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Classification of ADAMTS binding sites: The first step toward selective ADAMTS7 inhibitors

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ABSTRACT

Genome-wide association studies identified *ADAMTS7* as a risk locus for coronary artery disease. In carotid arteries of rats, neointima formation after balloon-mediated injury goes along with enhanced Adamts7 expression. Vice versa, *Adamts7*-deficient mice display reduced neointima formation following vascular injury. Although a causal link between ADAMTS7 and coronary artery disease remains to be proven, inhibition of ADAMTS7 represents a potential new target for intervention in this disease.

ADAMTS7, a member of the 'a disintegrin and metalloproteinase with thrombospondin motifs' (ADAMTS) family of proteins, contains a catalytic zinc ion in the binding site of its metalloproteinase domain. The structure of ADAMTS7 and its inhibitors are unknown. In this study, we used *in silico* methods, including homology modeling and pharmacophore modeling, to analyze the ADAMTS7 metalloproteinase domain, particularly its binding site. The results revealed structural and sequence differences relative to the binding sites of the other ADAMTS proteins; these non-conserved regions represent potential binding regions for selective ADAMTS7 inhibitors. The main contribution of this study is the proposal of a pharmacophore for ADAMTS7. The characterization of the ADAMTS7 binding site and definition of a pharmacophore are the first step toward developing a new therapeutic target for coronary artery disease.

1. Introduction

Genome-wide association studies have demonstrated that the *ADAMTS7* gene resides within a risk locus for coronary artery disease (CAD) [1,2]. Additionally, in rat carotid arteries Adamts7 is markedly overexpressed in parallel with neointima formation after balloon-mediated injury [3]. By contrast, *Adamts7*-knockout mice show loss of neointima formation following vascular injury [4] and Bauer et al. [5] demonstrated in whole-body Adamts7-knockout mice fed a high cholesterol diet on a proatherogenic background that the deletion of Adamts7 significantly reduces atherosclerotic lesion formation. These findings suggest that ADAMTS7 might represent a novel target for CAD therapies [6]. ADAMTS7 has not been shown to influence mechanisms targeted by existing treatments. Therefore, inhibition of this protein is a promising target for intervention; however, neither inhibitors nor structural data for ADAMTS7 are currently available.

ADAMTS7, a 1686-amino acid protein, is a member of the ADAMTS (a disintegrin and metalloproteinase with thrombospondin motifs) family. Together with MMPs (matrix metalloproteinases) and ADAMs (a disintegrin and metalloproteinases), the ADAMTS proteins belong to the metzincins [7]. The human ADAMTS family consists of 19 members, all of which are proteolytically active [8]. Substrates have been identified for most ADAMTS proteins, with the exceptions of ADAMTS6, -16, -17, -18 and -19. ADAMTS7 and ADAMTS12 degrade COMP (cartilage

Abbrevations: ADAMTS, a disintegrin and metalloproteinase with thrombospondin motif; CAD, coronary artery disease.

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oligomeric matrix protein), and ADAMTS7 binds α_2-macroglobulin [8] and connective tissue growth factor (CTGF) [9]. Several family members are associated with diverse diseases: *ADAMTS7* with CAD, as noted above; *ADAMTS4* and *-5* with arthritis; *ADAMTS1*, *-9*, *-12* and *-15* with cancer; and *ADAMTS16* with hypertension [10].

The ADAMTS proteins share a common domain structure that can be separated into a proteinase domain and an ancillary domain (Fig. 1). The proteinase domain consists of signal, pro, metalloproteinase, and disintegrin-like domains. The ancillary domain, which exhibits more variability, consists of varying numbers of thrombospondin type 1 motifs, a cysteine-rich and spacer domain, and special domains present in subsets of ADAMTS proteins [10]. MMPs and ADAMs also contain signal, pro, and metalloproteinase domains. Like ADAMTS proteins, ADAMs have disintegrin and cysteine-rich domains [11]. In addition, ADAMs and MMPs contain C-terminal domains not present in ADAMTS proteins, e.g., the hemopexin-like domain of MMPs [12] and the EGF-like and cytosolic domains of ADAMs [11]. ADAMTS, ADAM, and MMP proteins all contain a conserved zinc-binding motif, HEXGHXXGXXH (where X represents any amino acid residue), in their metalloproteinase domains. The three histidine residues in this motif coordinate a catalytic zinc ion in the binding site of the metalloproteinase domain [13].

The Protein Data Bank (PDB, www.rcsb.org) [14] contains several X-ray substructures of ADAMTS proteins, but no complete structure of an ADAMTS protein is currently available. At the moment, 16 substructures of human ADAMTS1, -4, -5, and -13 have been published. ADAMTS13 3D structures cover the disintegrin-like to spacer domain (e.g., PDB: 3GHM [15]), but no structural data for the metalloproteinase domain of this protein are currently available. A few X-ray structures of the ADAMTS5 metalloproteinase domain have been reported (e.g., 3HYG [16] and 3B8Z [17]), one of which covers both the metalloproteinase and disintegrin-like domains (PDB: 2RJQ [18]); however, in all of these structures, inhibitor molecules are bound to the binding site. The published X-ray structures of ADAMTS1 and -4 cover the metalloproteinase and the disintegrin-like domain, and both apo (ADAMTS1-PDB: 2V4B [19]; ADAMTS4-PDB: 3B2Z [18]) and inhibitor-bound (e.g. ADAMTS1-PDB: 2JIH [19]; ADAMTS4-PDB: 2RJP [18]) structures are available for both proteins. In all inhibitor-bound X-ray structures of ADAMTS proteins, the inhibitor coordinates the binding site zinc ion, suggesting that inhibition of the ADAMTS7 via the metalloproteinase domain (specifically, by coordination of the catalytic zinc ion) represents a first step toward finding a new treatment for CAD.

Hence, definition of the ADAMTS7 binding site using *in silico* methods such as homology modeling and pharmacophore modeling should help to identify compounds that could bind to this region of the protein. Our aim in this study was to use these *in silico* methods to analyze the ADAMTS7 metalloproteinase domain, especially the binding site, and determine differences relative to the binding sites of the other ADAMTS proteins. Our results revealed non-conserved areas that represent candidate binding regions for selective ADAMTS7 inhibitors. On the basis of these findings, we propose a pharmacophore for ADAMTS7. Our characterization of the binding site and definition of the pharmacophore should facilitate development of a new therapeutic target for CAD.

2. Methods

2.1. Multiple sequence alignment

Sequence comparisons of ADAMTS metalloproteinase domains were performed by aligning the domains using the PROMALS3D web service [20]. ADAMTS metalloproteinase domain sequences were obtained from UniProt [21]. The sequence similarity of the metalloproteinase domains was determined using the SIAS web service (substitution matrix: BLOSUM62) [22].

2.2. Homology modeling

For structural comparisons of binding sites, homology models of ADAMTS7 and other ADAMTS metalloproteinase domains, that currently lack solved 3D structures, were built. Crystal structure coordinates of ADAMTS1, -4, and -5 metalloproteinase domains were used as templates. Apo-state structures of ADAMTS1 and ADAMTS4 are available and were used preferentially. The best template was chosen based on sequence identity and similarity. If different templates had identical values, the X-ray structure with the best resolution was selected. Models were built using the homology modeling module of Schrödinger Prime (version 3.3, Schrödinger, LLC, New York, NY, 2013) with default settings. Missing amino acid residues in the template structures were modeled using an *ab initio* procedure. We placed structural constraints on the interaction between the zinc ion and the three histidine residues in the zinc binding motif, called zero order bonds, because this coordination is essential in homology models and was sometimes not observed without these constraints. The models were refined by loop refinement (Schrödinger Prime version 3.3) for non-template loops. Additionally, the models were subjected to energy minimization using the OPLS2005 force field [23]. Validation of the models was done using Prosa2003 [24], PROCHECK [25], and MetaMQAPII [26].

In further analyses, the available X-ray structures of the metalloproteinase domains of ADAMTS1 (PDB: 2V4B [19], resolution: 2.0 Å), ADAMTS4 (PDB: 3B2Z [18], resolution: 2.8 Å), and ADAMTS5 (PDB: 3HYG [16], resolution: 1.4 Å) were used instead of homology models of these proteins. The binding site of ADAMTS was defined based on crystal structures of ligand-bound ADAMTS proteins, and was determined to consist of two discontinuous segments.

2.3. Pharmacophore and 3D binding site characterization

A pharmacophore model for the ADAMTS7 binding site was

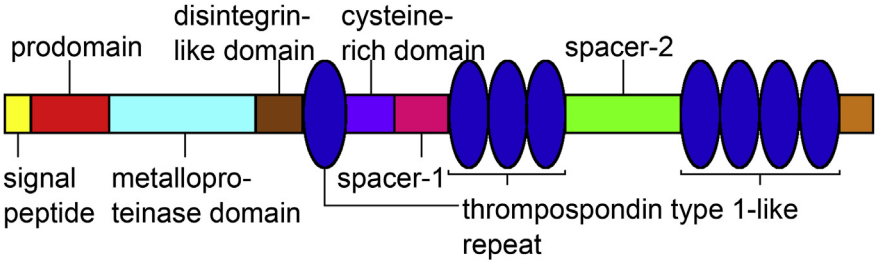

Fig. 1. ADAMTS7 domain structure exemplary for the ADAMTS domain structure adapted from Liu et al. [8].