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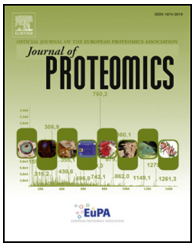

Dracula's children: Molecular evolution of vampire bat venom

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ABSTRACT

While vampire bat oral secretions have been the subject of intense research, efforts have concentrated only on two components: DSPA (*Desmodus rotundus* salivary plasminogen activator) and Draculin. The molecular evolutionary history of DSPA has been elucidated, while conversely draculin has long been known from only a very small fragment and thus even the basic protein class was not even established. Despite the fact that vampire bat venom has a multitude of effects unaccounted by the documented bioactivities of DSPA and draculin, efforts have not been made to establish what other bioactive proteins are secreted by their submaxillary gland. In addition, it has remained unclear whether the anatomically distinct anterior and posterior lobes of the submaxillary gland are evolving on separate gene expression trajectories or if they remain under the shared genetic control. Using a combined proteomic and transcriptomic approach, we show that identical proteins are simultaneously expressed in both lobes. In addition to recovering the known structural classes of DSPA, we recovered a novel DSPA isoform as well as obtained a very large sequence stretch of draculin and thus established that it is a mutated version of the lactotransferrin scaffold. This study reveals a much more complex secretion profile than previously recognised. In addition to obtaining novel versions of scaffolds convergently recruited into other venoms (allergen-like, CRiSP, kallikrein, Kunitz, lysozyme), we also documented novel expression of small peptides related to calcitonin, PACAP, and statherin. Other overexpressed protein types included BPI-fold, lacritin, and secretoglobin. Further, we investigate the molecular evolution of various vampire bat venom-components and highlight the dominant role of positive selection in the evolution of these proteins. Conspicuously many of the proteins identified in the proteome were found to be homologous to proteins with known activities affecting vasodilation and platelet aggregation. We show that vampire bat venom proteins possibly evade host immune response by the mutation of the

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surface chemistry through focal mutagenesis under the guidance of positive Darwinian selection. These results not only contribute to the body of knowledge regarding haematophagous venoms but also provide a rich resource for novel lead compounds for use in drug design and development.

Biological significance

These results have direct implications in understanding the molecular evolutionary history of vampire bat venom. The unusual peptides discovered reinforce the value of studying such neglected taxon for biodiscovery.

1. Introduction

Venom is defined as a secretion produced in a specialised gland in an animal, which is delivered to a target animal by inflicting a wound (regardless of size). Venom must also contain molecules (toxins) that disrupt normal physiological and/or biochemical processes so as to facilitate feeding or defence by the producing animal [1]. This definition includes secretions produced by blood feeding (haematophagous) specialists such as fleas, ticks, leeches or vampire bats that disrupt the haemostatic defences of prey/host organisms. These secretions which facilitate haematophagy or blood feeding are considered a particular subtype of venom.

With the level of infamy that extends far beyond the boundaries of science, blood-sucking vampire bats (Chiroptera, Desmodotinae) have been the subject of folk tales, superstitions and stories associated with the legendary Count Dracula for centuries [2,3]. All the three species of vampire bats are confined to Central and South America and typically live in the caves, tree hollows, and abandoned mines [4]. The relatively rare hairy-legged vampire bat (*Diphylla ecaudata*) feeds exclusively on avian hosts, while the white-winged vampire bat (*Diaemus youngi*) thrives on both the mammalian and avian blood, but most likely favouring the latter [2,5]. In contrast, the common vampire bat, *Desmodus rotundus* feeds overwhelmingly on mammals and has established itself in large colonies over an increasingly extensive distribution [2,6,7]. The expanding population of these bats is attributed to the increasing human population and the associated large number of domesticated animals and livestock, which provide a constant, high-density food supply [4,7].

All three species of vampire bats are highly specialised for a haematophagous lifestyle, especially *D. rotundus* [4]. Modifications of the teeth and limbs of vampire bats facilitate this lifestyle [6]. Reinforcement of limb strength permits *D. rotundus* and the other vampire bat species to approach their prey from the ground via quadrupedal walking and jumping [5,7,8]. *D. rotundus* is the most specialised of all vampire bats, and possesses specialised sensory capabilities for the detection of prey [9]. Close-range thermal and mechanical sensitivity is utilised for locating capillaries during feeding, while long-range vision, olfaction, acute hearing and echolocation are utilised for the discovery of potential prey [9]. They also have razor-like upper and lower incisors [5,7,10] that inflict a crater-shaped wound unique to *D. rotundus*. While feeding, their tongue darts in and out of the wound, releasing venom from the dorsal side of the tongue while drawing in blood via two straw-like ducts located on the ventral side of the tongue [7].

In order to facilitate blood-feeding, *D. rotundus* must be capable of interfering with their prey's natural haemostatic response to injury during feeding and digesting [11,12]. A typical haemostatic response produces a fibrin clot within minutes of the infliction of a wound, preventing further blood loss. The response commences with the constriction of blood vessels, restricting blood flow to the wound, and is followed by the adhesion of activated platelets to the site of injury and the conversion of fibrinogen to insoluble fibrin, forming a blood clot [12]. In contrast to this normal response to injury, bleeding from a wound induced by vampire bats may be prolonged from minutes to hours, ensuring a constant flow of blood for the bat to feed upon [12].

The gland predominantly associated with haematophagy in *D. rotundus* is the principle submaxillary gland, which consists of two separate anterior and posterior lobes [13]. This gland is responsible for the secretion of venom with strong anticoagulant and proteolytic activities [13]. The venom delays the onset of blood clotting by interfering with fibrin formation or acting upon fibrin as it is converted from fibrinogen. In addition it has a strong proteolytic action that breaks up any blood clots that may be formed. This proteolytic action is accomplished through the activation of the host's fibrinolytic system which converts plasminogen to plasmin, solubilising and removing fibrin clots to prevent excessive fibrin buildup at the site of the wound.

The venom of the vampire bat has been documented to disrupt the coagulation cascade via four distinct mechanisms: (i) inhibition of factor IXa, (ii) inhibition of factor Xa, (iii) activation of plasminogen and (iv) inhibition of platelets [1]. A venom component that has been researched considerably is draculin, an anticoagulant factor. It is an 88.5 kDa glycoprotein that inhibits activated factors IX (IXa) and X (Xa) of the coagulation mechanism [11,14,15]. The irreversibly bonded complex of Xa-Draculin forms immediately upon contact, inhibiting factors IXa and Xa [14]. The inhibition of IXa and Xa prevents the conversion of prothrombin to thrombin, which in turn prevents the conversion of fibrinogen to insoluble fibrin. Furthermore, the characteristic of non-competitive inhibition prevents the cleavage of draculin from Xa after binding, thus maintaining the toxin's anticoagulant activity during feeding and digestion [16]. Interestingly, a study conducted recently described the capacity of *D. rotundus* prey to develop immunity to draculin if they were targeted and fed upon over prolonged periods [17]. This kind of predator–prey arms race scenario is similar to that observed between other venomous animals and their prey.

The plasminogen activators or Desmokinase [12], better known as DSPA (*Desmodus rotundus* salivary plasminogen

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