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Title (English)

# Structural determinants for azathioprine activity of glutathione transferase identified by screening of mutant libraries

Title (Swedish)

Abstract

Screening of a glutathione transferase (GST) alpha class library identified chimeras with varying azathioprine activity and a structure-function relationship analysis ascribed part of the C-terminal helix as a determining factor for azathioprine activity. Azathioprine was recently shown to be activated by GSTs and enzymes with improved activity towards the prodrug azathioprine could be used in gene transfer applications. The library was constructed by shuffling of five parents, as well as a GST library with random H-site mutations. Screening of 1570 clones was performed by spectroscopic measurement of lysates at 320 nm during the reaction with azathioprine and a high-throughput method using 96-well plates for cultivation was optimized. Ten examined chimeras, with on average three parents, three crossovers and less than one point mutation, were purified by IMAC and their activities with azathioprine were shown not to be correlated with that of MCB, another GST substrate. The six most active enzymes had more than 20 % of the hA2-2 activity and a sequence identity of 85-99 %, with DNA originating from all parents. Thus, the first generation contained GSTs with enough activity and variation for continuing with a subsequent generation. Of nine recombined segments, the sequence of one segment was differing between the six most active enzymes and the other ten, and this segment, at positions 207-219, forms most of the C-terminal helix, with residues 208, 213 and 216 forming one wall of the H-site. By keeping this region constant, library sizes in later steps of directed evolution can be decreased while increasing the fraction of active GSTs. Alternatively, these key residues can be targeted for mutagenesis.

Keywords

Directed evolution, gene shuffling, glutathione transferase, prodrugs, azathioprine

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