Vinorine synthase (VS, EC 2.3.1.160) catalyzes the acetyl-CoA-dependent reversible biosynthesis of the ajmalan-type alkaloid vinorine from the sarpagine-type alkaloid 16-epi-vellosimine (Fig. 1) [1,2]. By connecting biosynthetically two different types of alkaloids, vinorine synthase occupies a central role in the metabolism of alkaloids of the genus *Rauvolfia*. Vinorine is a direct biosynthetic precursor along the complex pathway to the monoterpene indole alkaloid ajmaline, an antiarrhythmic drug from the Indian medicinal plant *Rauvolfia serpentina*.

**Figure 1.** Reaction catalyzed by vinorine synthase (VS).

Vinorine synthase has been enriched from hybrid cell suspension cultures of *R. serpentina x Rhazya stricta* and functionally expressed in *Escherichia coli* [1,2]. The synthase consists of 421 amino acids and has a molecular weight of 46.8 KDa. Sequence analysis and mutagenesis studies suggested that the enzyme is a novel member of the BAHD superfamily of acyltransferases. BAHD (benzylalcohol acetyl-, anthocyanin-O-hydroxy-cinnamoyl-, anthranilate-N-hydroxy-cinnamoyl/ benzoyl-, deacetylvinodoline acetyltransferase) is the coined name from the first four enzymes of the family isolated from plant species [3]. Most members with known functions of this family are involved in plant secondary metabolism, including floral scent production, anthocyanin biosynthesis and especially in the biosynthesis of a number of therapeutically significant alkaloids such as morphine, taxol, vindoline and ajmaline. Moreover, more than 70 BAHD gene family members are estimated to occur in the model plant *Arabidopsis*, but their functions still await to be determined. Prominent features of this family are the HxxxD motif (motif 1) in the center of the protein and a DFGWG motif (motif 2) near the C-terminus. Currently there is no recognizable sequence homology between this family and known protein structures. Thus, detailed information about the 3D-structure of VS would be important for both understanding the molecular mechanism of the catalytic process and the function of conserved residues in the BAHD superfamily.

Recently, VS and selenomethionyl VS has been crystallized with ammonium sulfate and PEG 400 as precipitant buffer [5,6]. Datasets were collected on beamlines of the EMBL-outstation in Hamburg. The crystals formed in space group P2_12_1 with two molecules in the asymmetric unit. The SeMet crystals diffraction to 3.2-Å resolution and native VS crystals diffraction to 2.6-Å. The structure of VS at 2.6-Å [7] has been solved by the multiwavelength anomalous diffraction (MAD) protocol of the EMBL-Hamburg automated crystal structure determination platform [8].
The structure of VS contains 14 β strands (β1-β14) and 13 helices (α1-α13), which can be arranged into two equally sized domains 1 and 2 (Fig. 2). Domain 1 and domain 2 share a very similar polypeptide backbone fold, however the topology is different. It can be aligned to within 3.1 Å over 85 amino acids. But this structural homology includes only the core of the two domains which consists of the six-stranded β sheet and two α helices (α2 in domain 1 and α9 in domain 2). The sequence identity among these aligned positions is rather low, with only 7 pairs of identical residues (8.2 %). On the basis of the structural alignment and sequence motifs present in the protein it is evident that VS is a novel member of the CoA-dependent acyltransferase family. The closest structure to VS is the polypeptide synthase associated protein 5 (Pap5, PDB code 1q9j) from *Mycobacterium tuberculosis*, which could be aligned to 2.58 Å r.m.s.d. over 277 amino acids with 14% sequence identity. Other proteins aligned to VS structure includes condensation domains of vibriobactin synthetase (VibH, PDB code 115a, 3.5 Å r.m.s.d. over 262 amino acids with 7 % sequence identity), Rat choline acetyltransferase (PDB code 1g6x, 3.7 Å r.m.s.d. over 244 amino acids with 10% sequence identity), and Human carnitine acetyltransferase (CRAT, PDB code 1nm8 3.6 Å r.m.s.d. over 236 amino acids with 10% sequence identity).

The solvent channel runs through the VS molecule and is formed between the two domains by two loops protruding from domain 2 to domain 1. The DFGWG and GN motif, respectively, in the first and second loop are absolutely conserved in the BAHD superfamily. The HxxxD motif in VS structure is located at the interface between the two domains and the catalytic His160 residue in HxxxD motif is accessible from both sides of the channel. Surprisingly, the DFGWG motif, which is indispensable for the catalyzed reaction and which is unique to the BAHD family, is located far from the active site and occupies a structural role.

VS represents the first structure solved belonging to the BAHD superfamily.

References: