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Review

Crystallographic characterization of functional sites of crotoxin and ammodytoxin, potent β -neurotoxins from *Viperidae* venom

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ABSTRACT

This review will focus on a description of the three-dimensional structures of two β -neurotoxins, the monomeric PLA₂ ammodytoxin from *Vipera ammodytes ammodytes*, and heterodimeric crotoxin from *Crotalus durissus terrificus*, and a detailed structural analysis of their multiple functional sites. We have recently determined at high resolution the crystal structures of two natural isoforms of ammodytoxin (AtxA and AtxC) (Saul et al., 2010) which exhibit different toxicity profiles and different anticoagulant properties. Comparative structural analysis of these two PLA₂ isoforms, which differ only by two amino acid residues, allowed us to detect local conformational changes and delineate the role of critical residues in the anticoagulant and neurotoxic functions of these PLA₂ (Saul et al., 2010).

We have also determined, at 1.35 Å resolution, the crystal structure of heterodimeric crotoxin (Faure et al., 2011). The three-dimensional structure of crotoxin revealed details of the binding interface between its acidic (CA) and basic (CB) subunits and allowed us to identify key residues involved in the stability and toxicity of this potent heterodimeric β -neurotoxin (Faure et al., 2011). The precise spatial orientation of the three covalently linked polypeptide chains in the mature CA subunit complexed with CB helps us to understand the role played by critical residues of the CA subunit in the increased toxicity of the crotoxin complex. Since the CA subunit is a natural inhibitor of the catalytic and anticoagulant activities of CB, identification of the CA–CB binding interface describes residues involved in this inhibition.

We propose future research directions based on knowledge of the recently reported 3D structures of crotoxin and ammodytoxin.

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1. Introduction

Ammodytoxin and crotoxin belong to the class of β -neurotoxins and represent monomeric and heterodimeric snake venom β -neurotoxins from the *Viperidae* family. These neurotoxic phospholipases A₂ (PLA₂, EC 3.1.1.4), homologous to the inflammatory, non-pancreatic mammalian sPLA₂ of group IIA, catalyze hydrolysis of the

2-acyl ester bond of 1,2-diacyl-3-*sn*-phosphoglycerides (Berg et al., 2001) and selectively interact with various targets such as soluble and membrane bound proteins and heparan sulfate proteoglycans (see review Faure et al., 2010). Ammodytoxin from the venom of long-nosed viper *Vipera ammodytes ammodytes* is a single chain PLA₂ which exhibits enzymatic, neurotoxic and anticoagulant activity. Heterodimeric crotoxin, the major toxin of the venom of the South American rattlesnake *Crotalus durissus terrificus*, consists of a non-covalent association of a basic and weakly toxic PLA₂ subunit (CB), and an acidic, non-enzymatic and non-toxic subunit (CA) made up of three covalently linked

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polypeptide chains (α , β , and γ) generated by post-translational maturation of a PLA₂-like pro-CA precursor (Bouchier et al., 1991; Faure et al., 1991). The overall 3D structure of ammodytoxin and the CB subunit of crotoxin display the highly conserved canonical structural features of group IIA PLA₂: the catalytic site (residues His48, Asp99, Tyr52, Tyr73), a Ca²⁺-binding loop, an interfacial binding surface (IBS), and seven conserved disulfide bonds (Saul et al., 2010; Marchi-Salvador et al., 2008).

Ammodytoxin and crotoxin act presynaptically, blocking the release of acetylcholine from peripheral neurons at the neuromuscular junction (Lee et al., 1984; Hawgood and Smith, 1977). The presynaptic neurotoxicity of β -neurotoxins leads to paralysis by disruption of the neuromuscular junction at the presynaptic membrane and results from interaction with specific intra- and extra-cellular protein receptors. Although the molecular mechanism of action of β -neurotoxins is not yet fully understood, it has been reported that after binding to a specific receptor at the plasma membrane β -neurotoxins enter the nerve cells and several high-affinity binding proteins contribute to their neurotoxic effect (see review Sribar and Krizaj, 2011). Although no direct correlation has been reported between neurotoxicity and enzymatic activity, β -neurotoxins with higher order quaternary structures display significantly higher levels of toxicity. Ammodytoxin and crotoxin are present in individual snake venom as natural isoforms. Three isoforms of ammodytoxin (AtxA, AtxB and AtxC) have been described in *Vipera ammodytes ammodytes* and 16 different crotoxin isoforms have been identified in *Crotalus durissus terrificus* venom (Thouin et al., 1982; Faure and Bon, 1987, 1988; Faure et al., 1993). Crotoxin variants result from the random association of four isoforms of the CA (CA1, CA2, CA3 and CA4) and four isoforms of the CB subunit (CBa₂, CBb, CBc and CBD) (Faure and Bon, 1988). Two classes of crotoxin complexes which differ in enzymatic activity, toxicity and stability have been described (Faure and Bon, 1988; Faure et al., 1993, 1994). Purification of homogenous isoforms of ammodytoxin and crotoxin directly from snake venom or expressed in *Escherichia coli* is critical for crystallization to solve the 3D structure of these β -neurotoxins (Saul et al., 2010; Faure et al., 2011). Small differences in exposed surface residues and in the interface between subunits play an important role in determining functional specificities of the different isoforms (Saul et al., 2010; Faure et al., 2011).

Previously it was shown that the anticoagulant activity of Atx and the CB subunit of crotoxin results from specific interaction with human coagulation factor Xa (hFXa) by inhibiting formation of the prothrombinase complex (Prijatelj et al., 2006; Faure et al., 2007). Interestingly, the different PLA₂ isoforms exhibit different anticoagulant potency. Recently, we analyzed by structural and functional studies several *Viperidae* venom sPLA₂s that exert an anticoagulant effect by a non-enzymatic PL-independent mechanism through direct binding to hFXa (Faure et al., 2010; Faure and Saul, 2011).

In this short review we focus on a description of the X-ray structures of two isoforms of ammodytoxin (AtxA and AtxC) and a single isoform of crotoxin (CA₂CBb), and present a detailed structural analysis of their important functional sites.

2. Crystal structures of ammodytoxin and crotoxin

Recently, we have determined the crystal structures of two isoforms of ammodytoxin (AtxA and AtxC) from *Vipera ammodytes ammodytes* (Saul et al., 2010). For crystallization assays, a single isoform of recombinant AtxA and the natural isoform AtxC from snake venom were used (collaboration with the group of Prof. Igor Krizaj, Jozef Stefan Institute, Ljubljana, Slovenia). Crystals of recombinant AtxA (Fig. 1a) were obtained using 1.26 M (NH₄)₂SO₄, 0.1 M Tris, pH 8.5 and 0.2 M Li₂SO₄ solution at a final protein concentration of 3.8 mg/ml. The crystals of AtxC were obtained with 1.2 M (NH₄)₂SO₄, 0.1 M imidazole, pH 8.0 and 0.2 M Li₂SO₄ at a final protein concentration of 5 mg/ml (Saul et al., 2010). X-ray diffraction extends to 1.7 Å resolution for crystals of rAtxA and to 1.35 Å for AtxC (Saul et al., 2010).

As shown in Fig. 1b, AtxA and AtxC display the highly conserved canonical structural features of group IIA PLA₂s with seven disulfide bridges at the expected group IIA

Fig. 1. Crystallization and determination of the crystal structure of AtxA and AtxC, two isoforms of ammodytoxin from *Vipera ammodytes ammodytes* venom. (a) Crystals of AtxA (left) and AtxC (right); (b) Structure of AtxA (PDB ID: 3G8G) and AtxC (PDB ID: 3G8H) (Saul et al., 2010). The positions of mutations Phe124 and Lys128 in AtxA are shown in red and Ile124 and Glu128 in AtxC are in green. The seven conserved disulfide bonds are shown in yellow.

positions : an N-terminal α -helix A (residues 1–14), a short helix B (residues 16–22), a Ca^{2+} -binding loop (residues 25–35), a long α -helix C (residues 39–57), a loop preceding an anti-parallel two-stranded beta sheet (β -wing, residues 75–78 and 81–84), a long α -helix D (anti-parallel to helix C, residues 89–109) and a C-terminal extension (residues 110–133) with very short helical turns (Saul et al., 2010).

We have also succeeded in determining the crystal structure of a single isoform (CA_2CBb) of crotoxin purified from the South American rattlesnake (*Crotalus durissus terrificus*) (Faure et al., 2011). This non-covalent heterodimeric complex belongs to crotoxin complexes of class I (more stable, with high toxicity and low enzymatic activity) (Faure et al., 1993). Crystals of the crotoxin heterodimer were obtained with a solution containing 30% PEG 4000, 0.1 M Na Acetate, pH 4.6, 0.1M MgCl_2 , at a final protein concentration of 2.0 mg/ml. The crystals diffracted to 1.35 Å resolution (Faure et al., 2011). A summary of the crystallographic refinement parameters for AtxA, AtxC and crotoxin is shown in Table 1.

The crotoxin heterodimer is formed by a basic CB PLA_2 subunit (122 residues) non-covalently bound to an acidic CA subunit comprising three covalently linked polypeptide chains (α , 39 residues, β , 35 residues and γ , 14 residues) (Fig. 2). The overall structure of crotoxin reveals a unique scaffold for the association of the CA and CB subunits, with the acidic three-chain subunit CA_2 rotated by 180° with respect to the main axis and canonical front face orientation of the CB subunit (Faure et al., 2011). The α and β -chains both contribute to the buried surface interface with CB (25 residues of the α - and β -chains make direct contacts at distances less than 4.5 Å with 22 residues of the CBB subunit) (Faure et al., 2011).

3. Functional sites of ammodytoxin

Atx interacts with various high-affinity binding proteins, including the neuronal M-type sPLA_2 receptor (R180) (Prijetelj et al., 2008), the cytosolic Ca^{2+} -sensor calmodulin (CaM) (Kovacic et al., 2009, 2010), the

mitochondrial membrane receptor R25, not yet identified (Prijetelj et al., 2008) and other proteins involved in the process of presynaptic neurotoxicity (Sribar and Krizaj, 2011; Prijetelj-Znidarsic and Pungarcar, 2012). To date, co-crystallization experiments of Atx with FXa or with CaM and other high-affinity binding proteins (potential presynaptic receptors) have been not successful and determination of the three-dimensional structure of these PLA_2 -protein receptor complexes presents a major challenge. Several approaches have been developed to study the structure–function relationship of neurotoxic and anticoagulant PLA_2 with their soluble receptors (Prijetelj et al., 2006; Saul et al., 2010; Faure and Saul, 2011). Direct interactions and the formation of high-affinity complexes between Atx and FXa or CaM have been observed by SPR experiments (see review Faure and Saul, 2011). Fig. 3a and c shows theoretical models (based on mutagenesis experiments and molecular docking calculations) of potential complexes between AtxA and the two soluble receptors hFXa and CaM. Competition experiments confirmed that the C-terminal region of Atx is common to these interactions (Saul et al., 2010). Additional critical residues implicated in interaction with FXa and located in the β -wing region also contribute to the anticoagulant effect of AtxA (Prijetelj et al., 2006; Faure et al., 2007). Fig. 3b and d shows the overall three-dimensional structure of AtxA and the side chains of residues involved in anticoagulant activity determined by mutagenesis studies and SPR affinity binding studies (Prijetelj et al., 2006), and residues important for binding of CaM (Prijetelj et al., 2003). It is important to note that the catalytic activity of Atx is not implicated in anticoagulant function (Faure et al., 2007), whereas CaM substantially increases catalytic activity (Kovacic et al., 2009). The enhancement of Atx activity by CaM is accompanied by conformational stabilization of the enzyme (Kovacic et al., 2010).

3.1. Impact of structural differences on anticoagulant and neurotoxic function of AtxA and AtxC revealed by their crystal structures

AtxA differs from AtxC by only two amino-acid substitutions (Phe124 > Ile and Lys128 > Glu), but displays significant differences in toxicity and anticoagulant activity (AtxC displays 10-fold lower affinity for hFXa (Prijetelj et al., 2006) and 15-fold lower toxicity than AtxA) (Prijetelj-Znidarsic and Pungarcar, 2012). Although it is known that a cationic surface in neurotoxic basic PLA_2 s is important for anticoagulant activity (Kini, 2005), comparison of the crystal structures of AtxA and AtxC allowed us to explain in greater detail that the observed 10-fold decrease in binding affinity of AtxC for hFXa (Saul et al., 2010) is also due to local conformational differences in these natural PLA_2 isoforms. As shown in Figs. 3b and 4a, Lys 127 and Lys128 in the C-terminal region of AtxA are fully exposed and accessible for binding to hFXa. These residues were previously identified by mutagenesis and molecular docking studies as important for interaction with hFXa (Prijetelj et al., 2006; Faure et al., 2007). The mutation Lys128 > Glu in AtxC leads to a shift in the side chain of unmutated Lys127 resulting from interactions between the side chain of Glu128 with the

Table 1

Summary of crystallographic refinement parameters for AtxA, AtxC and crotoxin.

	AtxA ^a	AtxC ^b	Crotoxin ^c
Resolution (Å)	1.70	1.35	1.35
R-value (working set)	0.172	0.171	0.165
R _{free}	0.216	0.196	0.189
Completeness (%)	99.9	96.5	98.8
No. of protein atoms	971	989	1537
No. of water molecules	150	198	265
RMS deviations from ideal			
Bond length (Å)	0.012	0.013	0.013
Bond angles (°)	1.193	1.464	1.38
Ramachandran plot			
Residues in most favored regions (%)	89.3	88.3	90.5
Residues in additional allowed regions (%)	10.7	11.7	9.5

^a Saul et al. (2010) PDB ID: 3G8G.

^b Saul et al. (2010) PDB ID: 3G8H.

^c Faure et al. (2011) PDB ID: 3R0L.

Fig. 2. Crystal structure of the heterodimeric CA₂CBb crotoxin complex from *Crotalus durissus terrificus* venom (PDB ID: 3R0L) showing critical residues in the CA₂–CBb binding interface (Faure et al., 2011). (a) The basic subunit CB is shown in blue. The side chains of buried residues contacting CA₂ of the CBb subunit are shown in yellow. (b) The heterodimeric crotoxin complex showing the hydrophobic interaction site between CA₂ and CBb. (c) The α , β , and γ chains of the acidic subunit CA are shown in orange, green, and red, respectively. The side chains of buried residues contacting CBb of the CA₂ subunit are shown in yellow.

Fig. 3. Ammodytoxin A and its two soluble receptors: hFXa and CaM. (a) Theoretical three-dimensional model of the complex between AtxA (in magenta) and the light and heavy chains of human coagulation FXa (in blue and yellow, respectively). Adapted from Faure and Saul (2010) and based on mutagenesis studies and molecular docking calculations (Faure et al., 2007). (b) FXa-binding site of AtxA. The crystal structure (Saul et al., 2010) reveals the orientation of side chains of residues (shown in green) previously identified by mutagenesis and affinity binding studies as important for anticoagulant activity (Prijetelj et al., 2006). (c) Theoretical three-dimensional model of the complex between AtxA (in magenta) and CaM (in yellow). Adapted from Faure and Saul (2010) and based on docking calculations (Kovacic et al., 2010). (d) CaM-binding site of AtxA. The crystal structure of AtxA (Saul et al., 2010) shows side chains of residues (in blue) previously identified by site-directed mutagenesis as important for binding to CaM (Prijetelj et al., 2003).

Fig. 4. View of the C-terminal region of (a) AtxA and (b) AtxC, showing differences in shape complementarity for potential receptors and residues responsible for differences in anticoagulant and neurotoxic activity. The mutation Lys128 > Glu in AtxC leads to a significant decrease in binding affinity for FXa and the mutation Phe124 > Ile leads to differences in neurotoxicity. Adapted from Faure and Saul (2010) and based on the crystal structure (Saul et al., 2010).

main chain nitrogen atom of Thr35 (Saul et al., 2010). This interaction leads to a displacement of the main polypeptide chain at position 127–128 (Fig. 4a and b) and contributes to the observed decrease in affinity for hFXa (Priatelj et al., 2006). The detection of different conformations of Lys 127, a conserved residue in AtxA and AtxC, was possible only by comparison of the crystal structures at atomic level between the two isoforms. These results demonstrate the importance of crystallographic structural studies to investigate the precise role of natural mutations and should be useful in other biological systems.

The residues identified by mutagenesis studies as important for presynaptic neurotoxicity were located in the C-terminal region of AtxA (Tyr 115, Ile116, Arg118, Asn119, Phe124), a few residues in the N-terminal helix A and at position Phe24 (Priatelj et al., 2008; Pungercar et al., 2011). Differences in toxicity between AtxA and AtxC could be

explained by differences in surface complementarity resulting from the mutation Phe124 > Ile in the C-terminal region of AtxA and AtxC (Saul et al., 2010) (Fig. 4).

The crystal structures of AtxA and AtxC revealed a novel homodimeric PLA₂ quaternary conformation in which residue Met7 (unique to Atx) in both molecules of the homodimer makes strong hydrophobic interactions between the N-terminal α -helices of the two monomers (Saul et al., 2010). The functional importance of this dimer is unknown, but we and others proposed that formation of the PLA₂ dimer could create more accessible surface area for interaction with protein receptors by increasing the number of binding sites (Saul et al., 2010; Marchi-Salvador et al., 2008). In particular, for the Atx homodimer the two well identified neurotoxic binding sites are exposed and accessible for interaction with the presynaptic receptor (Saul et al., 2010).

Fig. 5. View of the CA-CB binding interface in the crystal structure of crotoxin. The mutation His1 > Ser in the binding interface of crotoxin is critical and could lead to differences in the stability of crotoxin isoforms. (Adapted from Faure et al., 2011). The mutation His1 > Ser in isoform CB₂ leads to a significant displacement of Trp70 and Lys69 in an adjacent loop linking helices C and D that could lead to decreased binding affinity for hFXa (Faure and Saul, 2011).

4. Functional sites of crotoxin

Although the molecular mechanism of presynaptic neurotoxicity of crotoxin is not yet fully understood, it was shown previously that the non-toxic CA subunit increases the lethal potency of the basic subunit CB by directing the heterodimeric complex to a specific crotoxin receptor (Krizaj et al., 1996, 1997; Faure et al., 2003). Binding studies have shown that the CA subunit is temporarily involved in the binding of crotoxin to the membrane receptor (Delot and Bon, 1992; Faure et al., 2003), and at equilibrium, CA is released while the CB subunit remains bound to the receptor. The crystal structure of crotoxin revealed the hydrophobic CA-CB interaction site and showed that the CA subunit physically occludes the hydrophobic interfacial binding surface (IBS) of CB (Fig. 2). The CA subunit masks IBS residues important for PLA₂ interaction with phospholipids (PL) and decreases the adsorption of CB to PL membranes, targeting the crotoxin complex to reach the specific crotoxin presynaptic receptor CAPT (R48) at the neuromuscular junction. Since CA participates together with CB in binding to the receptor, forming a transient ternary CA-CB-R48 complex followed by the release of CA, it is possible that the fully exposed region around Trp80 of

CBb in the crotoxin complex located on the opposite side of the CA–CB interface, could constitute an important binding site of crotoxin to its specific receptor (Faure et al., 2011).

Identification of the binding interface of crotoxin is crucial to better understand the mechanism of action of crotoxin.

4.1. Catalytic site

The crystal structure of crotoxin showed that the conserved canonical PLA₂ catalytic site and Ca²⁺-binding site are located only in the CB subunit (Faure et al., 2011) and the conformation of the Ca²⁺-binding loop in the CBb subunit is preserved in the presence of Na⁺. However, despite conservation of the primary sequence in the predicted Ca²⁺-binding loop of CA and the presence of critical residues (Tyr28, Gly30, Gly32 and Asp49), the crystal structure revealed the absence of a Ca²⁺-binding loop in the CA subunit (Faure et al., 2011).

4.2. Identification of the CA–CB binding interface

Identification of the CA–CB binding interface revealed key residues involved in the stability, toxicity and decreased PLA₂ activity of the heterodimeric crotoxin complex (Faure et al., 2011). The CA–CB complex is stabilized by hydrophobic and polar contacts, with a total buried surface area of 1870 Å² (Faure et al., 2011). Critical residues Trp31 and Trp70 of the CB subunit (isoform CBb) make important non-covalent interactions with Asp99 and Asp89, respectively, of the β-chain of CA₂. These interactions play an important role in stability of the highly toxic and enzymatically less active class I CA₂CBb complex (Faure et al., 1993). Structural analysis of three isoforms of CB (CBb, CBc, and CBA₂) showed how the mutation His1 > Ser in α-helix A (located in the CA–CB interface) is important for the stability of crotoxin complexes (Faure et al., 2011). The mutation His1 > Ser in isoform CBA₂ (Faure et al., 1994) is associated with a shift of up to 9.2 Å in an adjacent loop (residues 67–71) (Faure et al., 2011; Marchi-Salvador et al., 2008). The observed orientation of the side chain at Trp70 and displacement of the loop 67–71 in the CBA₂ isoform is incompatible with formation of a stabilizing hydrogen bond with Asp89 of the CA₂ subunit (Faure et al., 2011). These differences also lead to greater substrate access to catalytic site residues His48 and Asp99, thus explaining the greater PLA₂ activity of class II CA₂CBA₂ crotoxin complexes. The role of Trp31 in the CB subunit is also important for the stability of the crotoxin complex. In the free PLA₂ CBc isoform, the side chain of Trp31 is disordered, whereas in the CA₂CBb class I crotoxin complex, Trp31 of the CBb subunit makes an important stabilizing hydrogen bond with Asp99 in the β-chain of CA₂ (Faure et al., 2011).

4.3. The CA subunit is a natural inhibitor of the catalytic and anticoagulant activities of CB

The overall structure of the CB subunit of crotoxin (10-fold less toxic than CA–CB complex) is similar to human and other group IIA snake venom *Viperidae* PLA₂. It displays the canonical PLA₂ protein fold with four α-helices (A, B, C

and D), the beta-wing, the Ca²⁺-binding loop and the C-terminal region, covalently linked by seven disulfide bonds (Marchi-Salvador et al., 2008; Faure et al., 2011). The highly conserved active site residues (the His48/Asp99 dyad), clustered on helices C and D, interact via Asp49 with the Ca²⁺-binding loop. The Interfacial Binding Site (IBS), which interacts with phospholipids, is formed by a hydrophobic channel surrounding the entrance to the active site.

The significantly reduced anticoagulant activity of isoform CBA₂ is due to its lower binding affinity for FXa resulting from the mutation Glu128 > Lys (Faure et al., 2007, 2010). Additionally, the mutation His1 > Ser in CBA₂ is associated with a displacement of Lys69 and Trp70, compared with isoform CBc (Faure and Saul, 2011), which could also inhibit interaction with FXa (Faure and Saul, 2011). Further structural studies involving crystallization of the PLA₂–FXa complex are required to provide a detailed view of interactions at the binding interface and to confirm our proposal for the anticoagulant site of the CB subunit.

4.4. Future research directions based on identification of the CA–CB binding interface

We and others have shown previously that the anti-venom protein CNF/CICS identified in the blood of the snake *Crotalus durissus terrificus* (Perales et al., 1995; Fortes-Dias et al., 1994) specifically neutralizes the neurotoxicity and enzymatic activity of crotoxin and its individual CB subunit. The mechanism of action of CICS on crotoxin is similar to the interaction of crotoxin with its specific presynaptic receptor (Perales et al., 1995; Faure, 2000), inducing the dissociation of the crotoxin heterodimer and release of the CA subunit. CICS competes with the CA subunit for binding to CB (Faure, 2000; Faure et al., 2000). To date, no co-crystallization experiments of PLA₂ with CICS have been reported. Identification of the binding interface of crotoxin is crucial and will help in identifying the binding site of the natural crotoxin inhibitor CICS.

The monoclonal anti-CA antibody A56.36, specifically directed against the non-toxic subunit CA, was previously shown to neutralize crotoxin toxicity by dissociating the crotoxin complex (Choumet et al., 1992, 1998). Competition experiments performed by ELISA and SPR showed that CB inhibits the binding of mAb to the CA subunit (Choumet et al., 1992; Faure and Fourier, 2006) and that mAb A56.36 competes with the CB subunit for binding to CA and blocks interaction between CA and CB (Faure and Fourier, 2006). Knowledge of the CA–CB binding interface of crotoxin could help to map the epitope of this neutralizing mAb.

5. Conclusions

Knowledge of the spatial arrangement of the sites of interaction of snake venom PLA₂ with different targets (the neurotoxic receptor, hFXa, CaM, natural inhibitors and neutralizing antibodies) is important for the understanding of fundamental processes such as neurotoxicity, hemostasis, inhibition of PLA₂ activity, and neutralization of toxicity by monoclonal antibodies and natural PLA₂ inhibitors. The mechanism of these different processes at the

atomic level remains unknown, but extensive structure-function studies by X-ray crystallography and molecular modeling provide a progressively clearer picture.

In recent work (collaboration with the group of Prof. Aleksander Edelman INSERM U845, Paris France) we discovered a new target and new function of crotoxin (see abstract presented in 17th Word Congress of the IST in Hawaii) Faure et al., 2012. This new target is human Cystic Fibrosis Transmembrane conductance Regulator (CFTR). Interestingly, crotoxin and its CB subunit effectively increase the Cl^- channel current of the human F508delCFTR mutant responsible for cystic fibrosis, and show a potentiation or correcting effect on the mutated CFTR. These results provide an original perspective to investigate the structure of the CFTR-crotoxin complex and the pharmacotherapy of cystic fibrosis.

Thus, new anticoagulant, anti-neurotoxic and anti-cystic fibrosis drug leads could be elaborated based on the 3D structure of β -neurotoxins and their interaction sites with various biological targets. The identification of novel functions of PLA_2 , including new protein receptor binding sites, constitutes a promising area of research for future studies.

Conflict of interest statement

The authors declare that there is no conflict of interest.

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