Synergistic insights from crystallographic and microcalorimetric studies in solution: a case of *R. etli* asparaginases

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L-Asparaginases hydrolyze asparagine into ammonia and aspartic acid. They are divided into 3 structural classes and can exhibit substrate affinity ranging from low micromolar to high millimolar, as well as co-activities towards other substrates, such as L-glutamine or β-aspartyl peptides. There are also reports about their ability to process substrates like urea or acrylamide. Certain asparaginases are of medicinal or industrial importance as powerful drugs in the treatment of acute lymphoblastic leukemia or for reducing harmful acrylamide levels in processed food by decreasing the asparagine pool before heat processing. The focus of our studies is on rhizobial representatives of the novel Class 3 asparaginases (the constitutive ReaIV and inducible ReAV), which are metalloenzymes with no similarity to other asparaginases. As research tools, we combine X-ray crystallography with microcalorimetry (ITC), which has become a widespread method for studying not only molecular interactions but also enzyme kinetics. Such approach not only helped to structurally explain the nature of serendipitous acrylamide reaction of ReAIV monitored by ITC, but also helped to establish conditions sufficient to obtain a crystalline complex of ReAV with a substrate or explain the differences in the effect of the reaction products on the kinetics of both isoforms.