Toxicon 56 (2010) 1120–1129

Contents lists available at ScienceDirect

Toxicon

journal homepage: www.elsevier.com/locate/toxicon

Review

Structure and mechanism in salivary proteins from blood-feeding arthropods

John F. Andersen*

Laboratory of Malaria and Vector Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, 2E-32B Twinbrook 3 Bldg, 12735 Twinbrook Parkway, Rockville, MD 20852, USA

ARTICLE INFO

Article history:
Received 2 October 2009
Accepted 9 November 2009
Available online 26 November 2009

Keywords:
Lipocalin
Odorant-binding protein
D7
Nitrophorin
Tick
Mosquito
Diptera
X-ray crystallography

ABSTRACT

The saliva of blood-feeding arthropods contains rich mixtures of ligand binding proteins targeted at inhibiting hemostasis and inflammation in the host. Since blood feeding has evolved many times, different taxonomic groups utilize completely different families of proteins to perform similar tasks. Structural studies performed on a number of these proteins have revealed biologically novel and sophisticated mechanisms used to perform their functions. Here, the results of these structural and mechanistic studies are reviewed.
Published by Elsevier Ltd.

Blood feeding has evolved many times in arthropods, and each time the blood sucker has been faced with an evolutionary battle to overcome the host defenses against blood loss (Francischetti et al., 2009). Independent evolution has resulted in a huge diversity of biologically active proteins in the salivary secretions of these organisms. Often, proteins from different structural classes take on the same or similar functions in different taxa, implying that these are the result of independent recruitments into the salivary proteome of each (Francischetti et al., 2009; Fry et al., 2009). Within a given species, additional diversity is created by gene duplication events that produce groups of related proteins expressed in a single salivary mixture. Members of these groups commonly diverge further to take on new functions in blood feeding. These changes can result in relatively small modifications of a single function or produce completely new functions. Diversification of single salivary protein families within a species has been described for many species. For a few groups such as the nitrophorins in *Rhodnius prolixus* (Andersen et al., 2000, Ribeiro et al., 1995), lipocalins in soft ticks (Mans et al., 2008; Paesen et al., 1999), and the D7 type ligand binding proteins in mosquitoes (Calvo et al., 2006), the modification of existing functions and acquisition of new functions has been described.

While a few types of enzymes have been found in salivas of blood feeders, the majority of functionally characterized salivary proteins are inhibitors of host physiological processes that act by binding proteins or small molecules. The targets of these proteins include enzymes of the coagulation cascade, collagen (Calvo et al., 2007), and small molecule agonists of platelet activation, inflammation and vasoconstriction/vasodilation (Andersen et al., 2003; Francischetti et al., 2000; Paesen et al., 1999; Ribeiro and Walker, 1994; Sangamnatdej et al., 2002). Blood-feeding Diptera, triatomine bugs, and ticks have all been shown to produce salivary proteins that bind agonists of hemostasis

Abbreviations: NO, nitric oxide; NP, nitrophorin; OBP, odorant-binding protein.

* Tel.: +1 301 402 5871; fax: +1 301 402 2201.
E-mail address: jandersen@niaid.nih.gov

0041-0101/$ – see front matter Published by Elsevier Ltd.
doi:10.1016/j.toxicon.2009.11.002

and inflammation. Among the molecules bound are serotonin (Andersen et al., 2003; Calvo et al., 2006; Sangamnatdej et al., 2002), norepinephrine, epinephrine (Andersen et al., 2003; Calvo et al., 2006), histamine (Paesen et al., 1999; Ribeiro and Walker, 1994), adenosine diphosphate (Francischetti et al., 2000), cysteinyl leukotrienes (Calvo et al., 2009; Mans and Ribeiro, 2008), leukotriene B_4, thromboxanes (Mans and Ribeiro, 2008), nitric oxide (Ribeiro et al., 1993) and collagen (Calvo et al., 2007). Two major structural families, the lipocalins and the odorant-binding proteins, have been extensively characterized in performing these tasks. Lipocalins serve this function in triatomine bugs, hard ticks and soft ticks, while odorant-binding proteins serve the function in mosquitoes.

This review will focus on structural studies of ligand binding proteins from the saliva of blood feeders. Most of the examples fall within the lipocalin and D7 (odorant-binding protein) families, and demonstrate the structural basis behind the remarkable functional diversity seen in these molecules. A number of coagulation inhibitors having Kunitz and Kazal domains have also been structurally characterized, but these studies have been recently reviewed (Corral-Rodriguez et al., 2009; Koh and Kini, 2009) and other than the lipocalin thrombin inhibitor triabin, will not be covered here.

1. Nitrophorins

1.1. Lipocalin NO carriers from R. prolixus

Triatomine bugs are important as vectors of the new world trypanosomiasis known as Chagas' disease. While the pathogen is transmitted in the feces rather than in the saliva, host feeding is an important element of the transmission cycle. The salivas of these insects are extremely rich in proteins, with dozens of distinct secreted forms being described in the transcriptomes of *Rhodnius prolixus* or *Triatoma infestans* salivary glands (Assumpcao et al., 2008; Ribeiro et al., 2004). Lipocalins are by far the most abundant and diverse group of proteins in triatomine saliva (Andersen et al., 2005). This structural diversity translates into functional diversity, with lipocalins serving as vasodilators, platelet aggregation inhibitors and anticoagulants. Some of the first studies of triatomine lipocalins were perfomed with the nitrophorins (NPs), an extremely interesting group of nitric oxide carrier proteins from *R. prolixus*.

NPs were originally described as four distinct heme proteins from the salivary gland extract of *R. prolixus* that were each found to carry a single molecule of Fe^{III}-coordinated nitric oxide (NO). (Ribeiro et al., 1993). Further analysis of the salivary transcriptome of this species revealed the existence of a number of additional forms, some of which were subsequently isolated from salivary gland extracts.

NO is an endogenous effector in vertebrates produced by endothelial cells, macrophages, and in the nervous system. Its vasodilatory and antiplatelet effects are a result of cellular signaling involving the activation of soluble guanylate cyclase. Nitric oxide is labile in the blood and tissues where it is rapidly oxidized with a half life of <1 s. Binding of NO with NPs protects it from oxidation, allowing its intact delivery to the host. When nitrophorins are injected during feeding, bound NO is released in a pH-dependent manner, making it available to affect host hemostatic and inflammatory processes. NO binds more tightly in the, acidic saliva (pH $\sim$6.0) than at vertebrate physiological pH. When the protein is diluted in the host blood and tissues, and the pH is raised to 7.4, NO is released and causes vasodilation and inhibition of platelet aggregation. Interestingly, after release of NO, nitrophorins are able to bind a molecule of histamine with high affinity in the distal pocket vacated by the NO molecule (Ribeiro and Walker, 1994). Binding of this mast cell-derived inflammatory mediator limits the itching response that results from recognition of salivary antigens by IgE.

The crystal structure of recombinant NO-free NP1 was solved using multiple isomorphous replacement methods and found to have an eight-stranded antiparallel β-barrel structure having a single heme moiety located in a central binding pocket (Fig. 1) (Weichsel et al., 1998). The heme is tethered to the protein by coordination of the iron atom to the imidazole group of His 57 (Fig. 1A). Although sequence comparisons at the time did not reveal relationship with the lipocalin protein family, the structure clearly showed the protein to be identical in fold, and quite similar in structure to the prototypic insect lipocalins that bind the pigment biliverdin (Fig. 1A). Two disulfide bonds are present in the nitrophorins, with one linking the N-terminal portion to the β-barrel and the second linking the C-terminal segment to the barrel. Entry to the distal (ligand-binding) pocket of the protein is regulated by loops which surround the entry to the binding pocket and undergo conformational changes on NO binding (Fig. 1A,B) (Weichsel et al., 2000). The NO ligand is observed in structures of the NP4 complex in a somewhat unusual (for an Fe^{III} complex) bent geometry (Roberts et al., 2001). After release of NO, histamine enters the distal pocket where its imidazole group coordinates with the heme iron, forming a bisimidazole complex (Weichsel et al., 1998). Histamine is also stabilized by interaction of its ammonium group with acidic and polar residues lining the distal pocket (Fig. 1C).

The structure of NP4 was critical to determining the ligand binding mechanism. It crystallizes under a wide range of pH conditions, diffracts to very high resolution, and unlike NP1 and 2, the recombinant form is free of an N-terminal methionine residue (Andersen et al., 1998). Soaking of NP4 crystals in solutions of NO causes extensive ligand-dependent conformational changes involving the loops surrounding the distal pocket as well as the N-terminal segment of the protein (Fig. 1A,B) (Weichsel et al., 2000). When NP4 binds NO, movements involving loop AB (connecting strands A and B of the β-barrel) and GH, act to close the binding pocket, expel ordered water, and form a new and extensive hydrogen bonding network. This results in a shift in the side chains of Val 36, Leu 130 and Leu 133 toward the lipophilic NO ligand essentially packing it in a hydrophobic environment (Fig. 1B). The hydrogen bonding network involving backbone and side chain atoms from Asp 129, Leu 130, Asp 35, Asp 30, Glu 32