Poster 6

Active Sites and Substrates Binding Mode of Malonyl-CoA Synthetase Determined by Transferred Nuclear Overhauser Effect Spectroscopy, Site-directed Mutagenesis and Comparative Modeling Studies

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Active sites and substrate bindings of *Rhizobium trifolii* molonyl-CoA synthetase (MCS) catalyzing the malonyl-CoA formation from malonate and CoA with the hydrolysis of ATP has been determined based on nuclear magnetic resonance (NMR) spectroscopy, site-directed mutagenesis and comparative modeling calculations. The enzyme-bound conformation of malonyl-CoA was calculated from NOE data collected from 2D-transferred NOESY spectra. MCS model for homology modeling consisted of 16-helices and 24-strands including active site loops. The core activity site of MCS was determined in a wide cleft close to N-terminal domain. The catalytic substrate malonate is also found between ATP and His206 in MCS/ATP/malonate complex structure, supporting the catalytic role of His206 for generating reaction intermediate, malonyl-AMP. These findings are strongly supported by site-directed mutagenesis data and explain the structural role of conservative residues of adenylate-forming enzymes including the specific mechanism of substrate bindings.