localized in upper left of the back of the protein. Most functional sites scattered on the surface of the protein(Figure 4).

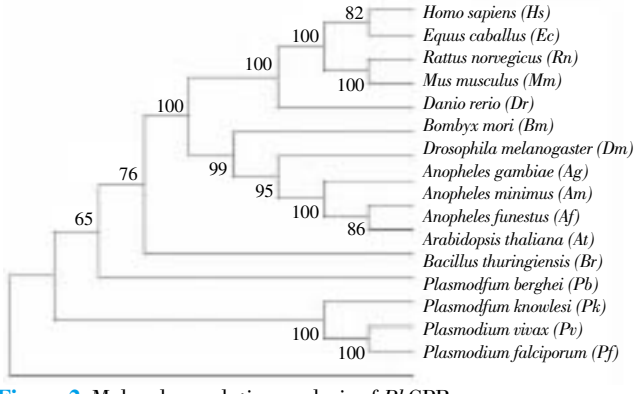

Figure 2. Molecular evolution analysis of *Pb*CPR.

3.5. Comparison of *Pb*CPR and *Mm*CPR

*Pb*CPR was in cell nuclei, while *Mm*CPR was in cytoplasm. 89aa–233aa of *Mm*CPR and 1aa–144aa of *Pb*CPR lied in conserved domain of FMN–red superfamily with 49 homolougous highly conserved amino acids. The rates of consistency and similarity were 31% and 54% between *Pb*CPR and *Pv*CPR. 343–677aa of *Mm*CPR and 323aa– 684aa of *Pb*CPR lied in conservative structure domain of FNR–like suoerfamily with 103 homologous highly conserved amino acids. The rates of consistency and similarity were 26% and 47% between *Pb*CPR and *Pv*CPR. The tertiary structure of *Pb*CPR showed forceps with wings, but no wings in the tertiary structure of *Mm*CPR (Figure 5).

Figure 3. Secondary structure of *Pb*CPR.
Red alphabet represent conserved amino acid residue, ▭ =conserved domain, H= α helics, E= β strands, e = exposed, N=NORs, ▭= N–glycosylation site, ▭= cAMP– and cGMP–dependent protein kinase phosphorylation site, ▭ and ▭ = Protein kinase C phosphorylation site, ▭= Casein kinase II phosphorylation site, ▭= Tyrosine kinase phosphorylation site, ▭= N–myristoylation site.

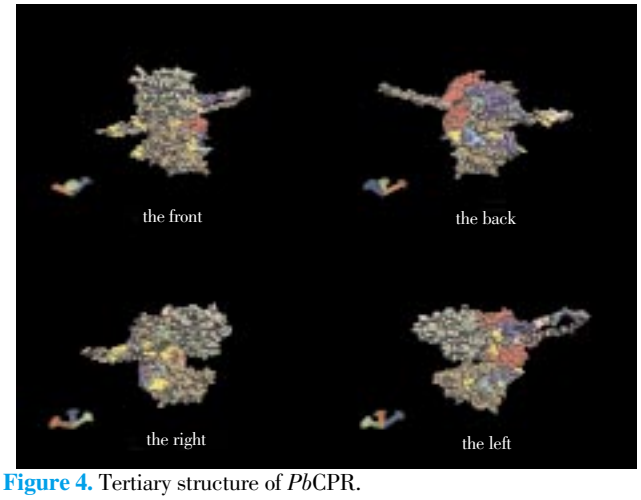

Figure 4. Tertiary structure of *Pb*CPR.
Red = NORs, tan = FMN–red superfamily, dchre = FNR–like superfamily, blue = non domain, pink = N–glycosylation site, grass green = cAMP– and cGMP–dependent protein kinase phosphorylation site, yellow = Protein kinase C phosphorylation site, black green = Casein kinase II phosphorylation site, gray = Tyrosine kinase phosphorylation site, cyan = N–myristoylation site.

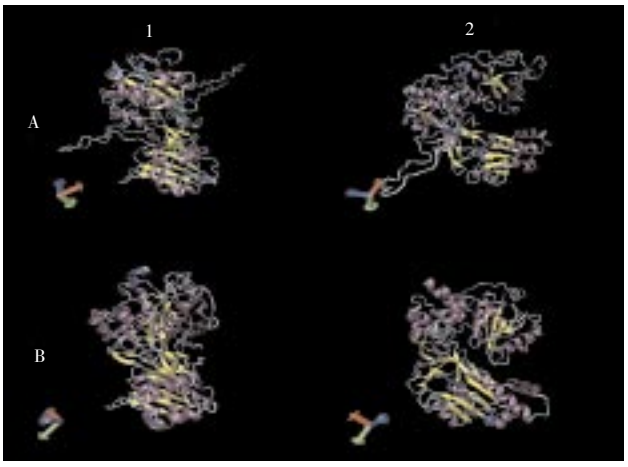

Figure 5. Comparison between the structure of *Pb*CPR and *Mm*CPR. A and B represent *Pb*CPR and *Mm*CPR, respectively 1 and 2 represent the front and the side, respectively.

3.6. Unique sites of *Pb*CPR

137aa–200aa, 201aa–218aa, 220aa–230aa, 232aa–248 aa, 269aa–323aa, 478aa–501aa and 592aa–606aa of *Pb*CPR were expose on the surface of the protein, and had no homology to *Mm*CPR. 137aa–200aa, 201aa–218aa and 220aa–230aa were located in NORs. 232aa–248aa and 269aa– 323aa were in front of the left winglike structure of the protein, 478aa–501aa was in the right, 592aa–606 aa was in the undersurface(Figure 3 and 6).

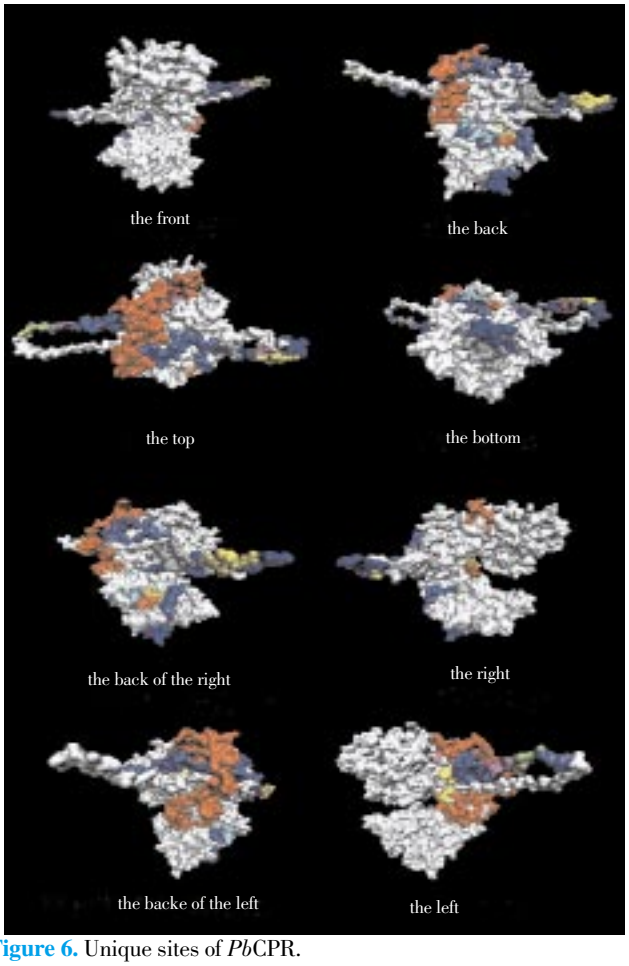

Figure 6. Unique sites of *Pb*CPR.

White = highly similar with other species; other colors= the unique sites of *Pb*CPR; red = NORs, blue = non functional sites; purple = N-glycosylation site; yellow = Protein kinase C phosphorylation site; green = Casein kinase II phosphorylation site; grey = Tyrosine kinase phosphorylation site; cyan= N-myristoylation site.

4. Discussion

NO widely exists in organisms. It is an important signal molecule and the key factor in the anti-malarial mechanism[2]. NOS is an important enzyme in NO synthesis process, and mainly provide the NO which participates in inhabiting or killing *Plasmodium*[3,4]. NOS can be found in both normal red blood cell (RBC)[5] and *Plasmodium* infected RBC[6]. Recently, NO has been found in *Plasmodium*. NOS of *Pb* and other *Plasmodium* species are not available in bioinformatics retrieval, whereas CPR partly similar to *Hs*NOS and *Mm*NOS can be gained. Therefore, *Plasmodium* including *Pb* may have no NOS gene, and NO in *Plasmodium* may originate mainly from other sources.

Both CPR and NOS belong to the family of diflavin reductases[7]. The main function of CPR are transporting electron and taking part in drug metabolism. CPR provides electron transport to cytochrome P450 monooxygenase system, cytochrome C, cytochrome b5, heme oxidase, squalen monooxygenase and 7-dehydrocholesterole reductase. It can catalyze reductive metabolism of prodrugs[8], and produce reactive oxygen species (ROS) by oxidation of NADPH[9]. Gene mutation of CPR causes Antley-Bixler syndrome (ABS) that is an autosomal recessive hereditary disease, developmental disability, disorders of drug metabolism, *etc*[10-13].

It is predicted by bioinformatics analysis that different from *Mm*CPR which is in cytoplasm, *Pb*CPR is located in cell nucleus, and has 14% sequence locating in NORs forming section. It is still possible that the subcellular localization of *Pb*CPR may be one immune evasion mechanism.

Amino acids sequence alignment and evolution analysis indicates that *Pb*CPR has closest relationship with *Pv*CPR, but distant from *Pf*CPR. Therefore, the study on *Plasmodium berghei* may be more suitable to *Plasodium vivax* than *Plasmodium falciparum*.

It is still unclear now what kind of role CPR of *Plasmodium* species play in the growth and reproduce of *Plasmodium* species, and how CPR gene mutation affect the growth and reproduce of *Plasmodium* species. Considering the role of CPR in electron transporting and drugs metabolism, it can't rule out the possibility that CPR of *Plasmodium* species may be target for potential anti-malaria drug and vaccine, and the possible resistance sites of anti-malaria drug. According to amino acids sequence, subcellular localization of *Pb*CPR and *Mm*CPR, the function of CPR and these diseases due to CPR gene mutation in mammals, it can be concluded that *Pb*CPR is a possible target for drugs and vaccines, with137aa-200aa, 201aa-218aa, 220aa-230aa, 232aa-248, 269aa-323aa, 478aa-501 aa and 592aa -606aa being the ideal target domains.

Role of founding source

This work was supported in part by the Research Program in higher educational institutes of the Education Department in Hainan (No.Hjkj2009-50).

Conflict of interest statement

We declare that we have no conflict of interest.

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