EFFECTS OF HALOPHILIC PEPTIDE FUSION ON SOLUBILITY AND STABILITY OF D-PHENYLGLYCINE AMINOTRANSFERASE

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ABSTRACT

D-Phenylglycine aminotransferase (D-PhgAT) from *Pseudomonas stutzeri* ST-201 is useful for enzymatic synthesis of enantiomerically pure D-phenylglycine. However, its low protein solubility prevents its application at high substrate concentrations. With the aim of increasing protein solubility, N-terminus of D-PhgAT was genetically fused with short peptides (A_1 α-helix, A_2 α-helix, and ALAL which is a hybrid of A1 and A2) from a ferredoxin enzyme of a halophilic archaeon, *Halobacterium salinarum*. The fused enzymes A_1-D-PhgAT, A_2-D-PhgAT and ALAL-D-PhgAT displayed reduced p*I* and increased in solubility by 6.1-, 5.3-, and 8.1-fold in TEMP pH 7.6 storage, respectively, and 5-, 4.5-, and 5.9-fold in CAPSO pH 9.5 reaction buffers, respectively, compared to the wild-type enzyme (WT-D-PhgAT). In addition, all the fused D-PhgAT displayed a higher enzymatic reaction rate than the WT-D-PhgAT at all concentrations of L-glutamate monosodium salt used. The highest one, 23.82 ± 1.47 mM h^{-1}, was that obtained from having ALAL-D-PhgAT reacted with 1500 mM of the substrate. Moreover, the halophilic fusion significantly increased the tolerance of D-PhgAT in the presence of NaCl and KCl, slightly in favor of KCl, where under the same condition at 3.5 M NaCl or KCl all halophilic fused variants showed higher activity than WT-D-PhgAT. In addition a higher thermal stability has been seen in halophilic fused variants as the hydrophobicity (log *p*) of miscible organic solvents increased.

KEY WORDS: D-PHENYLGLYCINE AMINOTRANSFERASE / HALOPHILIC PEPTIDE FUSION / SOLUBILITY / STABILITY

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