

位是不同的。它不需要金属离子的存在。

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Activity of Superoxide Dismutase on Cleaving Supercoiled Double Stranded DNA. LING Jun,

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Abstract Several superoxide dismutases (SODs), such as porcine and bovine erythrocyte Cu/Zn-SODs and *N. tabacum* Mn-SOD, were found to exhibit the activity to cleave supercoiled DNA *in vitro*. They converted supercoiled DNA into nicked and further into linear form. They did not act on linear double-stranded DNA. Activity assays after they were inactivated by H₂O₂ or guanidine and hydrolyzed by proteases suggested that these two activities of dismuting O₂⁻ and cleaving supercoiled DNA involved in different sites in SODs.

Key words porcine erythrocyte Cu/Zn-SOD, bovine erythrocyte Cu/Zn-SOD, *N. tabacum* Mn-SOD, supercoiled DNA-cleaving activity

五步蛇蛇毒类凝血酶 N 端的部分氨基酸序列

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摘要 从五步蛇蛇毒中纯化得到的类凝血酶, 在 SDS-PAGE 及 IEF 均为一条带, 且分子质量约 38 ku, 等电点约为 4.0。测定该酶 N 端 15 个氨基酸的序列是 VIGGVECDINEHRFL, 与其他的蛇毒类凝血酶有高度同源性。

关键词 五步蛇, 类凝血酶, N 端, 序列

蛇毒中的类凝血酶, 在体外引起血浆纤维蛋白原的凝聚, 起凝血作用; 但在体内因它不激活凝血因子 XIII, 所以由它水解而生成的纤维蛋白凝块, 没有侧链交联, 容易被纤维蛋白溶酶降解, 造成体内纤维蛋白原浓度降低而表现为抗凝效应^[1]。在临床上蛇毒类凝血酶已成为防治血栓栓塞性疾病的有效药物, 国外已有商品生产, 如 Ancrod, Batroxobin 等, 据统计至今为止已注册作为诊断和药用的有 7 个纯酶。

我们从江西产五步蛇 (*Agkistrodon acutus*) 蛇毒中分离得到纯的类凝血酶, 本文主要报道用 Edman 降解法在 PE 491 Protein Sequencer 中测定的该类凝血酶 N 端 15 个氨基

酸的序列。

1 五步蛇蛇毒类凝血酶的制备

五步蛇蛇毒经过 DEAE-Sepharose CL-6B 后, 收集具有精氨酸酯酶活力及凝血活力的部分, 依次再上 FPLC 的 Superose 12 柱及 Mono Q 柱, 得到了如图 1 所示对称的层析峰, 主峰面积大于 98%。此样品经 SDS-PAGE 检测为单一条带, 分子质量约为 38 ku。等电聚焦的结果也是一条带, 等电点约为 4.0。

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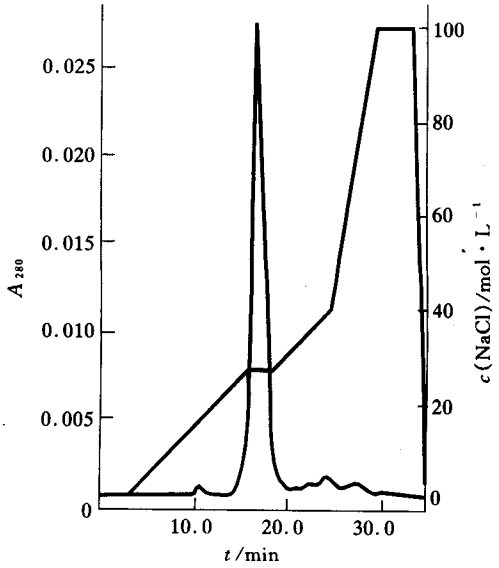


图 1 FPLC 上的离子交换层析

离子交换柱: Mono Q HR5/5; 流速: 0.8 ml/min;
洗脱液 A: 0.05 mol/L Tris-HCl, pH 7.8; 洗脱液
B: 0.05 mol/L Tris-HCl pH 7.8 + 1 mol/L NaCl.

2 该类凝血酶 N 端部分氨基酸序列

纯化的样品在 PE 491 Protein Sequencer
经过 15 个循环, 得到如下的 N 端序列:

1 5 10

Val Ile Gly Gly Val Glu Cys Asp Ile Asn Glu His

15

Arg Phe Lys

比较来自蝰科不同蛇种的类凝血酶 N 端
20 个氨基酸序列, 发现五步蛇蛇毒类凝血酶
与蝮亚科的红口蝮、矛头蝮及响尾蛇的同源性
最高; 与蝰亚科中角蝰、加蓬蝰蛇的同源性也
比较高, 尤其是 Glu6、Cys7 和 Ile9、His12 最
保守 (表 1)。

Ouyang^[2]早在 50 年代就发现五步蛇蛇毒
类凝血酶在体外高浓度时有促凝作用, 而在低
浓度时有抗凝作用, 同时指出类凝血酶对纤维
蛋白原作用后造成凝血, 此后国内也有关于五
步蛇毒中含有类凝血酶的报道^[3], 但他们都
侧重于研究其生物学效应及有关的药理、毒理
试验。另外, 国内在临床上广泛使用的蕲蛇
酶、清栓酶都是利用五步蛇蛇毒类凝血酶为主
要成分的抗栓药物, 至今为止还未见到对其一
级结构以及结构与功能关系的研究报道。

表 1 一些蛇毒类凝血酶的部分氨基酸序列

来 源	商品名称	残基数			
		5	10	15	20
五步蛇	Defibrase	VIGGVECDINEHRFL			
矛头蝮	Batroxobin	VIGGDECDINEHPFLAFMY			
红口蝮	Ancrod	VIGGDECNINEHRFLVALYN			
西部菱斑 响尾蛇	Crotalase	VIGGDECNINEHRFLVALY			
角蝰	Carastobin	VIGGAECNINEHRSIVLLY			
加蓬蝰蛇	Gabonase	VVGGAECKIDCHRCALLY			

我们实验室首次测定了五步蛇毒类凝血酶
N 端的一段氨基酸序列, 并将以此为起点深入
研究类凝血酶结构与功能的关系, 此外, 也为
从事分子生物学工作, 表达出高效无副作用的
基因工程产品打下基础。

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**Partial Amino Acid Sequence of N-terminal of
Thrombin-like Enzyme From *Agkistrodon
acutus*.** DU Xiaoyan, ZHU Hong, JIANG
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Abstract A thrombin-like enzyme was purified
from the venom of the snake *Agkistrodon
acutus*. It was shown to be a single band both in
SDS-PAGE and IEF. Its molecular weight is
about 38 ku and isoelectric point is near 4.0.
Also, its 15 amino acid sequence of N-terminal
was obtained. It is VIGGVECDINEHRFL. It
has high homology with thrombin-like enzymes
from other snake venoms.

Key Words *Agkistrodon acutus*, thrombin-like
enzyme, N-terminal, sequence