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Identification and characterization of a glycosaminoglycan binding site on interleukin-10 via molecular simulation methods

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ABSTRACT

The biological function of the pleiotropic cytokine interleukin-10 (IL-10), which has an essential role in inflammatory processes, is known to be affected by glycosaminoglycans (GAGs). GAGs are essential constituents of the extracellular matrix with an important role in modulating the biological function of many proteins. The molecular mechanisms governing the IL-10–GAG interaction, though, are unclear so far. In particular, detailed knowledge about GAG binding sites and recognition mode on IL-10 is lacking, despite of its imminent importance for understanding the functional consequences of IL-10–GAG interaction. In the present work, we report a GAG binding site on IL-10 identified by applying computational methods based on Coulomb potential calculations and specialized molecular dynamics simulations. The identified GAG binding site is constituted of several positively charged residues, which are conserved among species. Exhaustive conformational space sampling of a series of GAG ligands binding to IL-10 led to the observation of two GAG binding modes in the predicted binding site, and to the identification of IL-10 residues R104, R106, R107, and K119 as being most important for molecular GAG recognition. *In silico* mutation as well as single-residue energy decomposition and detailed analysis of hydrogen-bonding behavior led to the conclusion that R107 is most essential and assumes a unique role in IL-10–GAG interaction. This structural and dynamic characterization of GAG-binding to IL-10 represents an important step for further understanding the modulation of the biological function of IL-10.

1. Introduction

Interleukin-10 (IL-10) is an immunoregulatory cytokine whose biological relevance has been extensively reviewed [1]. Its most prominent function is to limit and eventually terminate inflammatory responses via its ability to inhibit effector functions of T cells, monocytes, and macrophages, and by inhibiting the synthesis of inflammatory cytokines such as IFN-γ and TNF-α [2–4]. Malfunction of IL-10 leads to improperly regulated immune reactions: IL-10-deficient mice spontaneously develop acute and chronic inflammation [5]. On the macroscopic scale, IL-10 has a crucial impact on tissue repair [6]. Despite the observed anti-inflammatory effects of IL-10, the attempts to use it directly as a therapeutic agent in various inflammatory conditions yielded disappointing results [7]. The IL-10 system turned out to be more complex than initially assumed, and it was found that its functions largely depend on its structural micro-environment [8], and the specific immune environment in which it is released [9]. Furthermore, it was found that IL-10 also has pro-inflammatory effects in certain conditions [10], pointing towards an incredibly multifaceted role of IL-10 in biology. Likewise, IL-10 is often called a *pleiotropic* cytokine.

IL-10 has been shown to bind glycosaminoglycans (GAGs) [11,12]. GAGs are essential building blocks of the extracellular matrix (ECM) and can be characterized as unbranched, negatively charged polysaccharides composed of repeating disaccharide units [13]. Based on the configuration of these disaccharide building blocks, GAGs are grouped into five classes: hyaluronan, chondroitin sulfate, dermatan sulfate, keratan sulfate, and heparan sulfate/heparin. GAGs play a critical role in many biological processes. Their multifarious biological activity arises from their ability to interact with and directly affect the biological activity of many cytokines, chemokines and growth factors [14–18]. Salek-Ardakani et al. demonstrated that GAGs may modulate IL-10 function by showing that soluble GAGs inhibit the IL-10-induced expression of CD16 as well as CD64 on monocytes and macrophages, revealing a dependency of the inhibition strength on the GAG sulfation degree. Interestingly, they also showed that sulfated cell surface-bound GAGs were required for IL-10 to trigger its biological function towards corresponding cells [11]. A recent NMR study showed that GAG sulfation is prerequisite for binding to IL-10: heparin was found to be the strongest binder, and the binding affinity of different GAG types decreased with the sulfation degree. No

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binding was detected for hyaluronan [12]. In essence, the molecular mechanisms of these effects are unclear so far. Thus, insights into the structural principles underlying the interaction between IL-10 and GAGs are required for understanding the biological role of IL-10–GAG interaction.

Here, we report the identification of a GAG binding site on IL-10 and the detailed characterization of the molecular interaction between IL-10 and GAGs in this site based on simulation methods. The calculation and analysis of the Coulomb potential of IL-10 in solution allowed us to locate a GAG binding region on its surface. We then employed Dynamic Molecular Docking (DMD), a recently published specialized method based on molecular dynamics (MD) simulations [20], to investigate the IL-10–GAG interaction in further detail. With DMD we performed exhaustive conformational space sampling while GAG and protein were treated as entirely flexible, and solvent was taken into account explicitly. The DMD calculations yielded two putative GAG binding pose models as well as ensemble-derived single-residue energetics and hydrogen bonding data. These data allowed the identification of those IL-10 residues being most important for binding GAGs. Our results shed light on possible molecular mechanisms governing GAG-mediated IL-10 function modulation.

2. Materials and methods

2.1. IL-10 and GAG structures

The analysis described in the present work was based on the X-ray structure of human IL-10 with PDB ID 2ILK (1.6 Å resolution) [20]. The biologically active unit of IL-10 is known to be a homodimer comprised of two intercalated IL-10 monomers and characterized by a twofold rotational symmetry [21,22]. We have built this homodimer by extending the monomeric 2ILK structure with a copy of itself, rotated by 180° around the crystallographic twofold axis as defined in the PDB entry.

The GAG molecules heparin (HP) dp4, HP dp6, hyaluronan (HA) dp4, HA dp6, chondroitin-4-sulfate (CS4) dp6, chondroitin-6-sulfate (CS6) dp6 ("dp" denoting the degree of polymerization; i.e. the number of sugar rings per molecule) were built with LEAP [23] and parameterized using GLYCAM 06 [24] as described previously [20]. MD snapshots of these molecules are depicted in SI Fig. S1.

2.2. IL-10 Coulomb potential simulation

The electrostatic potential of IL-10 was calculated with a finite-difference numerical solver applied to the linearized Poisson–Boltzmann (PB) equation, using the PBSA program shipped with AmberTools 13 [23]. In the PB model applied here, IL-10 is represented as a dielectric body with vacuum permittivity whose shape is defined by atomic coordinates and radii. The solvent is treated as a continuum with a relative permittivity of 80. The net electrostatic potential of IL-10 was calculated as the sum of its vacuum Coulomb potential and the corresponding solvent reaction field, using PBSA default parameters and a finite element grid spacing of 1 Å. IL-10 atomic coordinates were taken from the IL-10 dimer structure described above. The atomic radii and point charges of the protein were parameterized according to the FF99SB molecular mechanics force field [23]. Source code modification of the PBSA software was required for writing the discretized scalar data in compliance with the OpenDX data format and in units of kcal/(mol e) (whereas 1 kcal/(mol e) $\triangleq$4.18 kJ/(mol e) for appropriate post-processing in VMD [25]. Corresponding software patches were contributed back to the AmberTools project (see SI for further information about these modifications).

2.3. Dynamic Molecular Docking of GAGs to IL-10 and data analysis

The interaction between IL-10 and GAG molecules was investigated via Dynamic Molecular Docking (DMD), a recently published targeted molecular dynamics (tMD)-based docking method especially accounting for the effects of receptor flexibility and system solvation [20]. The DMD principle is schematically visualized in Fig. 1. Its cornerstone is the creation of an ensemble of MD trajectories by repetitively pulling a ligand molecule towards a predefined binding region on a receptor protein. Subsequent analysis of the trajectory data and the ensemble of ligand poses provides atomic information about the ligand–receptor interaction.

The geometrical DMD parameterization requires definition of a so-called *core atom*, an atom in the protein core with as little mobility relative to the bulk of the protein as possible, as well as a *focus point* near the protein surface at the center of the anticipated binding region. The straight line connecting *focus point* and *core atom* determines the directionality of the relative ligand–receptor movement when the ligand is being pulled towards the receptor. The distance between *core atom* and *focus point* corresponds to the so-called *target distance*. For a more complete definition of these terms see SI Fig. S2 and the previously published DMD methodology [20].

In the first stage of a so-called DMD run, the ligand molecule is slowly pulled towards the receptor molecule, starting from a distal position, as schematically visualized in Fig. 1. This pulling process is implemented with a time-dependent harmonic potential applied to the distance between the *core atom* and a central ligand atom. The pulling potential competes with the canonical molecular mechanics potentials and with the thermal fluctuations applied in the simulation. Hence, while being directed, the trajectory of relative movement between receptor and ligand remains random. When the *target distance* is reached, the pulling potential is switched off, and the second stage of the DMD run is performed, which is an unrestrained ("free") molecular dynamics simulation for relaxing the system and for collecting data. A DMD study consists of a large number of DMD run repetitions (see Section 3 for the

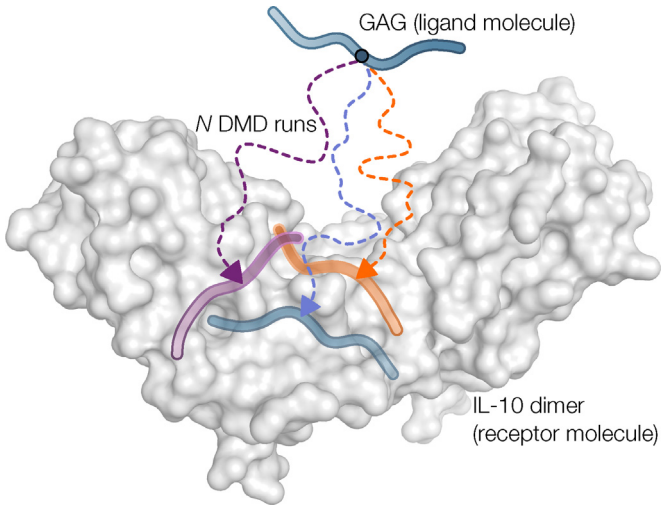

Fig. 1. Schematic representation of a DMD study. A DMD study is comprised of N DMD runs, performed in independent simulations. Each run begins with a pulling process: starting from a distal position, the GAG molecule is pulled towards IL-10 (shown in gray surface representation). During this process, the GAG translates and rotates along a random path and samples its conformational space. The pulling process stops when the GAG is in proximity to the surface of IL-10 and is followed by a long unrestrained MD simulation. This results in an ensemble of MD trajectories as well as in a collection of GAG poses in contact to the receptor surface, as schematically indicated here in purple, blue, orange. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)