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Structural features of the reprolysin atrolysin C and tissue inhibitors of metalloproteinases (TIMPs) interaction

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Abstract

Atrolysin C is a P-I snake venom metalloproteinase (SVMP) from *Crotalus atrox* venom, which efficiently degrades capillary basement membranes, extracellular matrix, and cell surface proteins to produce hemorrhage. The tissue inhibitors of metalloproteinases (TIMPs) are effective inhibitors of matrix metalloproteinases which share some structural similarity with the SVMPs. In this work, we evaluated the inhibitory profile of TIMP-1, TIMP-2, and the N-terminal domain of TIMP-3 (N-TIMP-3) on the proteolytic activity of atrolysin C and analyzed the structural requirements and molecular basis of inhibitor–enzyme interaction using molecular modeling. While TIMP-1 and TIMP-2 had no inhibitory activity upon atrolysin C, the N-terminal domain of TIMP-3 (N-TIMP-3) was a potent inhibitor with a K_i value of approximately 150 nM. The predicted docking structures of atrolysin C and TIMPs were submitted to molecular dynamics simulations and the complex atrolysin C/N-TIMP-3 was the only one that maintained the inhibitory conformation. This study is the first to shed light on the structural determinants required for the interaction between a SVMP and a TIMP, and suggests a structural basis for TIMP-3 inhibitory action and related proteins such as the ADAMs.

Keywords: TIMP; Atrolysin C; SVMP; Molecular docking; Molecular dynamics simulation; Binding affinity

Extracellular matrix components modulate cellular behavior and interactions with the surrounding environment. One class of endopeptidases involved in extracellular matrix degradation is the zinc metalloproteinases including the matrix metalloproteinases (MMPs), ADAMs (a disintegrin and metalloproteinases), and ADAMTSs (ADAMs with thrombospondin motifs). Many human diseases are related to disregulation of these metalloproteinase activities including arthritis, cancer, and atherosclerosis [1–3].

Snake venom metalloproteinases (SVMPs) are a subgroup of the reprolysins with an active role in the pathogenesis of snake envenomation. SVMPs have been shown to participate in the hemorrhagic process by proteolytically degrading extracellular matrix and capillary basement membranes which in part lead to the disruption of local capillary networks, hemorrhage, and edema [4]. The classification of these enzymes is based on their different domain structures, presented as follows: P-I (SVMPs with only metalloproteinase domain), P-II (SVMPs with metalloproteinase and disintegrin domains), P-III (SVMPs with metalloproteinase, disintegrin-like, and cysteine-rich domains), and P-IV (SVMPs with P-III domain structure plus two lectin-like domains connected by disulfide bonds) [5]. Atrolysin C is a P-I SVMP from *Crotalus atrox* venom which efficiently degrades basement membrane proteins surrounding capillaries, thus permitting the flux of capillary contents into the stroma and producing hemorrhage [6]. The three-dimensional structure of atrolysin C shows

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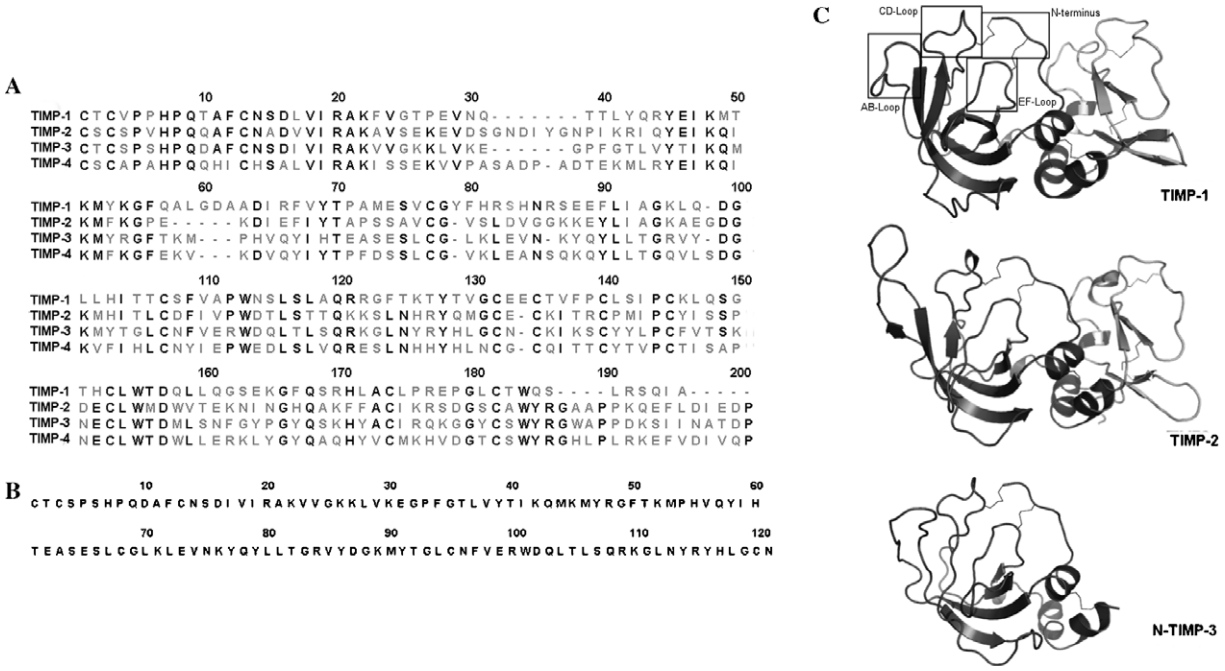

Fig. 1. TIMPs sequences and structures. (A) Sequence alignment of TIMPs showing identical residues in black. (B) Amino acid sequence of N-TIMP-3. (C) Crystallographic TIMP-1 (PDB code 1UEA) and TIMP-2 (PDB code 1BUV) structures were used as templates for modeling the N-TIMP-3 structure. N-terminal domains are shown in light grey and C-terminal in dark grey. Regions of TIMPs important for the interaction with metalloproteinases are boxed in the TIMP-1 structure.

a noticeable similarity with MMPs and human ADAMs [7], and displays the characteristic structural features including (1) an active-site helix with zinc ligands and (2) an extended binding site defined by an antiparallel β-strand [6].

The activity of MMPs on extracellular matrix is regulated by a family of endogenous inhibitory proteins, the tissue inhibitors of metalloproteinases (TIMPs). The TIMP family comprises four members (TIMP-1 to -4) with 184–194 residues (mature protein) which share between 41% and 52% sequence identity including six conserved disulfide bonds (Fig. 1A). All TIMPs have two domains and the larger N-terminal domain is responsible for the metalloproteinase inhibition, while the smaller C-terminal domain mediates the interaction with the hemopexin domains of certain pro-MMPs (Fig. 1C) [8,9].

TIMPs inhibit MMPs with relatively low selectivity and some, but not all, ADAMs can be inhibited by TIMPs with dissociation constants in the subnanomolar range [8–10]. Some members of the ADAM family, for example ADAM10, 12, 17, 19, and ADAMTS-4 and -5, have been shown to be efficiently inhibited by TIMP-3 but significantly less susceptible to the inhibitory effect of TIMP-1 and TIMP-2, except by the inhibition of ADAM10 by TIMP-1 [9]. To date there are four known resolved structures of TIMPs complexed with active MMPs [11–13]. These structures show TIMPs interacting with the catalytic domain of the MMP through six separated chain segments forming a tight non-covalent 1:1 complex. The main contact is made by the first five residues of the inhibitor binding to the active site cleft in a quasi-substrate manner. Based on the similarities between tertiary structures and interactions of MMPs with TIMP-1 and TIMP-2, it is generally believed that TIMP-3 should display a more or less identical configuration.

Given the ability of the TIMPs to interact with and inhibit MMPs it is conceivable that they might also inhibit SVMPs. Here, we examined the inhibitory pattern of TIMPs upon the proteolytic activity of atrolysin C. The differences in the inhibitory capability of TIMPs upon atrolysin C were also studied by molecular modeling and molecular dynamics in order to investigate the molecular basis of TIMPs–atrolysin C interactions.

Materials and methods

Inhibition assays of TIMPs on atrolysin C. Human recombinant TIMP-1 and TIMP-2 were expressed in mammalian cells and purified from conditioned medium as described before [14,15]. N-terminal domain of human TIMP-3 with His-tag at C-terminus was expressed in *Escherichia coli*, folded *in vitro*, and purified as described previously [16]. Atrolysin C solution was prepared to be 20 nM in TNC buffer (50 mM Tris–HCl, pH7.5, 150 mM NaCl, 1 mM $CaCl_2$, and 0.02% Brij-35). The enzyme solution (100 μl) was mixed with equal volume of TIMP solutions prepared at various concentrations in the TNC buffer. The proteinase-inhibitor mixtures (200 μl) were incubated at 37 °C for 1 h prior to addition of substrate. Twenty microliters of Azocoll50 (Calbiochem) solution (8 mg/ml in the TNC buffer) was added to each reaction and incubated for 18 h at 37 °C in bioshaker at 180 rpm. After incubation the reaction mixture was centrifuged at 3000 rpm for 3 min to precipitate the remaining substrates, the supernatant was harvested and its absorbance at 520 nm was measured to estimate the proteinase activity.