ABSTRACT

Thymidylate synthetase is a key enzyme for DNA synthesis especially in the malarial parasites where dTMP is synthesized *de novo*. It is an enzyme closely linked in function with dihydrofolate reductase; tetrahydrofolate required for reductive methylation of dUMP by thymidylate synthetase to form dTMP must be obtained through NADPH-dependent reduction of dihydrofolate by dihydrofolate reductase.

In this study, thymidylate synthetase and dihydrofolate reductase were purified by using Sephacryl S-300 and Sephadex G-200 column chromatography to 9 and 22-fold with 35% and 85% yield, respectively. Both enzymes were copurified on Sephacryl S-300 and Sephadex G-200 column with apparent molecular weight of 132,000 daltons and they also comigrated on polyacrylamide gel electrophoresis under non-denaturing condition. The molecular weight of thymidylate synthetase was estimated to be 65,000 on SDS-polyacrylamide gel electrophoresis.

Thymidylate synthetase was very unstable at -20 °C or 4 °C when compared to dihydrofolate reductase and the enzyme activity could not be preserved in 1mg/ml BSA,10%glycerol,0.1mM PMSF,0.1% Triton X-100 or 20mM DTT. Both enzymes had similar pH optimum of 6.5-8.5. Thymidylate synthetase was inhibited by KCl,NaCl and urea whereas dihydrofolate reductase was activated by these agents. Both enzymes were inhibited by 5,5'-dithiobis-(2-nitrobenzoic acid),p-chloromercuribenzoate and N-ethylmaleimide, however, iodoacetamide had no effect on dihydrofolate reductase.

For kinetic studies, the mechanism of action of thymidylate synthetase appeared to be a random sequential type with K_m of 71 and 312 μM for dUMP and 5,10-methylenetetrahydrofolate, respectively. Dihydrofolate reductase displayed an ordered sequential type of mechanism with K_m of 4.4 and 12.5 μM for dihydrofolate and NADPH, respectively.

Inhibition of dihydrofolate reductase by pyrimethamine, methotrexate and trimethoprim were found to be competitive with dihydrofolate with K_{is} of 0.63,0.5 and 1.88 nM, respectively.

Thymidylate synthetase was partially inhibited by TMP and 5-methyltetrahydrofolate,substrate analogues. It was also partly inactivated by some methyltransferase inhibitors such as tubercidin, cordycepin and S-adenosyl-L-homocysteine. FdUMP was found to be a potent competitive inhibitor for thymidylate synthetase with K_{is} of 0.05 μM. Methotrexate also inhibited this enzyme, however, the inhibition was uncompetitive with dUMP or 5,10-methylenetetrahydrofolate, with K_{ii} of 103 and 23 μM, respectively. Inhibition of thymidylate synthetase by FdUMP was not potentiated by methotrexate and *vice versa*, but a slight antagonistic effect was observed.

Modification of dihydrofolate reductase active site by N-bromosuccinimide affected catalytic activity of thymidylate synthetase as well as that of dihydrofolate reductase. Similar finding was observed upon modification of thymidylate synthetase active site by phenylglyoxal.

BIOGRAPHY

Name: SA-NGA PATTANAKITSAKUL

Date of Birth: October 4, 1956

Place of Birth: Bangkok, Thailand

Institutions attended:

Sathorn Widhaya School, Bangkok,

March, 1973

Certificate of Mathayom Suksa III

Wat Dhat Thong School, Bangkok,

March, 1975

Certificate of Mathayom Suksa V

Mahidol University, Faculty of Medical Technology, Bangkok,

March, 1979

Bachelor of Science(Med.Tech.)