BITS :: Call for Abstracts 2021 - Poster

Type	Poster
Session	Protein structure and function
Title	HIV-1 Tat/heparin interaction: translating new insight from molecular modelling to the comprehension of its biological functions
All Authors	Milanesi M (1), Chiodelli P (1), Urbinati C (1), Manconi A (2), D'Ursi P (2) and Rusnati M (1)

Affiliation

(1) Macromolecular Interaction Analysis Unit, Department of Molecular and Translational Medicine, University of Brescia, Brescia
(2) Institute for Biomedical Technologies-National Research Council (ITB-CNR), Segrate, Milano

Motivation

Human immunodeficiency virus (HIV) is the ethological agent of the acquired immunodeficiency syndrome (AIDS). HIV+ lymphocytes (LCs) release the transactivating factor (Tat) which, in its mono- or dimeric form, bind to different host cell receptors including heparan sulfate proteoglycans (HSPGs), vascular endothelial receptor 2 (VEGFR2) and integrins, mediating a variety of biological effects involved in the onset of AIDS [1].
Various functional domains of Tat responsible for several functions have been identified: the cysteine rich domain (aa 22-37) for dimerization, the "basic domains" (aa 48-57) for HSPGs and VEGFR2 binding and a RGD motif (aa 78-80) for integrins interaction1, suggesting that Tat binds simultaneously different cell receptors. In effect: Tat dimers released by HIV+ LC associate to HSPGs of the same cell, retaining the ability to bind to HSPGs on facing endothelial cells (ECs), thus forming an in-trans (between two facing cells) HSPG/Tat-Tat/HSPG quaternary complexes that promotes LC extravasation [2]. Tat bound to LC's HSPGs also retains the ability to bind in trans VEGFR2 and integrins of ECs, inducing their inflammatory activation and vessel permeability [work in progress]. Tat tethers onto HSPGs of the extracellular matrix, promoting the formation of an in cis (on the same cell) trimeric Tat/VEGFR2/integrin complex that triggers EC cytoskeleton organization and proangiogenic activation [3]. Free Tat engages simultaneously HSPGs and integrins of uninfected LCs, forming an in-cis trimeric complex that stimulates their migration [4].
Thus, the HSPGs/Tat complex orchestrates the recruitment of other signaling receptors, regulating biological processes relevant to AIDS pathogenesis. It is therefore important to characterize at a molecular level the structure of the HSPGs/Tat complex, with particular attention to the exposure of its various functional domains, a task suitably approachable by computational molecular modelling.

Methods

Heparin is used both experimentally and computationally as an analog of HSPGs.
We have so far modelled the monomeric HIV Tat/heparin complex and the Tat homodimer to simulate the starting units of the different Tat complexes described above.
Tat structure (isolate BRU/LAI, UniProt P04610) was used as template to create a 3D model of Tat isolate HXB2 (UniProt P04608) using the MODELLER program. From the ten conformations obtained, the lowest energy model was selected. 4-mer heparin probes was prepared and used in docking simulations to promote the 1→4 glycosidic linkage. To model the Tat dimer, the automatic protein-protein docking ClusPro web-server [5] was used that ranks the interactions according to the best cluster size and four different sets of energy coefficients; the resulting best complexes were filtered by visual inspection.
Heparin path identification: Blind docking simulations were performed by ClusPro using 4-mer heparin as ligand option to identify heparin-binding regions on Tat that were then filtered by best score, cluster size, visual inspection and finally positioned onto Tat to achieve a traced heparin path.
Incremental docking and heparin modelling: The 4-mer heparin probe was used in local docking simulation along the traced heparin path in Tat by Vina-Carb [6]. The "sliding window method" was set up to create a sequence of overlapping sliding grids. Local docking poses were filtered for free energy of binding, clusters size and correct orientation. The aligned 4-mer heparin probes were joined by 1→ 4 glycosidic linkages using Pymol [7]. Gasteiger-Hückel charges were assigned to the sugar and then minimized by Chimera [8], obtaining heparin chains of increasing length. The two previous methods have been developed and applied in heparin protein interaction studies [9].
Molecular dynamic simulations (MDs) are ongoing for Tat monomer in complex with 12-mer heparin and for homodimer Tat complex using Amber18 package [10].

Results

A first model has been obtained with a 12-mer heparin chain that binds several regions of monomeric Tat,

including the heparin binding but not the cysteine rich motif, suggesting that Tat heparin binding does not impaired protein dimerization.
A second model has been obtained using two Tat monomers to predict its dimeric form in which the heparin regions remains solvent exposed and available to interact with 12-mer heparin chain.
Moreover, in the first model (Tat monomer) but not in the second (Tat dimer), the integrin-binding RGD motif remains fully accessible.
MDs of the Tat monomer and dimer are ongoing to evaluate their stability. Stable complexes will be used to model the biological relevant heparin/Tat complexes and to identify the functional HSPG/Tat/Integrin complex.

Info

References
1. Rusnati M et al. Angiogenesis 2002, 5:141-51
2. Urbinati C et al. Blood 2009, 114:3335-42
3. Urbinati C et al. Arter. Throm. & Vas. Biol. 2012, 32:e25-34
4. Urbinati C et al. Oncogene 2017, 36:2609-18
5. Kozakov D et al. Nature Protocols 2017, 12:255-78
6. Nivedha AK et al. J. Chem. Theory & Comp. 2016, 12:892-901
7. DeLano WL Curr. Opinion Struc. Biol. 2020, 12:14-20
8. Pettersen EF et al. J. Comp. Chem. 2004, 25:1605-12
9. Bugatti A. et al. Sci. Rep. 2019, 9:15768
10. Case DA et al. J. Comp. Chem. 2005, 26:1668-88

Figure

-

Availability -

Corresponding Author

Name, Surname Maria, Milanesi
Email m.milanesi006@unibs.it
Submitted on 30.04.2021