Investigation of the interleukin-10-GAG interaction using molecular simulation methods

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Summary

Glycosaminoglycans (GAGs) are linear polysaccharides, built of periodically occurring disaccharide units. GAGs are ubiquitous in the extracellular matrix (ECM), where they exhibit multifarious biological activities. This diversity arises from — among others — their ability to interact with and regulate a large number of proteins, such as cytokines, chemokines, and growth factors. As of the huge variety in their chemical configuration, GAGs are further sub-classified into different types (heparin, for instance, is one of these sub-classes). Hence, GAGs are a diverse class of molecules, which surely contributes to the broadness of their spectrum of biological functions. Through varying arrangements of sulfate groups and different types of saccharide units, individual GAG molecules can establish specific atomic contacts to proteins. One of the best-studied examples is antithrombin-heparin, whose biologically relevant interaction requires a specific pentasaccharide sequence [1]. It is valid to assume, however, that various proteins are yet to be discovered whose biological functions are in some way affected by GAGs. In other cases, and this is true for the cytokine interleukin-10 (IL-10), there are already experimental indications for a biologically relevant protein-GAG interaction, but the details are still obscure and the fundamental molecular interaction mechanism has still not been clarified.

IL-10 has been shown to bind GAGs [2]. So far, however, no structural detail about IL-10-GAG interaction is known. Function-wise, IL-10 is mainly considered to be immunosuppressive and therefore anti-inflammatory, but it in fact has the pleiotropic ability to influence the immune system in both directions, i.e. it constitutes a complex regulation system on its own. Therefore, the role of GAGs in this system is potentially substantial, but is yet to be clarified. *In vitro*

experiments have yielded indications for GAGs being able to modulate IL-10's biological function [2], and obviously IL-10 and GAGs are simultaneously present in the ECM. This gives rise to the assumption that IL-10-GAG interaction is of biological significance, and that understanding the impact of GAGs on IL-10 biology is important — from the basic research point of view, but also for the development of therapies, potentially involving artificially designed ECMs.

A promising approach for obtaining knowledge about the nature of IL-10-GAG interaction is its investigation on the structural level, i.e. the identification and characterization of the molecular interaction mechanisms that govern the IL-10-GAG system. In this PhD project it was my goal to reveal structural and molecular details about IL-10-GAG interaction with theoretical and computational means, and with the help of experiments performed by collaborators in the framework of the Collaborative Research Centre DFG Transregio 67. For achieving this, I developed three methods for the *in silico* investigation of protein-GAG systems in general and subsequently applied them to the IL-10-GAG system. Parts of that work have been published in scientific journals, as outlined further below.

I proposed and validated a systematic approach for predicting GAG binding regions on a given protein, based on the numerical simulation and analysis of its Coulomb potential. One advantage of this method is its intrinsic ability to provide clues about the reliability of the resulting prediction. Application of this approach to IL-10 lead to the observation that its Coulomb attraction for GAGs is significantly weaker than in case of exemplary protein-GAG systems (such as FGF2-heparin). Still, a distinct IL-10-GAG binding region centered on the residues R102, R104, R106, R107 of the human IL-10 sequence was identified. This region can be assumed to play a major role in IL-10-GAG interaction, as described in chapter 3.

Molecular docking methods are used to generate binding mode predictions for a given receptor-ligand system. In chapter 4, I clarify the importance of data clustering as an essential step for post-processing docking results and present a clustering methodology optimized for GAG molecules. It allows for a reproducible analysis, enabling systematic comparisons among different docking studies. The approach has become standard procedure in our research group. It has been applied in a variety of studies [3, 4, 5, 6, 7], and served as an essential tool for

studying IL-10-GAG interaction, as described in chapter 6.

Motivated by the shortcomings of classical docking approaches, especially with respect to protein-GAG systems, I worked on the development of a molecular dynamics-based docking method with less radical approximations than usually applied in classical docking. The goal was to make the computational model properly account for the special physical properties of GAGs, and to include the effects of receptor flexibility and solvation. The methodology was named Dynamic Molecular Docking (DMD) and published in the *Journal of Chemical Information and Modeling* [8] — together with a validation study.

The subsequent application of DMD in a variety of studies required enormous amounts of computational resources. For tackling this challenge, I established a graphics processing unit-based high-performance computing environment in our research group and developed a software framework for reliably performing DMD studies on this hardware, as well as on other computing resources of the TU Dresden. The investigation of the IL-10-GAG system via DMD was focused on the IL-10-GAG binding region predicted earlier, and made heavy usage of the optimized clustering approach named above. An important result of this endeavor is that IL-10's amino acid residue R107 significantly stands out compared to all other residues and supposedly plays a particularly important role in IL-10-GAG recognition. The collaboration with the NMR laboratory of Prof. Daniel Huster at the Universität Leipzig was fruitful: I post-processed nuclear Overhauser effect data and obtained heparin structure models, which revealed that IL-10-heparin interaction has a measurable impact on the backbone structure of the heparin molecule. These results were published in *Glycobiology* [9]. In chapter 8, I propose two different scenarios about how GAG-binding to IL-10 might affect its biological function, based on the findings made in this thesis project.

In conclusion, a set of methods has been developed, all of which are generically applicable for the investigation of protein-GAG systems. Regarding the IL-10-GAG system, valuable structural insights for increasing the understanding about its molecular mechanisms were derived. These observations pave the way towards unraveling GAG-mediated bioactivity of IL-10, which may then be specifically exploited, for instance in artificial ECMs for improved wound healing.