STUDY OF SPECIFIC PROTEIN KINASE FROM MALARIA PARASITE (*PLASMODIUM BERGHEI*)

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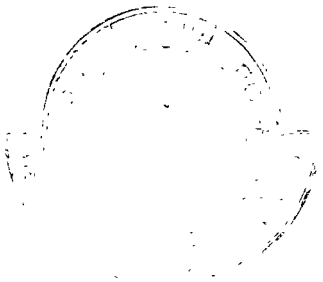

ABSTRACT

P. berghei was prepared from infected mouse erythrocytes both by N_2-cavitation technique and saponin lysis free from host cell contamination. High protein kinase activity was detected in parasite cytosol in the presence of exogenous protein substrates. Casein was shown to be the most preferential substrate while protamine was slightly less. However, the enzyme activity toward endogenous protein substrates was quite low as compare to the exogenous activity. Phosphorylation of exogenous substrates was stimulated 3-4 fold by cAMP while addition of 1 mM Ca^{2+} showed no effect on enzyme activity. The results indicated that both cAMP-dependent and cAMP-independent protein kinase were present in the parasite cytosol. It also showed that the enzyme was not affected by Ca^{2+}. Desaltation of the enzyme did not change the plasmodial protein kinase properties. However both cAMP and Ca^{2+} were without any effect on endogenous activity. These results suggested that some other factors might be involved in the regulation of plasmodium protein kinase activity, making it different from the enzyme in other systems.

Experiments designed to screen for effectors of the endogenous activity indicated that most divalent cations exerted inhibitory effect. The enzyme was not affected by compounds known to influence protein kinase activity. However, the parasite enzyme was strongly activated by Fe^{3+} and only slightly by Fe^{2+}. Almost 40 fold increase in endogenous activity in the presence of 1 mM Fe^{3+} was observed while only a 2 fold increase with

Fe^{2+}. The activation of parasite protein kinase by Fe^{3+} was quite specific. No other trivalent cations could mimic the Fe^{3+} effect. Protein kinase from other systems were not affected by Fe^{3+}. This pointed out a unique property of the parasite enzyme. The Fe^{3+} effect was reversible and could also be seen in the reduction of basal activity using specific iro chelators, desferoxamine and nitrilotriacetic acid. This specific response to Fe^{3+} might be physiological importance in parasite-nost interaction since the parasite constantly derives iron from digesting hemoglobin of the red cell as well as from the circulation. In contrast, the Fe^{3+} effect were not observed in exogenous activities assay except a slight activation of histone phosphorylation.

Characterization and identification of endogenous protein substrates were carried out by SDS-PAGE analysis. The major phosphorylated proteins in parasite cytosol had apparent molecular weight (M_r) of 59,000; 42,000; 34,000; 20,000; and 15,000 daltons respectively. These results were similar to those conducted on intact parasites. Phosphorylation of intact parasite with $^{32}P_i$ showed the phosphorylated proteins with M_r of 66,000; 55,000; 42,000; 34,000; and 25,000 daltons respectively.

Interaction of antimalarial drugs with plasmodial protein kinase was also investigated in this study. This study was undertaken to see if antimalarial drugs interfered with the regulatory processes in the parasite. This involved study the dose-response of parasite protein kinase to various antimalarial drugs. Quite a few of these drugs showed strong inhibition on the parasite enzyme in the range of 10^{-7}M. This was considered significant in terms of the potent effect at low concen-

tration of drugs. Among the most effective drugs were: pyrimethamine, 8-hydroxyquinoline, primaquine and artesunate. The effect of these drugs were quite specific since protein kinase from other sources were not affected. In addition, the effect of certain drugs could be re reversed by Fe^{3+}, a potent activator of the parasite enzyme. The non-specific action of antimalarial drugs might be ruled out from the study of two marker enzymes, Ca^{2+} -ATPase on mouse erythrocyte membrane and lactate dehydrogenase in parasite cytosol. Neither enzyme was affected by the drugs even at 10 times higher concentration. The significance of this study may have certain implication for the study of antimalarial action of drugs currently used as well as the ones to be developed.