

CHARACTERIZATION OF OROTATE PHOSPHORIBOSYLTRANSFERASE AND OROTIDINE 5'-MONOPHOSPHATE DECARBOXYLASE IN HUMAN MALARIA PARASITE

Sudaratana KRUNGKRAI

NRCT- 10222

Lecturer

Biochemistry Unit, Department of Medical Science, School of Science, Rangsit University

Japanese Advisor : Toshihiro HORII

Professor, Osaka University

Malaria remains one of the world's major public health problems. Chemotherapy of malaria is available but it is complicated by both drug toxicity and widespread resistance to most of the currently available antimalarial drugs. This study was undertaken to understand in more detail the *de novo* pyrimidine biosynthetic pathway in the human malaria parasite with the goal of elucidating chemotherapeutic targets for drug development. Because of the dependence of the parasite on the *de novo* biosynthetic pathway for pyrimidines, precursors for DNA and RNA, inhibitors targeting this malarial pathway could be effective antimalarial agents.

Orotate phosphoribosyltransferase (OPRT) and orotidine 5'-monophosphate decarboxylase (OMPDC), the fifth and six enzymes in *de novo* pathway catalyzing formation of uridine 5'-monophosphate (UMP), remain largely uncharacterized in the protozoan parasite; although their specific inhibitors, pyrazofurin and its 5'-monophosphate derivative, respectively, were shown to be effective antimalarial agents. Identification and characterization of OPRT and OMPDC would contribute largely to elucidating the unique metabolic features in the parasite. In this study the native enzymes from *P. falciparum* were purified and found to be a multienzyme complex of 140 kDa containing two subunits each of OPRT and OMPDC. Results show that the kinetic parameters and inhibitory constants of both enzymes were different from that of the bifunctional human red cell UMP synthase.

To conduct additional biochemical studies and gain further insights into these enzymes, the cDNA of both *PfOPRT* and *PfOMPDC* genes were cloned, sequenced and functionally expressed in soluble form. Recombinant *PfOPRT* exhibits a molecular mass of 33 kDa in SDS-PAGE and 67 kDa in analytical gel-filtration chromatography. Using dimethyl suberimidate to cross-link neighboring subunits of *PfOPRT*, the enzyme was confirmed to exist as a dimer. The steady state kinetics of initial velocity and product inhibition studies indicated that *PfOPRT* follows a random sequential kinetic mechanism. The recombinant

PfOPRT and *PfOMPDC* monofunctional enzymes were found to be kinetically different from the native multienzyme complex purified from *P. falciparum*. However, kinetics of the *PfOPRT-PfOMPDC* associated 140-kDa complex was similar to the native *P. falciparum* enzymes. Results demonstrate that the enzymes share characteristics of both the monofunctional and bifunctional counterparts in prokaryotes and eukaryotes. Furthermore, an inhibitor of the yeast OMPDC, 6-thiocarboxamido-uridine 5'-monophosphate, was about 5 orders of magnitude less effective on the *PfOMPDC* than on the yeast enzyme. The crystal structure of *PfOMPDC* exhibits trigonal symmetry (space group $R3$). With a dimer in the asymmetric unit, the solvent content is 46% ($V_M = 2.3 \text{ \AA}^3 \text{ Da}^{-1}$). Results on these studies will greatly facilitate antimalarial drug design based on the pyrimidine enzymes as chemotherapeutic targets.

