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Structural modeling of osteoarthritis ADAMTS4 complex with its cognate inhibitory protein TIMP3 and rational derivation of cyclic peptide inhibitors from the complex interface to target ADAMTS4

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ABSTRACT

The ADAMTS4 (a disintegrin and metalloproteinase with thrombospondin motifs 4) enzyme is a matrix-associated zinc metalloendopeptidase that plays an essential role in the degradation of cartilage aggrecan in arthritic diseases and has been recognized as one of the most primary targets for therapeutic intervention in osteoarthritis (OA). Here, we reported computational modeling of the atomic-level complex structure of ADAMTS4 with its cognate inhibitory protein TIMP3 based on high-resolution crystal template. By systematically examining the modeled complex structure we successfully identified a short inhibitory loop (^{62}EASESLC^{68}) in TIMP3 *N*-terminal inhibitory domain (NID) that directly participates in blocking the enzyme's active site, which, and its extended versions, were then broken from the full-length protein to serve as the peptide inhibitor candidates of ADAMTS4. Atomistic molecular dynamics simulation, binding energetic analysis, and fluorescence-based assay revealed that the TIMP3-derived linear peptides can only bind weakly to the enzyme (K_d = 74 ± 8 μM), which would incur a considerable entropy penalty due to the high conformational flexibility and intrinsic disorder of these linear peptides. In this respect, we proposed a cyclization strategy to improve enzyme–peptide binding affinity by, instead of traditionally maximizing enthalpy contribution, minimizing entropy cost of the binding, where a disulfide bond was added across the two terminal residues of linear peptides, resulting in a number of TIMP3-derived cyclic peptides. Our studies confirmed that the cyclization, as might be expected, can promote peptide binding capability against ADAMTS4 substantially, with affinity increase by 3-fold, 9-fold and 7-fold for cyclic peptides ^{62}CASESLC^{68}, ^{61}CEASESLAGC^{70} and ^{60}CTEASESLAGC^{70}, respectively.

1. Introduction

The ADAMTS (a disintegrin and metalloproteinase with thrombospondin motifs) enzymes are secreted, multi-domain matrix-associated zinc metalloendopeptidases that exhibit diverse functions in tissue morphogenesis and patho-physiological remodeling [1] and have been identified in distinct human diseases and genetic disorders [2]. The proteolytic enzyme family includes 19 members that can be sub-grouped on the basis of their known substrates, namely the aggrecanases or proteoglycanases (ADAMTS1, 4, 5, 8, 9, 15 and 20), the procollagen *N*-propeptidases (ADAMTS2, 3 and 14), the oligomeric matrix protein-cleaving enzymes (ADAMTS7 and 12), the von-willebrand factor proteinase (ADAMTS13), and a group of orphan enzymes (ADAMTS6, 10, 16, 17, 18 and 19) [3]. In particular, the two aggrecanase isoforms ADAMTS4 and ADAMTS5 play an essential role in the degradation of cartilage aggrecan in arthritic diseases, which are closely related to the pathogenesis of osteoarthritis (OA) [4], a progressive disease that results from irreversible collagen degradation in cartilage extracellular matrix by cleaving aggrecan at a unique site termed the "aggrecanase site" [5]. Therefore, the ADAMTS4 and ADAMTS5 have been recognized as new and promising targets for therapeutic intervention in OA and, in recent years, a number of chemical drugs and antibody agents have been developed to block the two enzymes [6–8].

The TIMP (human tissue inhibitors of metalloproteinases) is a family of the natural inhibitory proteins of metalloproteinases, which contains four members TIMP1, TIMP2, TIMP3, and TIMP4. The TIMP3 has been found to specifically inhibit the aggrecanase subfamily of ADAMTS [9] and some other metalloproteinases such as MMP3 [10] and TACE [11] through its *N*-terminal inhibitory domain (NID); reactive-site mutagenesis analysis and *in vitro* kinetic study revealed that the TIMP3 is a very strong, cognate

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inhibitor of ADAMTS4 and ADAMTS5 with inhibition constants K_i at subnanomolar level [12,13]. However, the structural basis and molecular mechanism of TIMP3 interaction with ADAMTS4 and ADAMTS5 still remain unelucidated to date, thus largely limiting its applications in development of OA therapeutics.

Previously, Yu et al. found that entropy penalty is the primary content of the indirect readout energy in protein–peptide recognition as compared to the deformation energy that is the main source of the indirect readout energy in classical biomolecular binding phenomena [14]. A number of chemical strategies have been used to improve protein–peptide binding affinity by reducing peptide flexibility and intrinsic disordering in isolated state. For example, Verdine and co-workers applied hydrocarbon stapling to restrict peptides in α-helix [15], and Arora et al. used hydrogen bond surrogate helices to stabilize peptide confirmation [16]. Here, based on the crystal structure TACE–TIMP3 complex we attempted to computationally construct the atomic-level structure model of TIMP3 NID domain in complex with ADAMTS4 catalytic domain by integrating bioinformatics modeling, molecular dynamics simulation, binding energetics analysis, and fluorescence polarization measurement. The constructed complex structure model was examined in detail by comparing to those of ADAMTS4 in *apo* conformation and bound with small-molecule inhibitors, which was then used to derive cyclic peptide inhibitors from the complex interface. In order to improve the binding affinity of linear peptides to ADAMTS4, instead of traditionally increasing favorable enthalpy contribution we herein proposed a peptide cyclization strategy to minimize the unfavorable entropy penalty in ADAMTS4–peptide recognition. We also explored the structural basis, energetic property and dynamic behavior of cyclic peptide binding to ADAMTS4.

2. Results and discussion

2.1. Structural modeling of ADAMTS4–TIMP3 complex

The TIMP3 is a cognate inhibitory protein of the aggrecanase subfamily of ADAMTS [9], which has been shown to exhibit nanomolar inhibition potency on ADAMTS4 (K_i = 3.3 nM) [12]. However, the inhibitory complex structure of TIMP3 against ADAMTS4 has not been solved to date. Fortunately, the crystal structure of TACE in complex with TIMP3 is currently available in the protein data bank (PDB) database [17] with accession 3CKI. The TACE (TNF alpha converting enzyme), also known as ADAMTS17 or MMP13, is a unique metalloproteinase with highly defined substrate selectivity [18,19], which can be potently inhibited by TIMP3. Here, we conducted structure superposition between the TIMP3-bound conformation of TACE catalytic domain (PDB: 3CKI) and the *apo* conformation of ADAMTS4 catalytic domain (PDB: 3B2Z) by using Swiss-PdbViewer program [20]. As can be seen in Fig. 1, the two enzymes share a generally homologous structural architecture and, in particular, their active sites are highly conserved, with a good conformational consistence in the four active-site components, *i.e.* β-strand, α-helix, *c*-loop, and Zn^{2+} ion chelator, although it seems a considerable variation at a loop region that precedes to the β-strand. The structural superposition analysis as well as visual survey of other metalloproteinase–TIMP complex crystal structures suggested that the TIMP3 should adopt a similar manner to interact with TACE and ADAMTS4. In this respect, a structural grafting strategy was employed to computationally model the ADAMTS4 inhibition by TIMP3 based on the crystal template of TACE–TIMP3 complex (PDB: 3CKI). Detailed description of the grafting procedure can be found in Section 3.1 and Fig. 10. Consequently, the coarse-grained complex structure of ADAMTS4 catalytic domain with TIMP3 NID domain was constructed, which was then subjected to 100-ns MD simulations for conformation relaxation and structural refinement. In fact, the complex system can fast reach at dynamics equilibrium after ~25-ns simulations, and only small conformational fluctuation and atomic thermal motion can be observed during rest of the simulations.

Next, the TIMP3-bound conformation (after MD simulation) of ADAMTS4 catalytic domain was compared to its initial *apo* conformation (before MD simulation) to detect the effect of TIMP3 on ADAMTS4 structure. As can be seen in Fig. 2, the two conformations are highly consistent, with root-mean-squares deviation (*rmsd*) of 0.57 Å between them, indicating that binding of TIMP3 to ADAMTS4 would not address substantial influence on the whole structure of the enzyme. This is not unexpected if considering that the inhibitory protein only adopt a flexible inhibitory site to interact with the enzyme's active site. However, a considerable difference in the loop region preceding β-strand can be observed

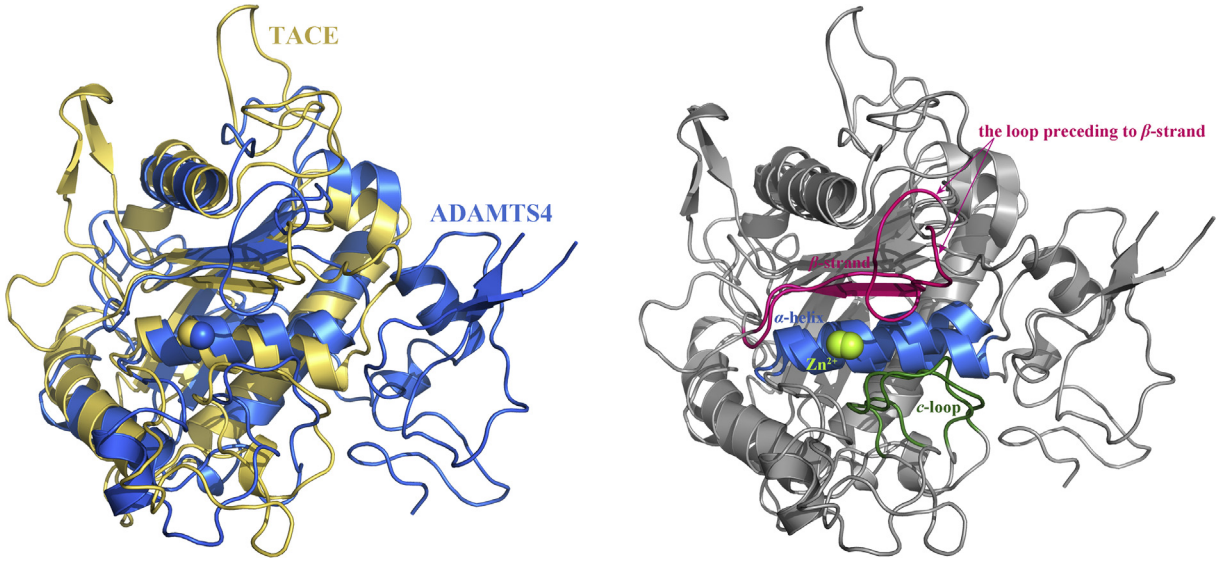

Fig. 1. Superposition between the crystal structures of TACE and ADAMTS4 catalytic domains, where the TACE catalytic domain is in TIMP3-bound conformation (PDB: 3CKI), while the ADAMTS4 catalytic domain is in *apo* conformation (PDB: 3B2Z). The enzyme's active site is highlighted, consisting of a β-strand, a α-helix and a *c*-loop as well as a Zn^{2+} ion chelator.