Sulfur SAD Structure of Heparin-Binding CRISP from *Naja atra* Reveals Protease and Ion Channel Blocking Domains

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Various cysteine-rich secretory proteins (CRISP) have been identified in diverse organisms with conserved sequences, including 16 of their cysteines. Although no clear evidence exists for a physiological function of mammalian CRISP found mainly in the epididymis and salivary glands, snake venom CRISP are known to inhibit smooth muscle contraction and cyclic nucleotide-gated (CNG) ion channels. The structure of CRISP-*a* from *Naja atra* is determined at 1.58-Å resolution using the sulfur-SAD method and consists of unique disulfide patterns and two distinct structural domains: a protease sandwich fold (N-terminal) and an ion channel-blocking BgK toxin fold (C-terminal). With one positively charged cluster found at water accessible helix regions next to the Ser-His-Glu triad of the protease domain, heparin binding plays a role in regulating CRISP-*a* activity. As important residues identified to block CNG and K^+ channels of other toxin homologues are located at one face of the ion channel-blocking domain, the structure provides a basis for rational design of a peptide blocker of the CNG channel. The ion channel-blocking domain and heparin-binding site of CRISP-*a* are suggested to play a chaperone role in leading it to the site of protease action for remodeling of the extracellular matrix in mammalian cells.

Keywords: sulfur-SAD, toxin CRISP structure, heparin