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Crystal Structure, Molecular Modeling and Catalytic Mechanism of the Highly Toxic Sphingomyelinases D from Spider Venoms. M.T. Murakami[1], M.F. Fernandes-Pedrosa[2], D.V. Tambourgi[2], R.K. Arni[1], [1]Dept. of Physics, IBILCE/UNESP, São José do Rio Preto, Brazil, [2]Immunochemistry Laboratory, Butantan Inst., São Paulo, Brazil.

Sphingomyelinases D (SMases D) isolated from *Loxoceles* spider venom are the main proteins responsible for the manifestation of dermonecrosis, intravascular hemolysis and acute renal failure which can eventually result in death. These enzymes catalyze the hydrolysis of sphingomyelin resulting in the formation of ceramide 1-phosphate and choline or the hydrolysis of lysophosphatidyl choline generating the lipid mediator lysophosphatidic acid. The crystal structure of a member of the sphingomyelinase D family from *L. laeta* (SMase I) has been determined at 1.75Å resolution using the `quick cryo-soaking´ technique and phases obtained from a single iodine derivative and data collected from a conventional rotating anode X-ray source. The crystal structure of this enzyme has been determined both in the presence and absence of SO^{4-} and also complexed with Mg^{2+}, Ni^{2+}, Gd^{2+} and phospholipid analogues. SMase I is a $(\alpha/\beta)_8$ barrel enzyme and the interfacial and catalytic sites encompass hydrophobic loops and a negatively charged surface. The structures of SMases D from *L. boneti* and *L. intermedia* which contain a loop stabilized by an additional disulfide bridge have been modeled. Substrate binding and/or the transition state are stabilized by an Mg^{2+} ion which is coordinated by E^{32}, D^{34}, D^{91} and solvent molecules. In the proposed acid base catalytic mechanism, H^{12} and H^{47} play key roles and are supported by a network of hydrogen bonds between D^{34}, D^{52}, W^{230}, D^{233} and N^{252}.