Structure-Function Relationships of C-Type Lectin-Related Proteins

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Key Words

C-type lectin-like protein · Three-dimensional domain swapping · Blood coagulation factor IX/factor X-binding protein (IX/X-bp) · EMS-16 · Collagen receptor antagonist

Abstract

The structural and functional studies of the first identified C-type lectin-like protein (CLP), blood coagulation factor IX/factor X-binding protein (IX/X-bp), have been instrumental in defining how new functionally heterodimeric CLPs are generated from monomeric carbohydrate recognition domain in C-type lectins by three-dimensional domain swapping. The crystal structures of γ -carboxyglutamic acid domains of coagulation factors X and IX have recently been clarified in structural studies of complexes between the γ -carboxyglutamic acid domain of factors X and X-bp (a venom CLP) and between the γ -carboxyglutamic acid domain of factors IX and IX-bp (a venom CLP).

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Introduction

Snake venoms contain proteins that both activate and inhibit blood coagulation factors. It was reported in 1972–1975 that the venoms of *Deinagkistrodon acutus* (formerly *Agkistrodon acutus*) [1] and *Trimeresurus stejnegeri* (formerly *Trimeresurus gramineus*) [2] contain proteins that inhibit prothrombin activation possibly by

nonenzymatic mechanisms [3, 4]. One of the anticoagulants in the venom of *D. acutus* was characterized as a factor X-binding protein (X-bp) in 1989, and the venom of *Trimeresurus flavoviridis* (Habu snake) was also found to contain another factor X-bp with anticoagulant activity [5]. Furthermore, this *T. flavoviridis* anticoagulant protein binds not only factors X and Xa, but also to factors IX and IXa [5].

IX/X-bp was identified as the first protein of the C-type lectin-like protein (CLP) class in 1991 [6]. RVV-X, factor X activator from Russell's viper venom, was sequenced in 1992 [7] and found to be a CLP with a C-type lectin-like domain within its light chain. The platelet agonist botrocetin was identified as a CLP with no lectin activity in 1993 [8]. Several more CLPs have been isolated from various snake venoms, sequenced, and found to have a variety of biological activities over the last 15 years [9–14]. It is now clear that the CLPs form a new protein family, and that they are useful tools for elucidating the complex mechanisms involved in clotting and platelet activation, as well as for determining the structure-function relationship of both blood clotting factors and human platelet glycoproteins [14].

Basic Structures of C-Type Lectin-Like Proteins

Table 1 shows a list of the representative CLPs affecting the hemostatic system, including anticoagulants, coagulants, and platelet modulators (agonist and antagonists) with no lectin activity. Many biologically active

Table 1. C-type lectin-like proteins (CLPs) of snake venom that modulate the hemostatic system

Function	CLP	Structure	Ligand protein or substrate
Anticoagulant	IX/X-bp, IX-bp IX/X-bp, X-bp bothrojaracin	$\alpha\beta$ $\alpha\beta$ $\alpha\beta$	Factor IX Factor X α -Thrombin
Coagulant	RVV-X carinactivase-1 (CA-1)	$\alpha\beta$ /metalloprotease $\alpha\beta$ /metalloprotease	Factor X Prothrombin
Agonist of platelet	botrocetin, bitiscetin alboaggregin-B aggregin/rhodocytin convulxin agglucetin	$\alpha\beta$ $\alpha\beta$ $\alpha\beta$ $(\alpha\beta)_4$ tetrameric structure*	vWF GPIb α GPIa/IIa GPVI GPIb α
Antagonist of platelet	EMS 16 echicetin flavocetin-A (FL-A)	$\alpha\beta$ $\alpha\beta$ $(\alpha\beta)_4$	GPIa/IIa GPIb α GPIb α

* Hypothetical construct may be tetrameric (α_1 - β_2 - α_2 - β_1) structure [24].

CLPs consist of one or more heterodimers of two C-type lectin-like subunits. These CLPs include the anticoagulant proteins blood coagulation factor IX/factor X-binding protein (IX/X-bp), IX-bp, and X-bp, which contain disulfide-linked heterodimers of C-type lectin-like subunits. Another CLP, bothrojaracin, is a hirudin-like thrombin inhibitor with anticoagulant activity [15]. Its biological activity is Ca^{2+} -independent, in contrast to the Ca^{2+} -dependent CLP anticoagulants. RVV-X and carinactivase-1 (CA-1) are metalloenzymes composed of two C-type lectin-like domains that recognize the γ -carboxyglutamic acid (Gla) domains of factor X and prothrombin, respectively [16, 17]. Other CLP effectors of platelet function include botrocetin [8] and bitiscetin [18, 19], which are von Willebrand factor (vWF)-binding aggregation inducers, and alboaggregin-B, a platelet GPIb-binding inducer of aggregation [20, 21].

The basic structure of the CLPs includes two homologous subunits: subunit α (A chain), of 14–15 kDa, and subunit β (B chain), of 13–14 kDa. The CLPs are composed of a variety of oligomeric forms, including $\alpha\beta$, $(\alpha\beta)_2$, or $(\alpha\beta)_4$ [6, 22–24]. In CLPs of snake venom, the basic $\alpha\beta$ dimer is formed by domain swapping (see next section). In contrast, C-type lectins, such as the lectin from the venom of *Crotalus atrox*, are made up of homodimers or homooligomers [25].

Dimerization of CLP by Domain Swapping Mechanism

One of the functional components of snake venom is now known to be a member of the C-type lectin-like protein family that includes IX/X-bp, CA-1, RVV-X, botrocetin, and flavocetin-A. The crystal structures of habu IX/X-bp [26], habu IX-bp [27] and acutus X-bp [28] indicate that each subunit of these proteins has a Ca^{2+} -binding site, different from that of C-type lectins such as mannanose-binding protein (MBP), and that dimerization involves swapping of the central loop of these subunits [26]. Dimerization of the carbohydrate recognition domain (CRD) in C-type lectins by three-dimensional (3D) domain swapping generates novel proteins with new functions such as coagulant-, anticoagulant-, or platelet-modulating activities.

The first IX/X-bp crystal structure revealed 3D domain swapping [26]. A domain from the α subunit replaces the essentially identical domain in the β subunit. At the same time, this domain from the β subunit is swapped for the same domain in the α subunit. A prominent feature of this structure is the projection of a central portion of the polypeptide chain towards the adjoining subunit, forming a tight dimer association. Excluding this feature, each subunit has a fold similar to the C-type CRD fold of MBP. The polypeptide chain then extends from the surface of the subunit, forming a large open loop that

project into the adjoining subunit, forming a loop-exchanged dimer, whereas the MBP chain in this region is folded back onto the subunit. In this way, the two subunits form an intertwined dimer with swapped domains.

The swapped and hinge region of the α subunit extends away from the surface of the subunits and forms a large open loop that projects into the adjoining subunit. This generates a loop-exchange dimer. In contrast, the equivalent loop in MBP folds back onto the subunit. Similar structural features occur in the β subunit of IX/X-bp. The extended loop of IX/X-bp returns to the main body of the subunit. Thus, subunit A generates a structure that is homologous to that of MBP by 'borrowing' the open loop of the adjoining subunit B. Similar structural features occur in subunit B. Thus, dimerization may be closely related to a function completely different from that of the 'monomer' motif C-type CRD, suggesting economic use of combinations of limited structural domains. Generally, the structures of both proteins are built up from a common frame. The open loop donated to the α subunit by the adjoining β subunit coincides structurally with the folded-back loop of MBP. Hence, the α subunit generates a structure that is homologous to that of MBP by borrowing the open loop of the adjoining β subunit. Most swapped domains of proteins are at either the N- or C-terminus. The only exception found to date is IX/X-bp in which the two subunits of IX/X-bp form a heterodimer by exchanging a loop in their central regions [29]. In addition, IX/X-bp is the only known example of a domain-swapped heterodimer; all other domain-swapped proteins are homodimers or homooligomers [29]. Thus, to be precise, IX/X-bp is quasi-domain-swapped.

This novel structure and function of proteins in snake venom serves as a strategy for survival against animal predators, and represents an evolutionary gain of function for the C-type CRD domain [26].

Structure-Function Relationship of CLP

The CLP family includes the anticoagulant proteins habu IX/X-bp (factor IX/factor X-binding protein), habu IX-bp (factor IX-binding protein), and acutus X-bp (factor X-binding protein), which contain disulfide-linked heterodimers of CLP subunits [5, 6, 26–28, 30–32]. Apparent dissociation constants for the binding of habu IX/X-bp to factor IX and factor X are 0.4 and 1.1 nM, respectively, in the presence of Ca^{2+} ions, indicating that these CLPs have quite high affinities for these coagulation

factors. These CLPs have strong anticoagulant activities due to their binding to Gla-containing domains of coagulation factors IX and X in the presence of calcium ions [32, 33]. The functionally important Gla domain binds to the concave surface in X-bp. Thus, the strong anticoagulant activity of X-bp is due to its binding at the Gla domain of factor X. The formation of this complex appears to block efficiently the membrane interaction of the Gla domain that is essential for amplification of the coagulation cascade [28].

There are several other unique CLPs that act as agonists and antagonists of platelet receptors, platelet glycoprotein Ib and glycoproteins Ia/IIa and VI, the collagen receptors. These CLPs may provide new insights into platelet function. EMS16, a CLP, is an antagonist of collagen receptor (GPIa/IIa), that is, a potent and selective inhibitor of the $\alpha 2\beta 1$ integrin [34–36]. The crystal structure of EMS16 in complex with the integrin $\alpha 2$ -I domain provides new information about the structure-function relationship of CLPs [37]. The structure of the EMS16 in complex with the integrin $\alpha 2$ -I domain at 1.9 Å resolution reveals that the collagen-binding site of the $\alpha 2$ -I domain is covered completely by the bound EMS16 [37]. This blockage by EMS16 inhibits collagen binding to the $\alpha 2$ -I domain. The complex structure reveals that the collagen-binding site of the $\alpha 2$ -I domain lies on the concave surface of EMS16, and there are direct binding sites at both ends of this surface. Binding takes place mainly through van der Waal's interactions, suggesting that EMS16 inhibits by blocking the collagen-binding site of the $\alpha 2$ -I domain. The crystal structure of EMS16 in complex with the integrin $\alpha 2$ -I domain may provide new information for the development of more effective antagonists against integrin $\alpha 2\beta 1$ [37].

Conclusions

CLPs of snake venom such as IX/X-bp and its structurally-related proteins adapt to recognize various ligands by the higher frequency of mutation in the open reading frames than in the noncoding regions after duplication of a gene. The existence of such accelerated duplication and mutation of genes could mean that similar processes for the A and B chains of IX/X-bp and of other CLPs might have resulted in the present family of heterodimeric CLPs that includes IX/X-bp and various other proteins that are structurally related to but yet functionally distinct from one another.

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