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Recombinant enzymes for biotransformation of cephalosporin antibiotics

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D-amino acid oxidases (DAO) and glutaryl-7-ACA acylases (GLA) are industrial enzymes widely used for production of semisynthetic cephalosporin antibiotics. We have developed highly efficient expression systems for production of *Trigonopsis variabilis* DAO and *Brevundimonas diminuta* GLA as fusions with N-terminal chitin binding domain enabling single-step affinity purification of recombinant enzymes. Generation of new GLA analogs with improved stability and activity is a promising route towards optimization of current biocatalytic processes. For the search of GLA mutants with altered substrate specificity bioinformatics analysis and molecular modelling were performed to identify amino acid positions responsible for substrate binding and formation of reactive substrate-enzyme complex. The mutant enzymes were expressed, purified and shown to possess catalytic activity towards cephalosporin C. In order to study the peculiarities of GLA quaternary structure formation amino acid residues forming inter subunit interface within the estimated enzyme tetramer were identified and mutated to alanines. Mutant enzymes had reduced activity and stability thus confirming the role of identified aminoacids in the formation of the presumed contact area between the two ab - dimers. Dynamic light-scattering and enzyme cross-linking studies have shown that in solution the wild-type enzyme and its "interface" mutants have complex oligomeric structure with predominance of tetrameric forms. Experiments with expressed individual GLA subunits have enabled us to suggest that formation of native quaternary enzyme structure occurs in the course of synthesis, processing and interaction of nascent enzyme subunits.

Biography

Michael Eldarov is the Group leader, Section of Fungal Genetic Engineering, Assistant Professor at the Department of Biotechnology, Biological faculty, Moscow State University. He is an expert in yeast and fungal genetic engineering, protein engineering, expression and purification, industrial enzymes, yeast genomics, plant virology, viral-like particles. He has published 66 scientific articles cited 386 times, Hi index-10.