Crystallographic Studies of D-Phenylglycine aminotransferase, an Enzyme with Possible Applications in Antibiotic Production


Beamline(s): X8C

Introduction: The D-Phenylglycine aminotransferase (D-PhgAT) from Pseudomonas stutzeri ST-201 catalyzes a transamination reaction between the stereochemically different amino acids, L-glutamate and D-phenylglycine (see Figure 1.). D-PhgAT, a homodimeric enzyme with a molecular mass of 49kDa per subunit, is a member of the pyridoxal-5'-phosphate-dependent enzyme family and is closely related to glutamate-1-semialdehyde aminotransferase (GSA-AT).

D-PhgAT can convert benzoylformate and p-OH-benzoylformate to D-phenylglycine and p-OH-D-phenylglycine, respectively. Chemical conjugation of D-phenylglycine to the penicillin nucleus, 6-aminopenicillanic acid (6-APA), produces ampicillin, while conjugation of p-OH-D-phenylglycine to 6-APA produces amoxicillin. Furthermore, conjugation of D-phenylglycine with the appropriate cephalosporin nucleus yields cephalixin, cephaloglycin, cefaclor, cefadroxil, or cefatrizine. Thus, the activity of D-PhgAT suits this enzyme for use in the semi-synthetic synthesis of β-lactam antibiotics. Penicillin G can be hydrolyzed enzymatically by penicillin G acylase to yield 6-APA and phenylacetic acid (PAA). PAA can then be subjected to a series of enzymatic conversions, including transamination by D-PhgAT, to give D-phenylglycine or p-OH-D-phenylglycine. Chemical means may then be employed to conjugate these side chains to the appropriate β-lactam nucleus. D-PhgAT, in conjunction with penicillin G acylase and other enzymes, could provide a cheaper, easier method for the semi-synthetic production of several β-lactam antibiotics, by taking advantage of enzymatic activities to prepare both the β-lactam nucleus and side chain. Currently, semi-synthetic production methods rely on difficult and costly organic synthesis of the drug side chain.

Results: D-PhgAT crystals (0.15 x 0.15 x 0.05mm) were obtained from phosphate buffer, pH 6.8 in the presence of 35% ammonium sulfate and 100mM NaCl. X-ray data collection was done during the RapiData 2001 Workshop at NSLS Beamline X8C, Brookhaven National Laboratory. The crystals belong to the primitive hexagonal space group P31, with a = b = 75.2, c = 142.8 Å, and they diffract to 2.1 Å resolution. In addition to the native data set, two additional data sets were collected. The first on crystals exposed to xenon pressurization for 30 minutes (λ=0.9971, 2.8Å resolution), and the second on a crystal soaked for 30 seconds in 1 M NaBr (λ=1.5Å, 3.5Å resolution). Initial phases have been obtained by the molecular replacement technique using the closely related paralogous enzyme, GSA-AT, as the search model. Also, SAD phasing techniques will be applied to the xenon and bromine data sets in an attempt to improve the phasing of the D-PhgAT structure.

Figure 1. Stereo-inverting aminotransferase reaction catalyzed by D-Phenylglycine aminotransferase (D-PhgAT) from Pseudomonas stutzeri ST-201.