jmb Article

Allosteric Switching of Agonist/Antagonist Activity by a Single Point Mutation in the Interluekin-1 Receptor Antagonist, IL-1Ra

Kendra L. Hailey[1], Dominique T. Capraro[1], Sulyman Barkho[1] and Patricia A. Jennings[1,2]

*1 - **Department of Chemistry and Biochemistry,** University of California, San Diego, San Diego, CA, USA*
*2 - **Department of Chemistry and Biochemistry,** University of California at San Diego (UCSD), 9500 Gilman Drive, La Jolla, CA 92093-0375, USA*

***Correspondence to Patricia A. Jennings:** Department of Chemistry and Biochemistry, University of California at San Diego (UCSD), 9500 Gilman Drive, La Jolla, CA 92093-0375, USA. pajennings@ucsd.edu*
http://dx.doi.org/10.1016/j.jmb.2013.03.016
Edited by C. Kalodimos

Abstract

The pleiotropic pro-inflammatory cytokine interleukin (IL)-1β has co-evolved with a competitive inhibitor, IL-1 receptor antagonist (IL-1Ra). IL-1β initiates cell signaling by binding the IL-1 receptor (IL-1R) whereas IL-1Ra acts as an antagonist, blocking receptor signaling. The current paradigm for agonist/antagonist functions for these two proteins is based on the receptor–ligand interaction observed in the crystal structures of the receptor–ligand complexes. While IL-1Ra and IL-1β are structurally homologous, IL-1Ra engages only two of the three extracellular domains of the receptor, whereas IL-1β engages all three. We find that an allosteric functional switch exists within a highly conserved pocket of residues, residues 111–120. This region is maintained across all IL-1 family members and serves as a hydrophobic mini-core for IL-1β folding. A key difference across species is a conserved aromatic residue at position 117 in IL-1β, *versus* a conserved cysteine in IL-1Ra at the analogous position, 116. We find that the replacement of C116 with a phenylalanine switches the protein from an antagonist to an agonist despite the distant location of C116 relative to receptor interaction sites. These results suggest new ways to develop designer cytokine activity into the β-trefoil fold and may be of general use in regulation of this large family of signaling proteins.

Introduction

Cytokines are one of the tightly regulated currencies of cellular communication.[1–3] Within the cytokine family, the interleukin (IL)-1 family of proteins is the only group to have identified natural antagonist proteins.[4] IL-1β has co-evolved with a competitive inhibitor, IL-1 receptor antagonist (IL-1Ra). IL-1β binds the IL-1 receptor (IL-1RI) and triggers a signal cascade whereas IL-1Ra competes for the same site and inhibits signaling.[5–7] The IL-1R belongs to the immunoglobulin (Ig) superfamily characterized by three extracellular Ig domains (I, II, and III), a single transmembrane spanning sequence, and a globular intracellular domain.[8–11] In addition, a soluble form of the receptor (sIL-1R), arising from alternative splicing and comprising residues 1–311 of the IL-1R, is found circulating in the bloodstream, inhibiting IL-1β signaling.[5,12] The structures of the complexes of sIL-1R with IL-1β and IL-1Ra, respectively, have been solved and reveal a novel interaction motif for a cytokine–receptor complex where the three Ig domains wrap around the trefoil-folded proteins with a 1:1 stoichiometry.[8,9,13] IL-1β has two known binding sites for the IL-1R, designated A and B (Fig. 1). Site A binds the first two Ig domains on the receptor while site B interacts with the third domain. Similarly, IL-1Ra maintains the interactions between site A and the IL-1R but differs in its interaction with Ig domain III. Comparison of the structures of the IL-1β/receptor and IL-1Ra/receptor complexes has led to the proposal that binding to the B-site of IL-1β induces a conformational change in the receptor, triggering the signal cascade[8,9,13] (Fig. 1a). The IL-1β/IL-1RI complex recruits

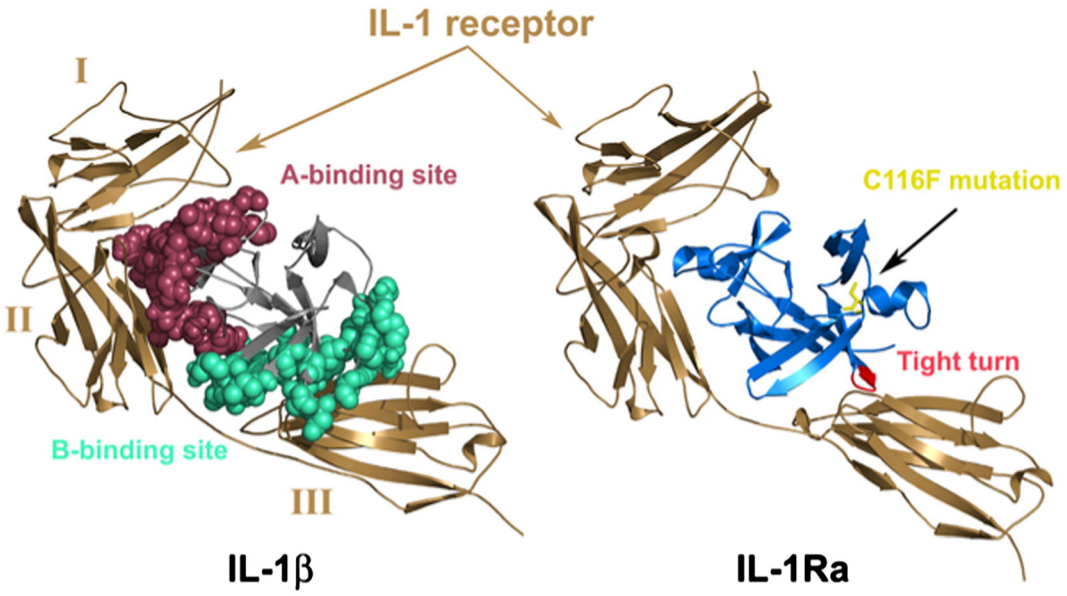

Fig. 1. (a) Comparison of IL-1β (left) and IL-1Ra (right) bound to the IL-1RI receptor. Ig domains I, II, and III of sIL-1RI are labeled on the complex on the left for clarity. IL-1β is thought to induce a conformational change (helical structure in the linker between domains II and III and a 20° rotation of Ig domain III with respect to that observed in the IL-1RI complex of IL-1Ra) and binds the third domain of the receptor. The binding sites in IL-1β are highlighted in red (binding site A) and cyan (binding site B), respectively. When bound to IL-1β, contacts in binding site B result in a closed conformation of the receptor domains while the receptor adopts an inactive open conformation when bound to IL-1Ra. (b) The heterotrimeric complex of IL-1RI (brown) and co-receptor, IL-1RAcP (magenta), modeled with IL-1Ra (blue). The C116F mutation (yellow) is highlighted to illustrate the location of the change with respect to the receptor as well as the barrel core of the protein.

a co-receptor, IL-1R accessory protein (IL-1RAcP), and this heterotrimeric complex is responsible for initialing cell signaling (Fig. 1b). The Nf-κB pathway is the major target for IL-1 signaling in various cell types, but especially in monocytes and macrophages.

The allosteric mechanism of IL-1RI activation by IL-1β binding is apparent when comparing the crystal structures of IL-1β and IL-1Ra bound to the cytoplasmic domain of the IL-1RI receptor protein.[8,9,13] When bound to IL-1β, contacts in binding site B result in a "closed" conformation of the receptor domains while the receptor adopts an inactive "open" conformation when bound to IL-1Ra (Fig. 1a). Functionally, proteins can act as allosteric effectors or inhibitors for other proteins, as is the case for IL-1Ra and IL-1β regulating the multidomain IL-1R. While allosteric regulation of activation/inhibition is unusual in single domain proteins that do not undergo obvious structural reorganization, a hallmark feature appears to be communication between distal residues. Recent studies reveal the occurrence of hot spot residues,[14] where allosteric signal propagation primarily involves conserved amino acids.[15–19] Additionally, there is emerging evidence for allosteric disulfide bonds in proteins that can control function by triggering a functional event when it breaks and/or forms.[20]

IL-1Ra and IL-1β share the same single-domain tertiary structure, the β-trefoil fold, despite having only 30% sequence identity. While IL-1Ra and IL-1β are structurally homologous, each makes a noticeably distinct number and type of contacts at the protein–receptor interface. Key structural differences are in loop regions, including the β-bulge "trigger loop" between β-strands 4 and 5 in IL-1β and the large loop between β-strands 11 and 12 in IL-1Ra. Interestingly, the most conserved sequence region, with the exception of a handful of residues, is from residue 111 to 121 in both proteins. These residues compose a hydrophobic "mini-core" important for folding in IL-1β[21] and maintaining the stability of the barrel–cap interface.[22] This stable mini-core bridges the barrel and cap and is located diametrically opposed to the receptor A and B (IL-1β) or A (IL-1Ra) interfaces (Fig. 1a) and the accessory protein binding site (Fig. 1b). Key differences between the agonist (IL-1β) and the antagonist (IL-1Ra) protein include the replacement of an aromatic residue at the structurally homologous position in agonist IL-1β (Phe117) with a cysteine at position 116 in IL-1Ra. Furthermore, this region bridges the early and late stages of folding[23] as cavity-changing mutations in the barrel core of IL-1β confirmed that core packing is rate limiting in folding.[24,25]

Transmission of information occurs through the hydrogen-bonding network and is significant for receptor binding and signaling for IL-1β.[26] The resistance to overt structural changes[24,26–28] despite potential changes in function suggests that the β-