Thesis Title	Purification and Characterization of a Stereospecific Transaminase for D-Phenylglycine Synthesis.
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ABSTRACT

A bacterium, identified as *Pseudomonas stutzeri* ST-201, was newly isolated from Thai soil under a screening program designed to search for microorganisms possessing an enzyme useful for synthesis of D-phenylglycine. When grown on a minimal medium containing D-phenylglycine as the sole carbon and nitrogen source, the bacterium produced D-phenylglycine aminotransferase (D-PhgAT) that converted D-phenylglycine to benzoylformate which was further degraded to other common metabolites. The D-PhgAT production by *Pseudomonas stutzeri* ST-201 was induced by

D-phenylglycine. Cell homogenate was prepared and the enzyme was purified by ammonium sulfate precipitation, isocratic phenyl agarose chromatography, LiChrospher TMAE 1000 anion-exchange chromatography and SigmaChrom HIC-Phenyl hydrophobic interaction chromatography. The purified D-PhgAT obtained was apparently homogeneous as analyzed by SDS-PAGE. The molecular weight (M_r) of the native enzyme was estimated to be 92,000. It was found to be composed of two identical subunits, each with a molecular weight (M_r) of 47,500. The isoelectric point (pI) of the native enzyme was 5.0. The enzyme catalyzed reversible ransamination reactions specific for D-phenylglycine or D-4-hydroxyphenylglycine in which 2-oxoglutarate was an exclusive amino group acceptor and was converted into L-glutamic acid. Neither the D- nor L-isomers of phenylalanine, tyrosine, alanine, valine, leucine, isoleucine or serine could serve as substrates. The enzyme was most active at alkaline pH with maximum activity at pH 9-10. The temperature for maximum activity was 35-45°C. The apparent K_M values for D-phenylglycine and for 2-oxoglutarate at 35°C, pH 9.5 were 1.1 mM and 2.4 mM, respectively. The transamination was found to proceed via a Ping Pong Bi Bi mechanism. The enzyme activity was strongly inhibited by typical inhibitors of pyridoxal phosphate-dependent enzymes.

The D-PhgAT obtained after the step of LiChrospher TMAE 1000 anion-exchange chromatography was relatively pure and free from other

interfering enzymes or substances, so it could be use for synthesis of D-phenylglycine. Experiments were set up for enzymatic synthesis of D-phenylglycine on the milligram scale using D-PhgAT. The reaction product could be isolated and purified by a simple non-chiral chromatographic method. The final product obtained was a white crystalline powder which was identified as enantiomerically pure D-phenylglycine.

The D-PhgAT found in the present study is a new enzyme that has not been purified and characterized before. This enzyme is of both academic and industrial interest because it possesses a characteristic "stereo-inverting" transamination activity which is unusual among aminotransferases known to date. It allows utilization of L-glutamate, a cheap amino-group donor, for enzymatic synthesis of enantiomerically pure D-phenylglycine or D-4-hydroxyphenylglycine in a single enzymatic transamination reaction step. Both D-phenylglycine and D-4-hydroxyphenylglycine are important side-chains that are in high demand for the β-lactam antibiotics industry.