Bioweapons synthesis and storage: The venom gland of front-fanged snakes ☆

Stephen P. Mackessy*, Louise M. Baxter

School of Biological Sciences, University of Northern Colorado, 501 20th St., CB 92, Greeley, CO 80639-0017, USA

Abstract

A paradoxical task of the venom gland of snakes is the synthesis and storage of an instantly available suite of toxins to immobilize prey and the protection of the snake against its own venom components. Furthermore, autolysis of the venom constituents due to the action of venom metalloproteases is an additional problem, particularly among viperid venoms, which are typically rich in lytic enzymatic proteins. To address questions concerning these problems, the structure of the venom gland was investigated using light microscopy, SEM and TEM. The composition of the venom originating from the intact venom apparatus or from the main venom gland alone was analyzed by electrophoresis, and the pH of freshly expressed venom as well as pH optima of several representative enzymes was evaluated. Results from several species of rattlesnakes demonstrated that the venom gland is structurally complex, particularly in its small rostral portion called the accessory gland, which may be a site of activation of venom components. Secreted venom is stable in extremes of temperature and dilution, and several proximate mechanisms, including pH and endogenous inhibitors, exist which inhibit enzymatic activity of the venom during storage within the venom gland but allow for spontaneous activation upon injection into prey. Whereas acid secretion by the parietal cells activates digestive enzymes in the stomach, within the venom gland acidification inhibits venom enzymes. We propose that the mitochondria-rich cells of the main venom gland, which are morphologically and histochemically very similar to the parietal cells of the mammalian gastric pit, play a central role in the stabilization of the venom by secreting acidic compounds into the venom and maintaining the stored venom at pH 5.4. Hence, our results indicate yet another trophic link between the processes of venom production and of digestion, and demonstrate that the venom glands of snakes may represent an excellent model for the study of protein stability and maintenance of toxic proteins.
© 2006 Elsevier GmbH. All rights reserved.

Keywords: Accessory gland; Autolysis; Enzymes; Gland; Mitochondria-rich cell; Parietal cell; pH; Rattlesnake; Stabilization; Toxin; Venom

1. Introduction

The biochemical ecology of venomous snakes involves a fascinating blend of protein chemistry, structural biochemistry, evolution and ecology. Venomous snakes utilize numerous protein and peptide enzymes and toxins to dispatch prey and deter predators, yet the compounds themselves are often highly toxic, inherently unstable, or both. Venomous snakes are thus confronted with a conundrum: How does one produce, store and deliver these substances, without inflicting toxic effects on oneself and without losing the biological potency due to autolysis and other degradative processes?

The venom glands of snakes, particularly those of rattlesnakes (Viperidae), represent an excellent model system for the study of the synthesis, secretion and long-term storage of toxic proteins. Following the depletion of stored venom by manual extraction, secretory epithelial cells lining the tubules of the venom gland initiate rapid protein synthesis (Carneiro et al. 1991; Kochva et al. 1980; Mackessy 1991; Warshawsky et al. 1973), apparently in response to stimulation by the autonomic nervous system (Kerchove et al. 2004; Yamanouye et al. 1997). Within 4–8 days, proliferation of the rough endoplasmic reticulum and mRNA levels have reached a maximum (Carneiro et al. 1991; Kochva et al. 1980; Mackessy 1991; Rotenberg et al. 1971; Yamanouye et al. 1997), and subsequent merocrine exocytosis results in the replenishment of venom in the epithelial ductules and large basal lumen. Completion of the synthesis and secretion stage occurs approximately 16 days after depletion of the gland, and during this period, cells cycle from cuboidal to columnar and back to cuboidal morphology (Kochva 1987; Kochva et al. 1975; Mackessy 1991). The venom is then stored in the basal lumen and ductules of the venom gland for varying periods of time and is available when needed. No evidence of venom protein turnover in the glandular lumen or ductules has been presented, but when secretory cells assume a cuboidal morphology, the rough endoplasmic reticulum resumes a minimal state, suggesting inactivity (Kochva 1987; Kochva et al. 1975; Mackessy 1991). Snakes in captivity, which have not had venom extracted in several years and which are maintained on dead prey, show a large amount of cellular debris in the venom, in contrast to snakes whose venom is extracted regularly (pers. obs.). The presence of cellular debris demonstrates that at least large particles are not reabsorbed within the gland, further demonstrating the static nature of the secreted and stored venom. Because snakes in general are adapted to withstand long fasting periods, the venom may be stored for months and in captivity even for several years and will remain active.

Redundant protective mechanisms exist to protect a snake from its own toxins and to maintain the potency of the stored venom, and the expressed venom remains remarkably stable under a wide variety of conditions (Munekiyo and Mackessy 1998). Numerous inhibitors of enzymes, which may be responsible for this stability, have been described, including peptides from venoms of several viperids (Francis et al. 1992; Huang et al. 1998, 2002; Munekiyo and Mackessy 2005; Robeva et al. 1991), zymogen activation of a metalloprotease via a "cysteine switch" mechanism (Grams et al. 1993), and enzyme inhibition by citrate (Fenton et al. 1995; Francis et al. 1992; Odell et al. 1998). Mechanisms that protect a snake from its own venom include the presence of antibodies in the blood to venom proteins (Straight et al. 1976) and structural modifications of specific receptors, such as the acetylcholine receptor of skeletal muscle, which inhibit toxin binding (Servent et al. 1998; Takacs et al. 2001). General protective mechanisms against circulating "rogue" enzymes, such as those produced by invasive microorganisms or resulting from activation of hemostatic mechanisms, are typified by the actions of α_2-macroglobulins against proteases, and these may also protect the snake. However, the mechanisms that allow the long-term storage of venom within the venom gland have not been fully addressed yet, nor is it fully understood how venom that has been released from the secretory cells may be stored in the glandular lumen and ductules for many months and yet remains active upon demand.

To address these questions, we analyzed both the structure of venom glands and the biochemistry of venoms of the Prairie Rattlesnake [*Crotalus viridis viridis* (Rafinesque, 1818)], the Northern Pacific Rattlesnake [*C. oreganus oreganus* (Holbrook, 1840)] and the Western Diamondback Rattlesnake [*C. atrox* (Baird & Girard, 1853)] as models of the viperid design of the venom gland. There are many variations in venom gland design and venom composition among viperids and all other venomous snakes, but the observations presented here may be common among venomous caenophidians generally, a conclusion that is supported by comparative observations of the histology of venom glands (e.g., Kochva and Gans 1966) and on the chemistry of snake venoms (Freitas et al. 1992; Munekiyo and Mackessy 2005; Odell et al. 1998).

2. Materials and methods

2.1. Collection of snakes and venoms

Rattlesnakes were collected in several locations in the western US with permission of appropriate local