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Studies of Mass Modulated Enzymes with Purified Methylenetetrahydrofolate

T. Abeysinghe[1*], A. Kohen[2]

The potential of the folic acid biosynthesis pathway as a target for the development of antibiotics and chemotherapeutic drugs has been recognized for many years and validated by the clinical use. One function of folic acid metabolism is the support of DNA synthesis and repair through the generation of nucleic acid building blocks such as thymidine triphosphate (dTTP). This process involves the last step of *de novo* synthesis of a precursor of DNA, 2'-deoxythymidine-5'-monophosphate (dTMP) with thymidylate synthase (TSase EC 2.1.1.45) using the cofactor (6*R*)-N^5,N^{10}-methylene-5,6,7,8-tetrahydrofolate (CH_2H_4folate). Therefore, the isotopically labeled R-[6-^{x}H]-CH_2H_4F (where ^{x}H: hydrogen, deuterium or tritium) allows studying the contribution of fast protein dynamics to a variety of kinetic steps along the catalytic cascade of natural vs. mass modulated *Escherichia coli* TSase. Ultimately, a better understanding of the catalytic mechanism of TSase can assist in developing more effective drugs that selectively act on cancerous cells therefore having reduced toxicity.

Generally, HPLC remains as the main purification method of the synthesized R-[6-^{x}H]-CH_2H_4F due to high sensitivity. However, it is quite laborious and the high salt content in the purified sample interferes with the NMR analysis. In this regard, we developed and optimized a simple procedure for the purification of the R-[6-^{D}H]-CH_2H_4F (D= deuterium) after its synthesis using solid-phase extraction with a strong anion exchange (SAX) cartridge followed by a C-18 cartridge. The purified 6R-^{D}H-CH_2H_4folate was used to investigate whether the mass modulation of the enzymes changes the rate-limiting step for the reaction. The kinetic studies indicated that the turn over number, k_{cat} is no longer rate limited by the hydride transfer in mass modulated TSase chemical cascade.

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[1] The Open University of Sri Lanka, Nugegoda, Sri Lanka *dtabe@ou.ac.lk
[2] The University of Iowa, Iowa City, USA