CALIFORNIA STATE SCIENCE FAIR
2002 PROJECT SUMMARY

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Project Title
Effect of Thiols on Transformation of dUMP into dTMP

Abstract

Objectives/Goals
Thymidylate synthase (TS) catalyzes the reaction transforming dUMP into dTMP in cells. In cancer cells, TS is expressed at an increased level. While usual drugs treat cancer by inhibiting TS, the NewBiotics# drugs are substrates of TS that produce toxic products upon TS catalyzed reaction. At NewBiotics different thiols were added to the reaction of (E)-5-(2-Bromovinyl)-2#-deoxy-5#uridyl monophosphate (BVdUMP) catalyzed by TS. The two non-natural ones, mercapotoethanol (ME) and dithiothreithol (DTT), worked to give desired toxic products. The natural thiol found in cells, glutathione (GSH), did not support the reaction. In the current work I studied the natural reaction of the conversion of dUMP into dTMP catalyzed by TS in the presence of different thiols to explain their effect on the BVdUMP reaction.

Methods/Materials
To evaluate the effect of thiols on the enzyme I studied the natural TS reaction converting dUMP into dTMP in the presence of six thiols: mercapotoethanol (ME), dithiothreithol (DTT), glutathione (GSH), N-Ac-cysteine (Ac-Cys), cysteine (Cys), and cysteine methyl ester (Cys-OMe). I used High Performance Liquid Chromatography to monitor the reaction.

Results
Four of the thiols (ME and DTT, and # to a lesser extent # Cys, and Cys-OMe) supported the reaction, and in the presence of the other two thiols (GSH and Ac-Cys) there was no reaction. After identifying the thiols that worked, I changed the enzyme concentrations to get more accurate results. I then chose to study Cys-OMe further, because it was natural and supported the reaction, and tested this thiol at different concentrations in the reaction. I found out that the higher concentration of the thiol, the less amount of dTMP was formed. I also preincubated the TS and Cys-OMe, and I found out the amount of dTMP formed was decreased.

Conclusions/Discussion
I concluded that all of the thiols I tested with the exception of DTT and ME inactivate TS.

Summary Statement
The TS catalyzed reaction of the NewBiotics' drug did not occur in the presence of GSH because certain thiols, including GSH, inactivate the enzyme.

Help Received
Used lab equipment at NewBiotics, Inc under the supervision of Dr. Maria Sergeeva