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NMR and CORCEMA Studies on Malonyl-CoA Synthetase and Its Substrate, Malonyl-CoA

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Rhizobium trifolii malonyl-CoA synthetase (MCS) which is encoded with a polypeptied of 504 residues, catalyzes malonyl-CoA formation directly from malonate and CoA with the hydrolysis of ATP. MCS plays a critical role in nitrogen flow from bacteroids to plant cells with malonamidases. In addition, the steady-state kinetic mechanism of MCS from R. trifolii and B. japonicum was determined as ordered in a Bi Uni Uni Bi Ter Ter system, based on studies of initial vilocity, product inhibition, and intermediate identification. The conformation of Malonyl-CoA bounded to MCS was determined by the transferred two-dimensional nuclear Overhauser effect spectroscopy (TRNOESY) and molecular dynamics simulations. The structure of Malonyl-CoA could be more refined through TRNOESY and NOESY than through NOESY only. And we undertook a more quantitative analysis of some of the intralignad tr-NOEs using CORCEMA program. These experimental conditions corresponded to null NOE for the free ligand, yielding a correlation time of 0.20 ns. For the complex, data set consisting of 4 tr-NOEs was used. Using complete mixing time curves of three intra-TrNOESY peaks between the MCS and malnoyl-CoA, this self-consistent analysis determined the correlation time of the bound species ($_B$ = 20 ns) and the exchange off-rate (k_{off}=0.65 ms) of the malnoyl-CoA. In addition, the analysis estimated the correlation time of the free species ($_F$ = 0.2 ns).