论文题目： 尖吻蝮蛇毒金属蛋白酶结构、酶失活及蛋白水解机理的研究

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中文摘要

尖吻蝮蛇毒金属蛋白酶 acutolysin A 具有强烈的出血作用，它是一种低分子量金属蛋白酶，分子量为 22kDa，以 adamalysin II 分子为模型用分子置换法测定了 acutolysin A 在 PH7.5 和 PH5.0 条件下的两种晶体结构并分别在 1.9Å 和 2.6Å 分辨率下进行了结构修正。最终晶体学 R 因子分别为 0.183 和 0.176 键长、键角均方偏差分别为 0.012Å,1.4° 和 0.014Å、1.5° 。根据其电子密度图并参考其它信息确定了 acutolysin A 的氨基酸残基序列。Acutolysin A 含三对二硫键，是国际上第一个三队二硫键型蛇毒金属蛋白酶的代表模型。与已知空间结构的设毒金属蛋白酶 adamalysin II 和 atrolysin C 不同的是 acutolysin A 分子中的锌离子为五配位，新发现的第五配基（一个水分子）为酶催化水解反应提供了必需的质子。这是在蛇毒金属蛋白酶中首次发现的质子供体，从而完整地阐明了作用机理。不同 PH 条件下 acutolysinA 空间结构的比较揭示出此类酶酸性条件下的可能原因。

同时，本文还用了分子置换法测定了同种蛇毒中碱性出血毒素 acutolysin C 的晶体结构并在 2.5Å 分辨率下进行了初步修整。 结果表明 acutolysin C 也属于间质金属蛋白酶超家族，但在钙离子的结合方面与其它蛇毒金属蛋白有着明显区别。

Abstract

Acutolysin A from the venom of Chinaese Five-Paced snake (Agkistrodon acutus),is a stronghemorrhagin and a small size zinc-metalloproteinase with molecular weight of 22kDa. Its two crystal structures were determined at pH5.0 and pH 7.5 by molecular replacement using the adamalysin II structure as the test model and refined at 1.9Å and 2.6 resolution to the crystallographic R factors of 0.183 and 0.176, r.m.s deviations of bond lengths of 0.012Å and 0.014Å, and r.m.s deviations of bond angles of 1.4 and 1.5°, respectively. Mainly based on the electron densities, the crystallographic sequence of acutolysin A consisting of residues was determined. The crystallographic studies showed that acutolysin A had three disulfide bonds and could be the first crystal structure of the three-disulfide-bridge snake venom metalloproteinase. Unlike adamalysin II and atrolysin C, the zinc ion ligand, could act as the hydrogen donor for the hydrolysis reaction. The comparison of two acutolysin A structures at different pH proposed the possible reason reason that acutolysin A is inactive at the acidic condition.

In the mean time, the crystal structure of an alkaline hemorrhagin acutolysin C from the same

snake venom was also determined by molecular replacement and preliminarily refined at 2.5Å. Acutolysin C has been shown to be a member of Matrix Metalloproteinase Supper-family, too. But it could have some obvious differences from other snake venom metalloproteinase in their structures.