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Research paper

Conformational analysis of the human chemokine receptor CXCR3

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ABSTRACT

In the last years, some studies showed the patho-genetic role of CXCR3 bound to its ligands in many human inflammatory diseases and cancers. Thus, the blockage of the CXCR3 interaction site to its ligands is seen as a possible therapeutic target for the treatment of cancer. The presence of flexible regions in the chemokine receptors determines their capability to develop specific mechanisms of action. We have recently focused on the features of the N-terminal region of human CXCR3 free in solution, where we demonstrate the presence of numerous conformational ensembles, dynamically stabilized by H-bonds. Since up to now no structure was experimentally determined for CXCR3, we decided to approach the study of its conformational behavior by molecular dynamics simulations, in a lipid bilayer, surrounded of water, at neutral pH and 300 K. Furthermore, we modeled the CXCR3/CXCL11 complex, where CXCL11 is one of its natural ligands. The aim of this work is to have a vision as realistic as possible in dynamic terms of the biological mechanism that drives the search for the ligand, its interaction and the formation of a stable complex between CXCR3 and CXCL11.

Overall, our approach has been able to describe the structural events which dynamically characterize the molecular mechanisms involved in the binding of CXCR3 to CXCL11 and the critical role exerted by its N-terminal region in "hunting" and capturing the ligand.

1. Introduction

Chemokine receptors are complex biological objects whose function is to transfer the information carried by the ligand molecules, from outside to inside the cell (Olson and Ley, 2002). The complexity resides in the fact that helical regions corresponding to the great part of their structures

are immersed in the membrane and the protruding terminal parts are involved in researching, recognizing and interacting of extracellular signaling molecules, as well as, in triggering the intracellular forwarding of the message (Clark-Lewis et al., 1995). These functions can only be explained dynamically (Raucci et al., 2014a, 2014b), but unfortunately the structural information that we possess by means of X-ray and NMR often does not allow a progressive reconstruction of all the structural events, which underpin the biological process, preventing us from understanding the complete molecular mechanism of receptors (Raucci et al., 2014a). One of the reasons is attributable to the presence of very mobile regions in these receptors that are not visible and determinable by X-rays and NMR (Raucci et al., 2014b). In fact, in Protein Data Bank (PDB) the experimental structures for human CXCR1 and CXCR4 obtained by NMR and X-Ray, respectively, are missing of their complete N-terminal regions (Park et al., 2012; Wu et al., 2010). Therefore, what we lack about chemokine receptor structures is the knowledge about the behavior of their highly fluctuating terminal segments, which certainly play a critical role in the mechanism of interaction with ligands.

Unfortunately, it has become apparent that the structural vision as a snapshot of the receptor functioning, as we have at present, even if accompanied by the knowledge of the physical determinants of the structural interaction with its ligand, is not adequate for a complete description of its molecular mechanism of action. This raises the important question of whether the motions of the terminal segments could represent a feature relevant for the knowledge of the molecular mechanism, because a classical globular vision leads to experimental approaches different from the ones needed for disordered elements or, in any case, highly fluctuating segments. Indeed, we have recently noted that the ends of the human chemokine receptors possess a considerable amount of intrinsic disorder, evolutionarily well conserved in mammals and, therefore, functionally important (Raucci et al., 2014a). This molecular characteristic is detected particularly in the form of high structural flexibility with a low tendency to create globular organizations by means of structures topologically fixed in time. For more

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information on these details, we focused on the 48 residues from the N-terminal peptide of the human CXCR3, an important pleiotropic receptor, often found involved in tumor diseases (Raucci et al., 2014b) that we synthesized and studied free in solution by spectroscopic methods as well as by molecular dynamics simulations. This peptide shows stretches of negative charges embedded in a very flexible sequence that generates numerous poly-structured globule-like ensembles, dynamically stabilized by H-bonds. Moreover, it is also important to underline that during molecular dynamics simulations some residues act as HUB residues because they interact through H-bonds with many other residues, playing a key role in stabilizing the structure (Raucci et al., 2014b). The behavior of the peptide suggests that we are in presence of a poly-electrolyte instead of a polyampholyte, specifically of a poly-anion, and its structural behavior can be well described by the polymer physics. Obviously these findings refer to the peptide free in solution, while the same peptide may have structurally different behaviors when bonded to his scaffolding of helices. What we have learned is that the structural behavior of the terminal segment cannot be assimilated to that characteristic of the globular proteins, because of its peculiar amino acid composition characterized by a prevalence of small and polar residues with a high percentage of charged residues, which confer to the peptide a high flexibility and high net charge (Trotta et al., 2009; Palladino et al., 2012).

In order to have an answer to this question we modeled the human CXCR3 through molecular dynamics simulations, embedded in a lipid bilayer with the outer surface negatively charged, like the natural one, and in the presence of explicit water molecules at physiological pH. Furthermore, we describe the structural events which dynamically characterize the molecular mechanisms involved in the binding to CXCL11, the natural ligand of CXCR3. In this article, we integrate the classical static view of the chemokine receptors with the data from MD to explain how the high flexibility of the terminal segment works. Furthermore, we discuss in detail the structural behavior of the N-terminal residues during the dynamics.

2. Methods

2.1. Molecular modeling of CXCR3

The three-dimensional model of human CXCR3 (Uniprot code: P49682, region 18–335) was recently published from our group (Trotta et al., 2009; Palladino et al., 2012). Since it lacked the first 17 residues in the N-terminus, we modeled the previous 3D structure adding the lacking residues. The new model was subjected to molecular dynamics (MD) simulations in a lipid bilayer and water molecules for 100 ns as described below in details.

2.2. Molecular dynamics simulation of CXCR3 embedded in lipid bilayer

Starting from the PDB file containing Cartesian coordinates of CXCR3 residues we generated a topology file using VMD psfgen plugin (Szpakowska et al., 2014). Then, by means of the “VMD membrane builder” plugin we assembled a lipid bilayer of POPC molecules in which we inserted the receptor taking into account not only the hydrophobicity of residues, but also data from the “Orientations of Protein in Membranes (OPM)” database (http://opm.phar.umich.edu) and of the program “Membrane Protein Explorer” (MPEx, http://blanco.biomol.uci.edu/mpex) in order to provide more rigorously, on energetics and thermodynamics basis, the correct insertion of the various receptor domains into the membrane. Overlaps between lipids and receptor were eliminated by two different criteria: a) by marking lipids whose phosphorus atom lies well within the region of receptor, and, b) by considering an overlap on the basis of an atom to atom distance under 0.8 Å. After the correct insertion of CXCR3 into the membrane, the lipid/protein system was solvated in a box of 16569 water molecules providing the correct ionization by using VMD. Then, it started a sequence of minimization/equilibration/dynamics steps.

The first step (Melting of Lipid Tails) was a simulation in which water, ions, protein, lipid head-groups(excluding lipid tails) were appropriately placed into disordered fluid-like bilayer. This result was obtained by running 1000 minimization steps by means of NAMD (Humphrey et al., 1996; Phillips et al., 2005), at 300 K, and reiterating a last step of dynamics for 0.5 ns with 2 fs of time-step.

After this step, we performed a “minimization” step to drive the system towards the nearest energetic minimum of its configurational space, followed by an equilibration of the constrained protein to permit an environmental relaxation. At this point we had a system with very well packed lipids around the protein, and water molecules not in forbidden regions. So, we could proceed with a last minimization step to release the harmonic constraints further balancing the entire system.

A molecular dynamics simulation for 100 ns, at 300 K, and pH 7 was launched for the entire system with GROMACS (Hess et al., 2008), where its routines were used to analyze the trajectories in terms of RMSD, RMSF, H-bonds, secondary structures, and gyration radius, to find correlated motions by Principal components analysis (PCA), and to identify and sample models similarly structured during the simulation for the cluster analysis. Intra-peptide interactions of models were studied considering their structure as a network (that is a graph) of interacting amino acid pairs, and focusing on the interactions (H-bonds, peptide bonds, etc.,represented as edges) between the different nodes (the single amino acid). In particular, we used two Cytoscape plugins, Network Analyzer and RINalyzer, for the standard and advanced analyses of network topologies, and a third plugin, CytoHubba, to explore an interaction network, also detecting the HUB residues. It is important to underline that HUB node is the component of a network with a high-degree node that is with a significantly larger number of links in comparison with other nodes in the graph. The existence of hubs is very important because they are indicative to distinguish between random networks and scale-free networks (biological networks). Moreover, we analyzed the network with different statistics according to Assenov et al. (2008), Doncheva et al. (2011) and Chin et al. (2014).

2.3. Molecular docking and molecular dynamics simulation of CXCR3/CXCL11 complex

The CXCL11/CXCR3 complex was modeled by molecular docking using as templates the NMR structure for CXCL11 (PDB: 1RJT) (Booth et al., 2004) and 84 clusters for CXCR3 obtained during MD. In details, we calculated the RMSD values of all clusters compared to the three most populated ones, where cluster 1 contains 6,2% of total conformations, cluster 2 the 5,5%, cluster 3 the 5%. This was required to select the clusters including not only the most represented conformations of the MD, but also those including the characteristics of interaction between chemokine and receptor already reported in literature.

Then, we extrapolated the CXCR3 conformations having RMSD values greater than 2 Å by focusing on those which represent at least 1% of total conformations, as well as on the three more presented clusters. All these structures were used for molecular docking studies between CXCR3 and CXCL11 by means of the Patchdock algorithm based on shape complementarity principles (Schneidman-Duhovny et al., 2005). The best CXCR3/CXCL11 complex was selected by visual inspection, where models of CXCL11 not properly docked to CXCR3 were discarded, and the best complex was selected driven by data from the literature. In particular, COCOMAPS application was used to identify the amino acids at the interface and to evaluate their solvent accessibility, and contact maps (Vangone et al., 2011), whereas Hbplus (McDonald and Thornton, 1994) and ESBRI (Costantini et al., 2008) programs were useful to evaluate the presence of H-bonds and salt bridges, respectively.

The best model of the CXCR3/CXCL11 complex was subjected to MD simulations for 60 ns at 300

K and neutral pH using the same protocol previously reported for