CALIFORNIA STATE SCIENCE FAIR
2003 PROJECT SUMMARY

Name(s)

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Project Number

S0418

Project Title

Effect of Natural and Synthetic Substances on Transformation of dUMP into dTMP

Abstract

Objectives/Goals

Thymidylate synthase (TS) is the enzyme that catalyzes the transformation of deoxyuridine monophosphate (dUMP) into deoxythymidine monophosphate (dTMP) in cells. In cancer cells, TS is overexpressed. While usual drugs treat cancer by inhibiting TS, the New-Biotics' drug is a substrate of TS that produces toxic products upon TS activation. When the drug enters the cell it is transformed into bromovinyl deoxyuridine monophosphate (BVdUMP), a substrate for TS. Both dUMP and BVdUMP reactions require certain thiols, although other thiols inactivate TS, as researched in previous project. The natural reaction requires a cofactor, methylene-tetrahydrofolate (CH(2)-THF), but the BVdUMP reaction does not. The effect of different natural compounds on TS while it catalyses dUMP transformation to mimic its intracellular BVdUMP activation during chemotherapy was studied.

Methods/Materials

Different concentrations of CH(2)-THF were used while changing the concentrations of BVdUMP and dUMP, to find the effect of CH(2)-THF on BVdUMP inhibition of TS. Then irriversible inactivation of TS by BVdUMP was studied by preincubation the BVdUMP and enzyme and adding it to the reaction mixture.

Results

It was found that BVdUMP and dUMP are competitive substrates, so BVdUMP appears to act as an inhibitor for the natural reaction. It was also discovered that the higher the concentration of CH(2)-THF, the less the effect of the BVdUMP on the dUMP reaction. The irreversibility of TS inactivation by BVdUMP was also studied and it was discovered that for the first 30 minutes the inactivation is reversible and does not harm the enzyme, TS. The inactivation becomes irreversible over time. In the presense of CH(2)-THF the irreversible inactivation of TS by BVdUMP was less extreme than its absense.

Conclusions/Discussion

Since BVdUMP and dUMP are competitve substrates, they bind at the From this it was concluded that at higher CH(2)-THF concentration, BVdUMP is bound less tightly to the enzyme, meaning that they have mutually exclusive binding sites on the enzyme. CH(2)-THF also acts as a protector against irreversible inactivation of TS by BVdUMP.

Summary Statement

New Biotics' drug, an alternative substrate of TS that competes with the natural substrate, is affected by CH(2)-THF and irreversibly inactivates TS only at long incubation times.

Help Received

Used lab equipment at New Biotics, Inc. under the supervision of Dr. Maria Sergeeva.