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 $\mu$M 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 $\mu$M for dihydrofolate and NADPH, respectively.

Inhibition of dihydrofolate reductase by pyrimethamine, methotrexate and trimethoprim were found to be competitive with dihydrofolate with $K_i$ 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 $K_i$ of 0.05 $\mu$M. Methotrexate also inhibited this enzyme, however, the inhibition was uncompetitive with dUMP or 5,10-methylenetetrahydrofolate, with $K_i$ of 103 and 23 $\mu$M, 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.
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