Chapter 1

Introduction

Chemokines (chemoattractant cytokines) are small 6-15 kDa secreted proteins, which were discovered through their major function, chemoattraction of leukocytes to the sites of inflammation[1]. They can be divided into four groups according to the position of highly conserved cysteine residues in their N-terminal region. The C-X-C chemokines or α-subfamily, where two of the conserved cysteines are separated by any single amino acid, generally attract neutrophils and lymphocytes[2]. The two most studied members of this subfamily are interleukin 8 (IL-8) and stromal derived factor 1 (SDF-1). The C-C or β-subfamily has two adjacent conserved cysteines and acts on monocytes, lymphocytes, eosinophils, basophils, and dendritic cells[3]. RANTES (Regulated on Activation, Normal T Cell Expressed and Secreted) is the member of this group. Lymphotactin is an example of the C- or γ-subfamily of chemokines and has only one conserved cysteine in the N-terminus[4]. The C-X_3-C or δ-subfamily of chemokines has two conserved cysteines separated by three amino acids and is defined by the single member, fractalkine. The nomenclature for chemokine receptors is linked to their ligands. For example, C-C chemokines bind C-C chemokine receptors, and so on. Apart from cytokines, chemokines bind G-protein coupled receptors (GPCRs). A chemokine can bind more than one receptor, and receptors can bind multiple ligands.

GPCRs form a large superfamily of membrane proteins that modulate sensory perception, chemotaxis, neurotransmission, cell communication, and many other vital physiological events. Characterized by their cell-surface localization and tissue-specificity, these protein receptors are the targets of 50-60% of all existing medicines including well-known ß-blockers and anti-histamine therapeutics[5]. It is generally accepted that a better understanding of the structure and function of these receptors will

help in the design of drugs for the treatment of GPCR-related diseases. Unfortunately, GPCRs are rather difficult to express and crystallize and to date there exists only one such crystal structure – that of bovine rhodopsin[6] (BR). Consequently, computational biologists have concentrated their efforts on using this experimental information to build crude structural models of other GPCRs[7]. A major shortcoming of this approach is that it fails to produce reliable structures of GPCRs which have low (< 25%) sequence identity to rhodopsin. Clearly, then, it is necessary to devise a general method which can generate reliable structures of GPCRs and thus accelerate the drug design process.

CCR1 was the first CC chemokine receptor to be identified[8-9], and is believed to be involved in the pathogenesis of numerous chronic inflammatory diseases such as rheumatoid arthritis[10], multiple sclerosis[11], and organ transplant rejection[12]. Recent studies have broadened this scope and have implicated human CCR1 (hCCR1) in seemingly diverse areas such as Alzheimer's disease[13], cancer[14], and HIV-2[15]. CCR1 signaling may also contribute to tissue damage and inflammation through the enhancement of T-cell activation, regulation of T-helper, and stimulation of macrophage function and protease secretion. These properties support the concept that CCR1 is an attractive therapeutic target to modulate leukocyte infiltration and decrease the associated tissue damage common to autoimmune diseases.

Clearly then, the need for small molecule antagonists of hCCR1 is imperative from a therapeutic standpoint. Previous work has elucidated the common features of typical antagonists: halogen-modified aromatic systems coupled with a basic region[16]. However, these antagonists have practical shortcomings, especially with respect to receptor cross-reactivity and reduced binding affinity to animal models[17]. One of the

major challenges in drug design for chemokine receptors is to find an animal model, where the drug response is similar to that of the corresponding human chemokine receptor.

In light of these issues, there exists a clear need to work toward the design of receptor specific antagonists. Since this approach necessitates understanding of receptor structure, we are currently limited by information available from homology models based on the crystal structure of BR[6]. Considering that the sequence homology of hCCR1 to BR is in the sub-twilight zone (<25%), this methodology proves to be largely ineffective for accurate structural modeling[18]. Consequently, we were motivated to develop a new approach to structure determination with minimal experimental information and no information from the atomic coordinates of the crystal structure of BR.

The structure of this thesis is as follows. Chapter 2 describes the use of the MembStruk procedure[19-21] to predict the structure of human CCR1. The human structure is subsequently validated through prediction of the antagonist binding site, to which a series of known antagonists are docked and scored for comparison to experimental structure-activity data. Our ligand binding energies are in excellent agreement with the experimentally known trend in binding affinities. Results from a virtual ligand screening calculation also support the validity of our structural model. This work was done in collaboration with Drs. Sabine Schlyer and Richard Horuk at Berlex (Schering AG). A condensed version of this chapter will be submitted as a manuscript to *Journal of the American Chemical Society*.

The work presented in Chapter 3 involves comparing the binding sites of a strong and weak affinity antagonist to hCCR1, with the goal of designing receptor mutants that

markedly improve the binding of the low-affinity antagonist. We docked to our validated hCCR1 structure two antagonists with completely different (3 orders of magnitude) known binding affinities. We then chose two sites (L260 and V263) for computational point mutation, and systematically made changes to a series of polar residues (Asn, Gln, Ser, Thr, and Tyr). In most cases we find a large improvement in binding energy, and have proposed these mutations to our experimental collaborators at Berlex (experiments are underway). This chapter will be submitted as a communication to *Nature Chemical Biology.*

The central theme of Chapter 4 is prediction of structure and antagonist binding site in the mouse CCR1. Structural differences with respect to BR and hCCR1 are discussed, and corollaries drawn where appropriate. We dock a library of three ligands in an effort to understand the effect of charged ligands, and compare the results with the hCCR1 binding sites presented in Chapter 2. Analysis of the BX471 binding cavity provides an interesting study of conserved residues in the binding site, showing the effect of helical rotation on antagonist binding. The results presented here are a starting point for subsequent docking of larger and more diverse antagonist libraries, with the goal of complete structural validation.

Determination of the rat CCR1 structure is the focus of Chapter 5. In order to explain binding differentials across highly homologous structures (rat and mouse), we docked BX471 and analyzed its binding site. We find that in rCCR1, two conserved aromatic residues (in the rodent structures) pi-stack much more favorably with the aromatic functionalities of the BX471 ligand. Additionally, we are able to quantitatively discern why BX471 binds better to hCCR1 than rCCR1 by examining differences in side

chain placement of a conserved tyrosine residue. Due to the lack of (published) experimental binding affinities for antagonists other than BX471, we have not yet been able to validate this structure as we did for hCCR1. However, this study provides much needed insight into what factors we believe are accountable for subtle differentials in antagonist recognition.

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