Structure of Strictosidine Synthase

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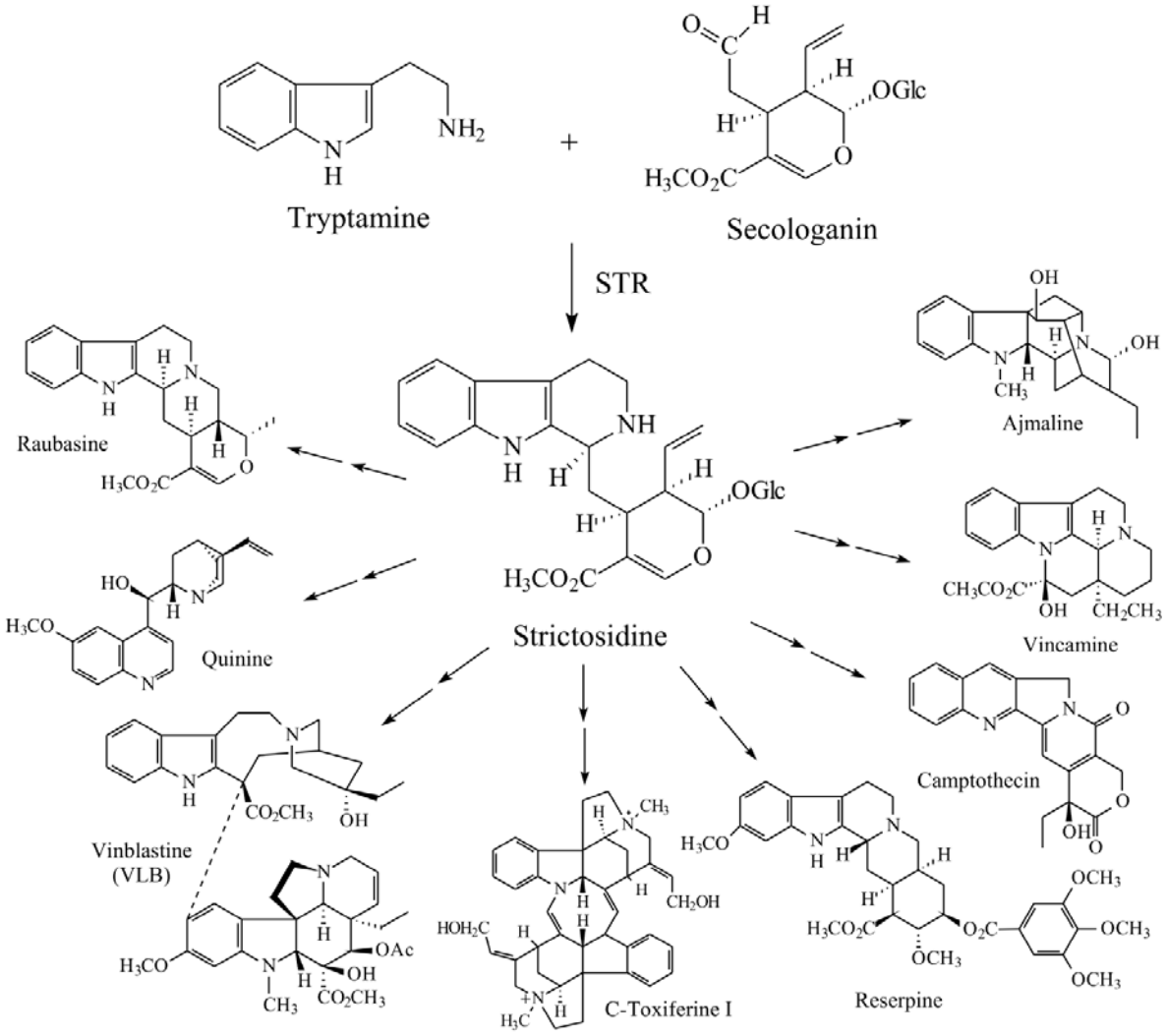
Strictosidine Synthase (STR1) is the first enzyme in the biosynthesis of the monoterpenoid indole alkaloid family consisting of about 2000 structurally most diverse plant compounds [1]. The synthase catalyzes the condensation reaction between the both early biogenetic precursors, the tryptophan decarboxylation product, tryptamine, and the monoterpene glucoside, secologanin [2]. The role of the enzyme product strictosidine in the biosynthesis of indole alkaloids is presented in the figure.
Our preliminary studies the crystals of STR1 diffracted to a resolution of 3.7 Å with unit-cell parameters a=b=147.3, c=122.3 Å. A self-rotational analysis of the native data set, performed with the program *CNS* yields a twofold rotational non-crystallographic symmetry (NCS) axis; thus the asymmetric unit contains two molecules of STR1 related by the found NCS axis with a relatively high solvent content of 67%. These data allowed to determine the rhombohedral space group R3 and gave first hints to the β-propeller fold of the enzyme [3,4]. The crystallization conditions were optimized further resulting in an enhanced resolution of 2.95 Å for the native enzyme [5]. Moreover, crystal complex with the substrate tryptamine were made and analyzed by X-ray [5]. The obtained crystals diffracted to 2.3 Å. All X-ray dataset were collected at EMBL Hamburg beamlines.

For solving the structure, the multiple wavelength anomalous dispersion (MAD) method was explored. Initial attempts to solve the structure of STR1 labelled with two selenomethiones failed. Thus, two mutants were constructed with four and six methionines and the crystals of these mutants were used for subsequent MAD experiments.

Our experiments in 2005 were concentrated on the final elucidation of the overall structure of STR1 and its analysis. The refinement of the structure resulted in R_{cryst}/R_{free} (%) of 18.9/23.6 for STR1-Native and 16.4/21.7 for the STR1 complex with tryptamine [6]. The structure with second substrate secologanin (STR1-SEL) was refined to a resolution of 3.0 Å with R_{cryst}/R_{free} (%) of 18.6/24.0.

The crystal structures of STR1 in complex with its natural substrates tryptamine and secologanin provided preliminary structural understanding of the observed substrate preference and identifying the residues lining the active site surface that contact the substrates. STR1 also represents the first 6-bladed β-propeller fold found in plant proteins. Structure-based sequence alignment revealed a common repetitive sequence motif (three hydrophilic residues are followed by a small residue and a hydrophobic residue) indicating a possible evolutionary relationship between STR1 and several sequence-unrelated six-bladed β-propeller structures [6]. Structural analysis and site-directed mutagenesis experiments have now demonstrated the essential role of Glu309 in catalysis.

The data obtained will provide an initial point for deciphering the details of the reaction mechanism of STR1 e.g. by generating further complex structures and mutation experiments. The data will help to describe other members of this enzyme family, especially because the STR1 structure is the first one in this particular protein family.



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