Abstract

Penicillin G acylases (PGAs) belong among enantioselective enzymes catalyzing a hydrolysis of stable amide bond in a broad spectrum of substrates, often having high application potential. PGA^{Ec} from *Escherichia coli* and PGA^{A} from microorganism *Achromobacter* sp. CCM 4824 were used to catalyze enantioselective hydrolyses of seven selected N-phenylacetylated (N-PhAc) α/β-amino acid racemates. The PGA^{A} showed higher stereoselectivity for three (*S*) enantiomers: N-PhAc-β-homoleucine, N-PhAc-α-*tert*-leucine and N-PhAc-β-leucine. We have constructed a homology model of PGA^{A} that was used in molecular docking experiments with the same substrates. *In-silico* experiments reproduced the data from experimental enzymatic resolutions confirming validity of employed modeling protocol. We employed this protocol to evaluate enantiopreference of PGA^{A} towards seven new substrates with application potential. For five of them, high enantioselectivity of PGA^{A} was predicted for.

PGA^{A} was further studied in kinetically controlled syntheses of β-lactam antibiotics (SSBA). The PGA^{A} was significantly more efficient at synthese of ampicillin and amoxicillin (higher S/H ratio and product accumulation) compared with PGA^{Ec}. Analogously to prediction of enantioselectivity of PGA^{A} towards new substrates this protocol was applied for SSBA syntheses catalyzed by PGA^{A}.

To improve parameters of SSBA syntheses, we performed *in-silico* directed modification of PGA^{A} at position Phe24β and Phe146α. As a promising modification appeared to be PGA^{A} Phe24β→Cys. The distance between the NH_2 group of nucleophile and carbonyl group of acyl-donor dropped from 4.1 Å to 3.3 Å for amoxicillin. We subsequently constructed pga^{A} gene encoding this amino acid modification. The gene was expressed and recombinant PGA^{A}Pheβ24Cys was purified. The enzyme exhibited very similar values of all tested parameters in syntheses of amoxicillin and ampicilin compared with PGA^{A}.

The recombinant PGA^{A}Pheβ24Cys was further tested in the synthesis of amoxicillin under industrial conditions 1) at a concentration of nucleophile above 400 mM and the molar ratio AD / N 1.05 and 2) at a concentration of nucleophile of 160 mM and the same molar ratio.

Efficiency of stabilized Pheβ24Cys as CLEA was studied in synthesis of amoxicillin at nucleophile concentrations above 400 mM and the molar ratio AD / N 1.05. The value of S / H has increased compared with the soluble form of approximately 8% (from the value of 4.9 to 5.0). The degree of conversion increased greatly, from 22 to 61%.