CHAPTER I

Introduction

1. Malaria

Malaria is a serious, sometimes fatal, disease caused by parasites of a genus of *Plasmodium*. At present, at least 300,000,000 peoples are affected by malaria globally, and there are between 1,000,000 and 1,500,000 malaria deaths per year (1). Malaria occurs in over 100 countries and territories : large areas of Central and South America, Hispaniola (Haiti and the Dominican Republic), Africa, the Indian subcontinent, Southeast Asia, the Middle East, and Oceania are considered malaria-risk areas. 80% of cases are in Africa, with the remainder clustered in nine countries: India, Brazil, Afghanistan, Sri-Lanka, Thailand, Indonesia, Vietnam, Cambodia and China. In Thailand, the highest incidence continues to be recorded in the border provinces of Trat (bordering Cambodia) and Tak (bordering Myanmar). These two provinces together with Chantaburi and Kanchanaburi register about 50 % of cases in Thailand (2).

There are approximately 156 species of *Plasmodium* which infect various species of vertebrates. Four are known to infect humans :

1. *P. falciparum*
2. *P. vivax*
3. *P. ovale*
4. *P. malariae*

Malaria parasites probably originated in Africa (along with mankind) and fossils of mosquitoes up to 30 million years old show that the vector for malaria was presented. From their origins in Africa, *P. vivax* and *P. malariae* were possibly brought to the New World by early trans-Pacific voyagers, and imported malaria continues to this day. *P. falciparum* may have come in consignments of slaves bound for the Spanish colonies.

Of the four, *P. falciparum* is the predominant species with 120,000,000 new cases and up to 1,000,000 deaths per year globally. It is the *P. falciparum* species which has caused malignant tertian malaria, cerebral malaria, intestinal malaria and Blackwater fever (malarial hemoglobinuria) (3). It's also given rise to the presently available drug resistant strains which causes a major health burden.

2. *P. falciparum* Uridine Phosphorylase as a Target of Antimalarial Drugs

As mentioned above, because *P. falciparum* kills more than 1,000,000 people each year, and the parasites are becoming resistant to existing drugs. Moreover, the development of vaccines against malarial has proven extremely difficult due to the complex life cycle of the parasite (4,5). Therefore, effective antimalarial drugs are still in need of the fight against the disease. This has started a renewed search for new types of drugs with novel targets.

In order to servive within the human host, the malarial parasite has to proliferate very rapidly. Therefore, the nucleotide metabolism offers new targets for inhibition of parasite growth.

It has been shown for sometimes that the parasite could only obtain purine nucleotides through the salvage pathway and pyrimidine nucleotides through *de novo* synthesis (6). The malarial parasite operates pyrimidine biosynthetic pathway for its growth and development in the human host. The first six enzymes, catalyzing the formation of uridine 5'-monophosphate from the starting precursors of HCO_3^-, ATP and L-glutamine, were partially characterized. The genes encoding these six enzymes were identified, in order from the first to the sixth step, as *CPS* II (carbamoyl phosphate synthetase II), *ATC* (aspartate transcarbamoylase), *DHO* (dihydroorotase), *DHOD* (dihydroorotate dehydrogenase), *OPRT* (orotate phosphoribosyltransferase), and *OMPDC* (orotidine 5'-monophosphate decarboxylase) (7,8). Recently, the enzyme activities inter-converted uracil to uridine and UMP of the pyrimidine salvage pathway have been demonstrated in this parasite but the responsible gene namely uridine phosphorylase gene has not been identified yet (9). Therefore, it is interesting to identify the gene encoding uridine phosphorylase activity.

3. Aims of Thesis

Many reports suggested that enzymes involved in pyrimidine metabolism, including uridine phosphorylase are potential targets for new antimalarial drugs. One way to achieve fundamental basis for target-directed

development of new antimalarials is to analyse these enzyme in biochemical detail. The sufficient amount of pure enzyme from *P. falciparum* is essential for such studies. Thus, the objectives of this thesis are as follow;

1. To clone *P. falciparum* uridine phosphorylase gene.
2. To heterologously express the enzyme in a bacterial system.
3. To perform kinetic analysis of the recombinant enzyme.