Section «Bioengineering Bioinformatics»

Bioinformatic analysis of glutaryl acylases reveals key residues responsible for substrate discrimination

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Glutaryl-7-aminocephalosporanic-acid acylases (GA) catalyse selective cleavage of the side chain while leaving cephalosporanic nucleus intact. Glutaryl-7-aminocephalosporanic acid (GACA) is the most specific substrate whereas structurally similar natural antibiotic cephalosporin C (CephC) is not hydrolyzed by this enzyme [1]. Analysis of structural determinants of substrate discrimination can help to understand molecular mechanisms of catalysis and design new functional properties.

Enzymes within Ntn-hydrolase superfamily homologous to *Brevundimonas diminuta* GA were identified in UniProt databank, resulting in 1074 sequences. Corresponding sequence alignment was built and studied. Bioinformatic analysis [2] was used to identify subfamily-specific positions (SSPs) in *B. diminuta* GA that seem to be responsible for the enzyme's substrate specificity. Molecular modeling was used to study the impact of SSP residues on binding and catalytic conversion of selected substrates. Glutaryl group of GACA was found to be bound in the site composed of SSP residues that outlines their relevance for substrate discrimination. These residues were used as hotspots to introduce specificity to CephC in the *B. diminuta* GA and the corresponding *in silico* library of 5645 variants containing single, double, triple or quadruple substitutions was constructed. Computer screening was applied to select reactive enzyme-substrate complexes that satisfy knowledge-based criteria of amidase catalytic activity. Consequently, GA variants with substitutions Y->E, F->L and F->V were selected to significantly improve affinity to CephC by providing favorable electrostatic interactions to accommodate D-α-aminoadipic group of the substrate.

Bioinformatic analysis was used to study molecular mechanism of substrate specificity in GA family of enzymes and identify a set of amino acid residues responsible for substrate discrimination in the active site. Mutants of *B. diminuta* GA with improved selectivity towards CephC were identified by molecular modeling and recommended for experimental evaluation.

References

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Words of gratitude

We thank our supervisor Prof. Dr. Sci. Vytas Švedas for fruitful discussions. This work was supported by the Russian Foundation for Basic Research grant # 12-08-01163-a.