

Structural and functional studies of Serine Palmitoyltransferase

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Sphingolipids are essential components of eukaryotic membranes and are potent signalling molecules. Serine palmitoyltransferase (SPT) is a pyridoxal 5'-phosphate (PLP)-dependent enzyme which catalyses the decarboxylative, Claisen condensation between L-serine and palmitoyl-CoA. This is the first enzyme in the biosynthetic pathway and the product, 3-ketodihydroshingosine (KDS), is further metabolized to various sphingolipids and ceramides via complex, multi-step pathways. Since ceramides have been shown to be important regulatory molecules they have attracted a great deal of interest as pharmaceutical targets. Recently we determined the high-resolution x-ray crystal structure of the first SPT from any organism – that of the bacterium *Sphingomonas paucimobilis* (1). This bacterial SPT is a soluble homodimer encoded by a single gene whereas the eukaryotic SPTs are membrane-associated heterodimers encoded by two genes (*lcb1* and *lcb2*). Of interest is the link between mutations in *lcb1* and a rare hereditary sphingolipid disorder, Hereditary Sensory and Autonomic Neuropathy type I (HSAN1) which leads to degradation of the distal nerves in the limbs. Analysis of HSAN1 patients revealed two mutational hotspots in *lcb1* at positions Cys133 (C133W or C133Y) and Val144 (V144D). We used our bacterial SPT structure to model where these mutations would map onto the human enzyme. The equivalent residue to Cys133 in the *S. paucimobilis* SPT is N100 – which is in the vicinity of the active site, but does not appear to play a role in catalysis. To probe the effect of mutations at this position we have isolated SPT N100C, N100W and N100Y mutants and have studied their catalytic activity, spectroscopic properties and high resolution structures. These results provide an interesting insight not only to the possible cause of the HSAN1 phenotype but of the SPT mechanism in general.

(1) Yard, et al., *J. Mol. Biol.*, (2007), **370**, 870-886.