CHAPTER VI

Discussion

At present, three enzyme activities (uridine phosphorylase, uridine kinase and uracil phosphoribosyltransferase) inter-converting uracil, uridine and UMP of the pyrimidine salvage pathway are demonstrated in *P. falciparum* (34). In other word, *P. falciparum* could operate the uracil salvage pathway and could have the genes encoding these three enzymes. In this study, we clone uridine phosphorylase gene homolog in *P. falciparum* by using PCR amplification technique with genomic data from bioinformatics underlying NCBI resources. Nucleotide sequence analysis of the gene homolog shows that it is single open reading frame containing 735 bp for deduced 245 amino acids. The gene has been expressed in *E. coli*. The expressed and purified protein has the activity of enzyme uridine phosphorylase. Its molecular mass is approximately 30 kDa, similar to the molecular mass of the protein that is predicted from the amino acid sequence of *P. falciparum* uridine phosphorylase gene.

We have used bioinformatics approaches to identify our candidate gene homolog. The uridine phosphorylase gene has been identified in *P. falciparum* from GenBank database by the BLAST program. The nucleotide sequence of the gene homolog has been found on chromosomes 5 and 7, which is 28 % identical to *E. coli* uridine phosphorylase. *P. falciparum* uridine phosphorylase shows higher homology with *E. coli*

uridine phosphorylase than that of *E. coli* purine nucleoside phosphorylase (35). The single open reading frame of *P. falciparum* uridine phosphorylase contains 735 bp starting with ATG and ending with TAA. The amino acid sequence of the protein is deduced with a molecular mass of 28 kDa. However, the molecular mass of *P. falciparum* enzyme is larger than *E. coli* uridine phosphorylase (36).

Using bioinformatics to design primers, we have succeeded in DNA amplification by PCR of *P. falciparum* uridine phosphorylase. The size of the PCR product of the candidate DNA was about 735 bp. The PCR product was cloned into a pDrive cloning vector and using *E. coli* strain *EZ*. The restriction analysis with *Bam*HI and *Sac*I was used to identify the positive clone for sequencing by an automated DNA sequencer, then the homology of the DNA sequence was analyzed by the BLAST program. The results of homology analysis showed that our candidate gene is *P. falciparum* uridine phosphorylase gene homolog with 99% identity to the sequence data deposited in GenBank database. Interestingly, there is only one base substitution (130/A-->G). This does not affect the amino acid substitution. The base substitution in the gene could occur from: a). single nucleotide polymorphism exists every 2.3 kb on chromosomes (37), b). *P. falciparum* in GenBank database (3D7) and our laboratory (T9) are different in their strains, and c). we use non-proofreading *Taq* polymerase instead of proofreading *Pfu* polymerase for DNA amplification by PCR.

Studies on the heterologous expression system by subcloning the insert to pQE30 expression vector in both of *E. coli* SG13009 and M15 competent cells are pursued. The recombinant protein is expressed after IPTG induction in both of cells. The expressed protein, as His_6-tagged at N-terminus, has been purified using the Ni-NTA affinity chromatography. The purified protein is then identifed on SDS-PAGE analysis as a major band with a molecular mass of 30 kDa which is very close to the calculated molecular mass of the amino acid sequence of this protein.

In order to verify the function of the recombinant protein obtained in IPTG-induced *E. coli* harboring the gene inserted in the pQE vector, the activity of the enzyme in the recombinant protein has been determined spectrophotometrically. The specific activity of uridine phosphorylase in the *E. coli* lysate is about 42 nmol/min/mg protein and in the eluate after the Ni-NTA affinity chromatography is 342 nmol/min/mg protein. This results indicated that the expressed uridine phosphorylase is functional active with a k_{cat} value of 1.18 min^{-1}. The relatively low k_{cat} value in the recombinant *P. falciparum* uridine phosphorylase is consistent to the very recent report on dual-function of uridine phosphorylase and purine nucleoside phosphorylase in the same polypeptide in which the uridine phosphorylase exhibits 10-fold lower k_{cat} than the purine nucleoside phosphorylase (35).

In addition, working with transcriptomic analysis by DNA microarray technique of the whole genes in *P. falciparum* (*http://www.PlasmoDB.org*), the gene encoding uridine phosphorylase located on chromosome 5 locus PFE0660c is actively transcribed during the asexual

erythrocytic stages, mostly at trophozoite stage (38). The gene is also expressed in other stages of *P. falciparum* development, e.g. gametocyte and sporozoite (39). Taken together, the gene, mRNA and protein of uridine phosphorylase enzyme have been identified in *P. falciparum.* The relatively low enzyme activity and catalytic efficiency may be important in parasite survival. The existence of the uracil salvage pathway of the pyrimidine biosynthesis, in addition to the *de novo* pathway, will highlight the chemotherapeutic targets in *P. falciparum*.