Thesis Title	Studies on Cloning and Expression of the Genes Coding *Plasmodium falciparum* Primase Small Subunit and Topoisomerase II
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ABSTRACT

Plasmodium falciparum, a protozoan parasite that causes the most severe form of human malaria, has become resistant to nearly all presently available antimalarial drugs. This has prompted investigation of potential new targets at the biochemical and molecular levels. Enzymes involved in DNA replication process, including primase and topoisomerase, have proven to be important targets for chemotherapy.

The gene coding *P. falciparum* (K1) primase small subunit was cloned and heterologously expressed in baculovirus-insect cell system. The entire gene consisted of 3265 base pairs (bp) which was interrupted by 15 introns, the largest number of introns ever found in *Plasmodium* genes. The transcript size of *P. falciparum* primase small subunit gene was about 2091 nucleotides, as indicated by Northern blotting which showed the positive hybridization band at the position of 18S rRNA. The complete cDNA encoding a protein of 452 amino acids with a calculated molecular weight of 53 kDa was isolated and sequenced. The protein contained eight conserved regions, similar to those present in five other eukaryotic primase small subunits. A putative catalytic center and a conserved C-terminus that might be involved in catalytic function were also

identified when the deduced amino acid sequence was compared with DNA polymerases, RNA polymerases, and reverse transcriptases. The cDNA clone was placed under control of the baculovirus polyhedrin promoter and successfully expressed as a fusion product containing an N-terminal hexahistidine tag in *Spodoptera frugiperda*, Sf9, insect cells. The availability of the enzyme will enable detailed studies on specific primase inhibitors to be carried out.

The gene coding *P. falciparum* topoisomerase II consists of 4194 bp, containing no intron, which encodes a protein of 1398 amino acids (161 kDa). In this study, the 5′-terminal part of topoisomerase II gene was identified on a 2.6 kb genomic fragment of *P. falciparum* (K1) DNA. The entire gene was constructed in the baculovirus transfer vector, pAcSGHisNT-B, by sequencial cloning of the 5′-PCR 210 bp fragment, the 1691 bp genomic fragment (a part of 2.6 kb genomic fragment), and the 3′-PCR 2677 bp fragment. The DNA sequence of the gene was also confirmed before cotransfecting with baculovirus DNA into Sf9 cells. Expression of the topoisomeraseII gene was conducted in the same system described for primase small subunit gene. However, the fusion protein with expected molecular weight of 165 kDa (161 kDa of topoisomerase II plus 4 kDa of hexahistidine) could not be detected by SDS-PAGE and Coomassie blue staining. The nature of the gene may be a limiting factor in its expression in this heterologous system.