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Full length nucleotide sequence of a Factor V-like subunit of oscutarin from *Oxyuranus scutellatus scutellatus* (coastal Taipan)

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Abstract

An *Oxyuranus scutellatus scutellatus* venom gland cDNA expression library was screened with antivenom. Positive clones were isolated and their nucleotide sequences determined. The complete sequence for a Factor V-like component from the Taipan venom prothrombin activator, oscutarin (EC 3.4.21.60) (Walker, F.J., Owen, W.G., Esmon, C.T., 1980. Characterization of the prothrombin activator from the venom of *Oxyuranus scutellatus scutellatus* (Taipan venom). Biochemistry, 19(5), 1020–1023; Speijer, H.G.R., Zwall, J., Robert, F.A., Rosing, J., 1986. Prothrombin acitvation by an activator from the venom of *Oxyuranus Scutellatus* (Taipan Snake). J. Biol. Chem. 261, 13258–13267) was determined from the sequencing of antigenic cDNA clones. The cDNA sequence encoded a protein of 1460 amino acid residues, including a 30-residue signal peptide. This sequence shared 95% sequence similarity with the non-enzymic subunit of the prothrombin activator (pseutarin C) from brown snake (*Pseudonaja textilis*) venom. This sequence in turn has been reported to share high similarity with mammalian Factor V. Sequence comparisons indicated the size, charge and cleavage sites were conserved across the two species. This is the first nucleotide sequence of a Factor V-like component from *Oxyuranus* venom and the second sequence within Elapidae to be reported.

Keywords: Factor V; Oxyuranus; Nucleotide sequence

1. Introduction

Venoms from many species of Australian elapids show a strong coagulant activity (Mirtschin et al., 1984; King and Smith, 1991; Southern et al., 1996; Lalloo et al., 1997). These venoms contain Factor Xa-like proteins and, to a lesser extent, Factor Va-like proteins, plus prothrombin activating enzymes which are responsible for most of the disruption of blood chemistry observed in snakebite patients (Rosing and Tans, 1988; Joseph and Kini, 2002).

The genus *Oxyuranus* includes three of the largest and most feared Australo-Papuan elapid snakes, *Oxyuranus scutellatus scutellatus* (coastal Taipan, *O. s. scutellatus*), *Oxyuranus microleptidotus* (inland Taipan) and *Oxyuranus scutellatus canni* (Papuan Taipan). One of the consequences of envenomation by *Oxyuranus* spp. can be the clinically significant disseminated intravascular coagulopathy (DIC) (Trinca, 1969; Sutherland, 1975; Masci et al., 1990; Lalloo et al., 1995; Sutherland and Tibbals, 2001). The venom of *O. s. scutellatus* contains a potent prothrombin activator (oscutarin; EC 3.4.21.60) as well as an activator of Factor VII (Walker et al., 1980; Speijer et al., 1986; Nakagaki et al., 1992). Scutelarin is a multimeric activator of approximately 260 kDa composed of four subunits:

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a 110 kDa subunit showing activity similar to Factor V, an 80 kDa subunit similar to Factor X and two disulphide linked polypeptides, of which one or both contain the active site, of 30 kDa each. Studies have shown that oscutarin coagulates citrated plasma, Factors V and X deficient plasmas and warfarin plasma, as well as converting purified human prothrombin to thrombin, and hydrolysing the peptide *p*-nitroanilide substrate S-2222 (Marshall and Herrmann, 1989; Masci et al., 1998; Tan and Ponnudurai, 1990).

Sequence and structure analyses of *Oxyuranus* spp. prothrombin activators have not previously been reported. This report provides the sequence of the Factor V component of oscutarin from *O. s. scutellatus*.

2. Materials

2.1. Reagents

All general laboratory reagents were obtained from Sigma, InVitrogen, ICN, Amersham or BioRad Laboratories.

3. Methods

Techniques not included below, such as restriction enzyme digestion, ligation and SDS-PAGE were carried out as described by Sambrook et al. (1989).

3.1. cDNA Library

Total RNA was isolated from a venom gland excised from *O. s. scutellatus* and stored in TRIzol (Gibco BRL). poly(A)+ mRNA was purified using a MicroPolyA Pure kit (Ambion) utilising an oligo-dT column. A cDNA library was constructed using a λZAP-cDNA® Cloning Kit (Stratagene).

3.1.1. Library screening using protein expression

Expressed proteins from the *O. s. scutellatus* venom gland cDNA library were screened with Taipan monovalent antivenom (CSL). Clones binding antivenom were isolated and the nucleotide sequences of in vivo-excised plasmids determined.

3.1.2. Manipulation of DNA in plasmid vectors

Plasmid mapping was undertaken to sequence this clone. Restriction enzyme deletions were constructed to facilitate the determination of the nucleotide sequence of the full-length clone. Where deletions could not be used oligonucleotide primers were constructed and PCR used in duplicate to complete the sequence. Primers were synthesised by Proligo Australia Pty Ltd (Southern Cross University, Lismore, NSW, Australia 2480).

3.1.3. Protein sequencing

Proteins in samples (80 μg) of *O. s. scutellatus* whole venom were fractionated by SDS-PAGE (7.5 or 12% reducing conditions) and blotted onto PVDF membrane. The protein bands on the membrane were visualised with Ponceau Red and a 120 kDa band was excised and protein sent for N-terminal Edman degradation sequencing (Institute of Biomolecular Sciences, University of Queensland).

3.1.4. DNA sequencing

DNA for sequence determination was amplified either using Templiphi (Amersham) or as a plasmid.

Forward and reverse sequences were determined, using either an ABI 310 capillary sequencer or an Amersham MegaBACE 1000. Samples were prepared using ABI-Big Dye terminator and ET terminator sequencing kits according the manufacturers' instructions.

3.1.5. Analysis of sequence data

DNA sequences were analysed using EditView, MacVector™ 7.0 and Sequencher programs. Partial sequences of clones were aligned manually due to numerous repeat regions within a sequence. Public database comparisons of *O. s. scutellatus* DNA sequences and inferred protein sequences were conducted using the National Centre for Biotechnology Information website (www.ncbi.nlm.nih.gov). The nucleotide, translated and protein sequence databases were screened for homologous sequences. Sequence analysis and domain searches were conducted using the BLASTx program and Conserved Domain Database, respectively, at the National Centre for Biotechnology Information website (http://www.ncbi.nlm.nih.gov). Sequence comparisons were performed using the Clustal alignment program within MacVector 7.0 and signal peptide and *N*-glycosylation site predictions were obtained using pSORT and NetNGlyc, respectively, at the ExPASy website (http://www.expasy.ch).

4. Results

Screening the venom gland cDNA library with Taipan monovalent antivenom produced a relatively large number of positive plaques. Analysis of the nucleotide sequence of a range of clones revealed similarities with Factor V (FV) sequences from *Homo sapiens* (GenBank Accession number NP000121) and *Bos taurus* (GenBank Accession number AAA30513). These clones were the most abundant positive clones isolated from the cDNA library and represented 13% of the total clones characterised from the cDNA library (120 total, results not shown).

The complete 4690 nucleotide sequence contained 4380 bp of ORF which translated into a 1460 amino acid residue protein including a 30-residue signal peptide. This ORF was preceded by a 3′-untranslated region of 284