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Research article

In silico characterization of the interaction between LSKL peptide, a LAP-TGF-beta derived peptide, and ADAMTS1

Marie-Amandine Laurent[a], Dominique Bonnier[b], Nathalie Théret[b], Pierre Tufféry[a], Gautier Moroy[a,*]

[a] Université Paris Diderot, Sorbonne Paris Cité, Molécules Thérapeutiques in silico (MTi), Inserm UMR-S 973, 35 rue Hélène Brion, 75013 Paris, France
[b] INSERM U1085, Institut de Recherche en Santé, Environnement et Travail (IRSET), Université de Rennes 1, 2 avenue Pr Léon Bernard, Rennes, France

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ABSTRACT

Metalloproteases involved in extracellular matrix remodeling play a pivotal role in cell response by regulating the bioavailability of cytokines and growth factors. Recently, the disintegrin and metalloprotease, ADAMTS1 has been demonstrated to be able to activate the transforming growth factor TGF-β, a major factor in fibrosis and cancer. The KTFR sequence from ADAMTS1 is responsible for the interaction with the LSKL peptide from the latent form of TGF-β, leading to its activation. While the atomic details of the interaction site can be the basis of the rational design of efficient inhibitory molecules, the binding mode of interaction is totally unknown.

In this study, we show that recombinant fragments of human ADAMTS1 containing KTFR sequence keep the ability to bind the latent form of TGF-β. The recombinant fragment with the best affinity is modeled to investigate the binding mode of LSKL peptide with ADAMTS1 at the atomic level. Using a combined approach with molecular docking and multiple independent molecular dynamics (MD) simulations, we provide the binding mode of LSKL peptide with ADAMTS1. The MD simulations starting with the two lowest energy model predicted by molecular docking shows stable interactions characterized by 3 salt bridges (K_3–NH_3^+ with E_{626}–COO^-; L_4–COO^- with K_{619}–NH_3^+; L_1–NH_3^+ with E_{624}–COO^-) and 2 hydrogen bonds (S_2–OH with E_{623}–COO^-; L_4–NH with E_{623}–COO^-). The knowledge of this interaction mechanism paves the way to the design of more potent and more specific inhibitors against the inappropriate activation of TGF-β by ADAMTS1 in liver diseases.

1. Introduction

The extracellular matrix (ECM) is the complex environment surrounding the cells in solid tissue. The ECM remodeling by metalloproteases is required for physiological events such as morphogenesis, development or tissue repair. However, alterations in expression and activities of these proteases are implicated in many diseases such as fibrosis and cancers (Egeblad and Werb, 2002).

Metalloproteases belonging to the ADAM (a disintegrin and metalloproteinase) protein family are characterized by a multi-domain organization that includes metalloprotease, disintegrin, cystein, transmembrane and cytoplasmic domains and are involved in ectodomain shedding, cell adhesion and cell signaling. While ADAMs are highly expressed in cancer cells and tissues, their functions are not still precisely understood (Mochizuki and Okada, 2007).

ADAMTS (ADAM with thrombospondin motifs) members are secreted-type ADAMs that associate with ECM components and are characterized by the presence of one or more thrombospondin type-1 repeats (TSP1). The ADAMTS1 gene was originally cloned from a colon carcinoma cell line and ADAMTS1 upregulation is associated with cell proliferation, inhibition of apoptosis and altered vascularization (Tan Ide et al., 2013). ADAMTS1 has been firstly described as a protease, which degrades mainly proteoglycans, such as aggrecan and versican. However, we have recently demonstrated a new proteolytic-independent role in liver fibrosis demonstrating that ADAMTS1 induces the activation of the transforming growth factor-β (TGF-β) (Bourd-Boittin et al., 2011).

Abbreviations: ADAM, a disintegrin and metalloproteinase; ADAMTS, ADAM with thrombospondin domains motifs; ECM, extracellular matrix; LAP, latency-associated protein; LGAL, amarckian genetic algorithm; K_d, dissociation constant; MD, molecular dynamics; RMSD, root mean square deviation; SLC, small latent complex; TGF-β, transforming growth factor; TSP1, thrombospondin type-1.

* Corresponding author. Fax: +331 57278372.
E-mail address: gautier.moroy@univ-paris-diderot.fr (G. Moroy).

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TGF-β is one of the most important regulators of tissue homeostasis and upregulation and activation of TGF-β has been linked to various diseases, including fibrosis and cancer through promotion of cell proliferation and invasion and of the epithelial-mesenchymal transition (Ikushima and Miyazono, 2010). To antagonize the increase TGF-β activity in diseases, many strategies have been developed including ligand traps, small inhibitory molecules and antisense based approaches (Korpal and Kang, 2010), however more specific targeting method are required to take into account the pathological activity of TGF-β.

TGF-β is synthesized as an inactive homodimeric large precursor molecule consisting of a self-inhibiting propeptide, the latency-associated protein (LAP), in addition to the covalently linked active form of TGF-β. Pro-TGF-β is then intracellularly cleaved by furin-type enzymes to generate mature TGF-β which remains non-covalently associated with LAP as the small latent complex (SLC). TGF-β, secreted as SLC, cannot bind to its cell surface receptors and the release of biologically active TGF-β requires its dissociation from LAP in a process termed latent TGF-β activation. Numerous protease- and non protease-dependent mechanisms, which differ according to cell type and to the physiological context, have been implicated in the conversion of latent TGF-β into its biologically active form, *i.e.* dissociation of TGF-β from LAP-TGF-β in the soluble SLC and/or from the ECM-bound SLC (Shi et al., 2011). Interestingly, we have recently demonstrated that ADAMTS1 binds LAP-TGF-β and induces the release of active TGF-β (Bourd-Boittin et al., 2011).

The TGF-β activation occurs through the interaction between a KTFR sequence of ADAMTS1 and a LSKL sequence of TGF-β (Bourd-Boittin et al., 2011). The KTFR motif is localized in ADAMTS1 cysteine-rich domain and the LSKL sequence in LAP subunit of latent TGF-β. Interestingly, LSKL peptide is able to reduce liver fibrosis by inhibiting TGF-β activation. To note, LSKL peptide has been shown to have an equivalent effect on liver fibrosis in rats (Kondou et al., 2003).

The knowledge of the binding mode of a targeted protein with a partner is often the cornerstone of the rational design of new therapeutic molecule. In this paper, the ability of two recombinant fragments of human ADAMTS1, containing the KTFR sequence, to bind LAP-TGF-β was assessed experimentally. Then, we have characterized the binding mode of LSKL peptide on the greater recombinant fragment of ADAMTS1, called F1, using molecular modeling methods. LSKL peptide was docked at ADAMTS1 surface and the stability of binding modes was investigated by multiple molecular dynamics (MD) simulations.

2. Methods and materials

2.1. Preparation of recombinant fragments and binding experiments

Recombinant fragments of human ADAMTS1 containing KTFR sequence, called F1 (from F_{554} to F_{849}, 33 kDa) and F2 (from F_{554} to K_{725}, 19 kDa) were produced as previously described (Leyme et al., 2012). While F1 is composed of TSP1-1 domain, the spacer domain and the cysteine-rich domain, F2 is only formed by TSP1-1 domain and the spacer domain.

Affinity measurements were performed using MicroScale Thermophoresis technology with a Nanotemper Monolith NT.115 (Nanotemper, Munich, Germany) and standard treated capillaries. Settings were: Red excitation and detection, 50% LED, 40% MST power and 25 °C temperature control setting. LAP-TGF-β (Biosciences, Cambridge, UK) was labeled using the Monolith NT Protein Labeling Kit RED (NanoTemper Technologies) according to the supplied protocol. The concentration of labeled LAP-TGF-β was kept constant at 62.5 nM. The corresponding unlabeled binding partners (F1 and F2) were titrated in 1:10 dilutions. As positive control, we use thrombospondin-1, the well-known interactant of LAP-TGF-β.

2.2. Structures

LSKL peptide was built in a fully extended model with N and C extremities charged, using c35b4 version of CHARMM program (Brooks et al., 1983, 2009).

The ADAMTS1 structure at the atomic level is not available in the Protein Data Bank (Berman et al., 2000; Bernstein et al., 1977). However, a homology model of the F2 recombinant fragment, containing the TSP1-1 domain, the spacer domain and the cysteine-rich domain, has been previously built (Bourd-Boittin et al., 2011). The sequence identity between the selected domains of the human ADAMTS1 sequence and the sequences of the related proteins structures (ADAMTS13, ADAMTS5 and proteins with TSP1-1 domain) is of 30.4 %. The structure of the model is stabilized by 8 disulfide bonds (3 in TSP1-1 domain and 5 in cysteine-rich domain), which are conserved between related proteins structures and ADAMTS1. Molecular dynamics (MD) simulations have been performed for over 12 ns to assess the stability of the model. The MD analysis has been shown a conformational stability of the region encompassing the KTFR motif and a preservation of its solvent exposure. KTFR sequence is in α-helical structure, which is a very stable structure, generally well-predicted and well-built using homology modeling approaches. K, T and F residues are exposed to the solvent and able to interact with a partner, in accordance with experimental results. R residue is buried due to its interaction with other parts of ADAMTS1. The protonation states of titratable residues have been calculated at physiological pH of 7.0, using PROPKA program (Bas et al., 2008).

2.3. Molecular docking

The molecular docking computations attempt to predict the structure of a complex formed between a ligand and a targeted protein. In the case of short peptides, classical molecular docking programs, such as AutoDock program (Hetenyi and van der Spoel, 2002; Moroy et al., 2009) or Glide program (Tubert-Brohman et al., 2013), that have been developed for the docking of small molecules have been proven to be efficient.

The molecular docking has been carried out using AutoDock 4.2 program (Morris et al., 1998, 2009). Gasteiger partial charges for both ADAMTS1 and LSKL peptide as well the generation of the input files necessary to the docking procedures have been performed using the AutoDockTools package. At first, docking computations have been done on the whole ADAMTS1 surface to verify whether AutoDock is able or not to find the expected binding site for the interaction, *i.e.*, KTFR sequence. The grid box size for this "blind docking" procedure was set to $126 \times 126 \times 126$ points with a grid spacing of 0.70 Å. Such spacing between the grid points is not sufficiently precise to provide accurate models at the atomic level. For the regions of interest identified during the blind docking, a narrower grid has been employed. This second grid box was centered on the position obtained previously during the "blind docking" step. The grid dimensions were set of $126 \times 126 \times 126$ points with a spacing of 0.22 Å. We have employed the Lamarckian genetic algorithm (LGA) for ligand conformational searching. Four independent runs of docking computations have been done with, for each, the following parameters: 250 LGA operations, a population size of 250 individuals, a maximum of 2,000,000 energy evaluations and a maximum of 2700 generations. The default parameters for Solis and Wet local search were used. During the docking computations, all the torsion angles of the LSKL peptide were allowed to be rotatable, except the ϖ dihedral angles of the peptide bonds, which were fixed to 180°.