

# 海南捕鸟蛛毒素-I (HNTX-I) 的<sup>1</sup>H-NMR信号归属和二级结构分析

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海南捕鸟蛛毒素-I (HNTX-I) 是从海南捕鸟蛛(*Ornithoctonus hainana*)的粗毒中纯化的一种新型神经毒素。应用二维<sup>1</sup>H-NMR技术研究HNTX-I的溶液结构特点,通过分析水和重水中的DQF-COSY、TOCSY和NOESY谱,识别出HNTX-I全部33个氨基酸残基自旋体系;通过NOESY谱中的 $d_{\alpha N}$ 、 $d_{\beta N}$ 、 $d_{NN}$ 和 $d_{\alpha\delta}$ 联系完成了序列专一的谱峰归属,从而确认了HNTX-I所有的主链质子和大于96%的侧链质子的化学位移。并通过分析 $^3J_{NH-C\alpha H}$ 耦合常数、序列间的NOE联系以及慢氢交换质子等,确定HNTX-I的二级结构主要是由三股反平行的 $\beta$ -折迭组成(Lys7-Cys9, Tyr20-Asn23和Trp28-Val31),这些结构特点与已经探明结构的其它蜘蛛毒素的基本相同。这些结果为完全解析HNTX-I的溶液三维结构奠定了基础。

## SEQUENCE-SPECIFIC ASSIGNMENT OF <sup>1</sup>H-NMR RESONANCE AND DETERMINATION OF THE SECONDARY STRUCTURE OF HAINANTOXIN-I

Hainantoxin-I (HNTX-I) is an insect blocker of voltage-gated sodium channel isolated from the venom of the Chinese bird spider *Ornithoctonus hainana* (early named *Selenocosmia hainana*). The three-dimensional conformation of HNTX-I in aqueous solution was determined using two-dimensional <sup>1</sup>H NMR techniques. The complete sequence-specific assignments of proton resonance in the <sup>1</sup>H-NMR spectra of HNTX-I were obtained by analyzing a series of 2D spectra, including double-quantum-filtered correlation spectroscopy (DQF-COSY), total correlated spectroscopy (TOCSY) and nuclear overhauser effect spectroscopy (NOESY) in H<sub>2</sub>O and D<sub>2</sub>O. All the backbone protons and more than 96% of the side-chain protons were identified by  $d_{\alpha N}$ ,  $d_{\beta N}$ ,  $d_{NN}$  and  $d_{\alpha\delta}$  connectivities in NOESY spectrum. Furthermore, it was found that the main element of the secondary structure of HNTX-I is a short triple-stranded antiparallel  $\beta$ -sheet with Lys7-Cys9, Tyr20-Asn23 and Trp28-Val31, based on  $^3J_{NH-C\alpha H}$  coupling constants, sequential NOE connectivities and slowly exchanging amide protons. These characters of the secondary structure of HNTX-I are similar to those of HWTX-I, SHL-I and HWTX-IV, which have known solution structures. These results provide a basis for the further determination of the solution conformation of HNTX-I.

### 关键词

海南捕鸟蛛毒素-I (HNTX-I); 二维核磁共振(2D-NMR); 序列专一归属(Sequence-specific assignment); 二级结构(Secondary structure)