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Molecular isoforms of cobra venom factor-like proteins in the venom of *Austrelaps superbus* ☆

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Abstract

Cobra venom factor (CVF) is characteristic of the elapid cobras and has not been reported from venoms of any other families of snakes. During our search for novel proteins, we isolated a polypeptide from the venom of the snake *Austrelaps superbus* (Lowland Copperhead) that showed structural similarity to C-terminal segment of the α-chain of CVF and hence named as AVFαc (AVF—*A. superbus venom factor*). cDNA sequence of AVFαc and its precursor indicated the presence of two isoforms of CVF-like proteins in *A. superbus* venom gland. This is the first report of molecular isoforms of CVF-like proteins in the venom of an Australian elapid snake. We have determined the complete cDNA sequence of both the isoforms (AVF-1 and AVF-2). They differ in their potential glycosylation sites and the characteristic thioester bond sequence. They display the overall domain structure of CVF and complement C3 proteins. By real-time quantitative analysis, we show that there is a 140-fold difference in the mRNA expression levels of the two isoforms in the venom gland of *A. superbus*. We also show the presence of AVF-1 and its variant (not AVF-2) in *A. superbus* venom by partial purification, dot blots, Western blots and peptide mapping using mass spectrometry. Partially purified proteins activate human Factor B in the presence of Factor D and Mg^{2+}, and deplete the complement activity in human and guinea pig serum. The bimolecular complex (AVFBb) formed activates complement C3 but not complement C5. Thus, AVF proteins may serve as potential candidates for therapeutic complement depletion without side effects. Thus, the discovery of CVF-like proteins in the venom of this Australian elapid snake provides an alternative source of research tools, and contributes to our understanding of the structure–function relationships and evolution of new members of CVF-like proteins.

Keywords: Complement; Thioester bond; Cobra venom factor; *Austrelaps superbus*; Real-time PCR; Convertase; Alternate pathway

Abbreviations: CVF, cobra venom factor; AVF, *Austrelaps superbus* venom factor; RP-HPLC, reversed-phase high-performance liquid chromatography; ESI-MS, electrospray ionization mass spectrometry; NTC, no template control; RT-PCR, reverse transcriptase-polymerase chain reaction; RACE, rapid amplification of cDNA ends; SNP, single nucleotide polymorphism; TSS, transcriptional start site; UTR, untranslated region; PVDF, polyvinylidene fluoride.

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1. Introduction

Complement system and its activation are important in the host defense against infectious agents and the inflammatory process. Complement protein C3 plays a pivotal role in the activation of both classical and alternative pathways and is indispensable to this cascade of activation. Cobra venom factor (CVF) is a three-chain glycoprotein in the cobra venom that activates the complement system (Eggertsen et al., 1981; Vogel, 1991; Vogel et al., 1996). It is a structural and functional analog of the complement component C3 (Lachmann and Nicol, 1973; Vogel et al., 1984). When added to human (or mammalian) serum, CVF binds to Factor B of the alternative pathway to form the bimolecular complex CVFB (Hensley et al., 1986). Bound Factor B is cleaved by Factor D (Cooper, 1973) in the plasma into Ba (the activation peptide) and Bb that remains bound to the CVF thereby forming a stable complex CVFBb (Vogel and Muller-Eberhard, 1982). The CVFBb complex cleaves C3 and C5 into C3a and C3b, and C5a and C5b, respectively. Thus, CVF functions both as C3 and C5 convertase (DiScipio et al., 1983; Vogel and Muller-Eberhard, 1982). Complement activation by CVF thus results in the consumption of complement components C5, C6, C7, C8, C9 and Factor B leading to their depletion (Birdsey et al., 1971).

Using screening methods based on the immunological cross-reactivity and complement consumption, it has been shown that CVF is present in the venoms of several cobras of three genera (*Naja naja*, *Naja haje*, *Naja nigricollis*, *Naja siamensis*, *Naja nivea*, *Naja melanoleuca*, *Naja atra*, *Naja kaouthia*, *Hemachatus hemachatus* and *Ophiophagus hannah*) (Birdsey et al., 1971; Eggertsen et al., 1981; Takahashi and Hayashi, 1982). However, CVF-like proteins were not found in the venoms of other elapids including *Bungarus multicinctus*, *Bungarus fasciatus*, *Bungarus caeruleus* and *Dendroaspis angusticeps* (Birdsey et al., 1971; Eggertsen et al., 1981; Takahashi and Hayashi, 1982). Similarly, no CVF-like proteins were found in the families of Crotalids, Viperids and Hydrophids (Birdsey et al., 1971; Takahashi and Hayashi, 1982). Thus, CVF or CVF-like proteins have never been found in non-cobra snakes. It is to be noted that so far none of the Australian elapids have been screened for the presence of CVF-like proteins.

We have been characterizing novel proteins from snake venoms (Banerjee et al., 2005; Nirthanan et al., 2002; Pung et al., 2005; Torres et al., 2003; Kuhn et al., 2000; Watanabe et al., 2002). In our quest for novel proteins in the venom of *A. superbus*, we made a comprehensive search for low molecular weight polypeptides. During this study, we identified a polypeptide that showed significant structural similarity to the C-terminal of the α-chain of CVF. Here, we describe the cDNA cloning and sequence analyses of its precursor proteins. The results show the presence of two CVF-like proteins, which were named as AVF-1 (*A. superbus* venom factor) and AVF-2 in *A. superbus*. Partial purification and Western blot analyses in combination with matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry revealed the presence of two variants of highly expressed isoform AVF-1 in the venom of *A. superbus*. These variants activate human Factor B and complement C3; however, unlike CVF they do not activate C5. Thus, AVF may be useful in developing therapeutic agents for complement depletion and the Australian snakes may provide an alternative source of CVF-like proteins.

2. Materials and methods

2.1. Materials

Venom glands and lyophilized crude venom of *A. superbus* and lyophilized crude venom of *N. kaouthia* were purchased from Venom Supplies Pty Ltd. (Tanunda, South Australia, Australia). Columns for reversed-phase high-performance liquid chromatography (RP-HPLC)—Jupiter™ C18 preparative (250 mm × 21 mm) and C18 semi-preparative (250 mm × 10 mm) columns were purchased from Phenomenex (Torrance, CA, USA). Superdex 200 Hiload™ (16/60) and Sephasil™ C18 (5 μm SC 2.1/10) narrow bore columns were purchased from Amersham Biosciences (Uppsala, Sweden). Primers were purchased from Research Biolabs (Singapore). RNeasy mini kit for isolation of total RNA, reverse transcriptase-polymerase chain reaction (RT-PCR) enzyme mix, gel extraction kit and cloning vectors were purchased from Qiagen (Valencia, CA, USA). *Eco*RI restriction enzyme was purchased from New England Biolabs (Beverly, MA, USA). Polyclonal anti-CVF antibodies were obtained as a generous gift from Dr. David Fritzinger, University of Hawaii, Hawaii, USA. Anti-goat IgG complexed to HRP was obtained from Sigma (St. Louis, MO, USA). Immobilon-polyvinylidene fluoride (PVDF) transfer