Crystals of soluble interleukin-1 receptor complexed with its natural antagonist reveal a 1:1 receptor–ligand complex

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Abstract Interleukin-1 is a cytokine involved in the acute phase response against infection and injury. We obtained crystals of a complex of soluble, recombinant human interleukin-1 receptor and recombinant human interleukin-1 receptor antagonist, a naturally occurring antagonist. The crystals are suitable for X-ray analysis and diffract to 2.7 Å resolution. Solvent content calculations indicate that the crystals contain one receptor and one antagonist molecule per asymmetric unit. Other receptor to antagonist ratios are highly unlikely. These results suggest that the interleukin-1 antagonist binds a single receptor molecule and does not cause receptor aggregation.

Key words: Interleukin-1 receptor; Interleukin-1 receptor antagonist; Cytokine; Protein crystal; X-Ray analysis; Human

1. Introduction

Interleukin-1 (IL-1) is a cytokine involved in the early phase of inflammation, the acute-phase response. Interleukin-1 is produced by activated macrophages, T-cells, and other cell types and triggers a wide variety of inflammatory reactions [1]. The inflammatory response protects the organism against pathogens, but interleukin-1 has also been implicated in a number of immune-related disorders such as arthritis, inflammatory bowel disease and septic shock [2]. Molecules which are able to inhibit the interleukin-1 response could potentially be used as drugs to treat these disorders.

Three naturally occurring IL-1 receptor ligands are known: two agonists, IL-1α and IL-1β, and one antagonist, the IL-1 receptor antagonist (IL-1ra). These three ligands are proteins of about 150 amino acids with a molecular mass of ~17.5 kDa. Crystal structures are available for all three ligands [3–8]. Two types of IL-1 receptors are known. The type I receptor is a glycoprotein of 552 amino acids with a molecular mass of ~80 kDa [9]. The type II receptor has 385 amino acids and a mass of ~60 kDa [10]. The IL-1 receptors consist of a ligand binding portion of ~316 amino acids (type I) or ~310 amino acids (type II), comprised of three immunoglobulin-like domains, a *trans*-membrane region and an intra-cellular domain. Whereas the intra-cellular domain of the type I receptor is large (~215 amino acids), the cytoplasmic domain of the type II receptor is small (only 29 amino acids). The extra-cellular domains of both receptor types are distantly related and share ~28% identity. The type I receptor has been linked to a biological response [11], but not the type II receptor. It has been proposed that the type II receptor functions as a decoy target, binding excess interleukin-1 [12].

Here we report the crystallization of a complex of the extra-cellular, ligand-binding portion of the type I IL-1 receptor, consisting of three immunoglobulin-like domains, and IL-1ra.

2. Experimental

Both the IL-1 receptor and the IL-1ra used are recombinant proteins. The IL-1ra was expressed in *E. coli* and purified as has been described before [8]. The soluble IL-1 receptor was cloned and expressed as follows.

The full-length and extra-cellular domains, as determined from hydrophobicity studies based on the sequence of the type I IL-1 receptor [13], were cloned by PCR from HepG2 Cell total RNA. PCR primers contained homology to the type I IL-1 receptor gene plus additional sequences to create restriction sites for cloning. The resulting PCR products were cloned into the vector pSRAlphaNeo [14].

The extra-cellular domain of the IL-1 type I receptor was edited by 5 cycles of PCR with the oligonucleotides ON-72 (5′-TAGGCGGCC-GCCATGAAAGTGTTACTCAGACTTATTTGTTTCATAG-3′) and ON-403 (5′-CGGAGAATTACTTCTGGAAATTAGTGACTG-G-3′). The amplified DNA was then digested with *Not*I and *Eco*RI and cloned into the baculovirus expression vector pVL1392 (Invitrogen). This plasmid was then digested with *Bgl*II and *Xho*I, the *Bgl*II site was filled in with Klenow and the receptor containing fragment was then ligated to the *Nhe*I (filled in) and *Xho*I digested fragment containing β-galactosidase of the BlueBac vector (Invitrogen). The resulting plasmid, BlueBac/IL-1R Exo, was then used to create a recombinant baculovirus and scaled up through standard procedures (Invitrogen) to express the secreted IL-1 type I receptor extra-cellular domain.

The soluble type I IL-1 receptor was expressed in baculovirus infected insect cells and purified from cell supernatants in a single step by affinity chromatography using MBP-IL-1ra sepharose. The resin was washed extensively with PBS and the receptor was eluted with 0.1 M glycine, 0.15 M NaCl pH 3.0 and neutralized with 1 M Tris base. Receptor fractions were concentrated to approximately 1 mg/ml using an Amicon stirred cell with a 10,000 kDa cut-off membrane. The purified receptor was then dialyzed 2 times, 4 h each against 2 liters of 50 mM $(NH_4)_2CO_3$. After dialysis the receptor was quickly frozen in dry-ice/methanol and lyophilized overnight.

The complex of the IL-1 receptor with the antagonist was prepared by mixing a solution of IL-1 receptor in 50 mM Tris (pH 7.5) and 150 mM NaCl, with a solution of IL-1ra in the same buffer. The receptor to antagonist ratio was 1:1. To get rid of aggregated material, the complex was passed through a membrane with a cut-off of 300 kDa using a filtron macrosep micro-concentrator and the complex was subsequently concentrated to a total protein concentration of about 18 mg/ml using a centricon micro concentrator with a cut-off of 3 kDa.

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Abbreviations: IL-1, interleukin-1; IL-1ra, interleukin-1 receptor antagonist.

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The complex was crystallized at 23°C using the hanging drop method. The drops were prepared by mixing 4 μl of the protein solution with 1 μl of reservoir solution containing 30% (w/v) PEG 3350, 400 mM $MgCl_2$ in 100 mM MOPS buffer (pH 7.0) and the drop was set to equilibrate against 1 ml of reservoir solution. Crystals with dimensions up to $0.2 \times 0.3 \times 0.5$ mm^3 grew between 2 weeks and 2 months. The crystals were analyzed with a Siemens area detector, mounted on a Siemens rotating anode generator using XDS software [15].

3. Results

Analysis of the diffraction pattern showed that the space group was $P2_12_12_1$, with a = 47.2Å, b = 84.6Å and c = 140.2Å. SDS-PAGE of dissolved crystals showed that the crystals contain both the IL-1 receptor and the antagonist. Assuming one receptor molecule and one antagonist molecule per asymmetric unit, we obtain a V_m of 2.22 Å^3/Da and a solvent content of 44.6% [16], which is in the middle of the range observed for protein crystals. Assuming two receptor molecules and one antagonist molecule, we would obtain a V_m of 1.28 Å^3/Da, corresponding to 3.9% solvent, which is extremely unlikely. We therefore conclude that the asymmetric unit of our crystals contains one receptor and one antagonist molecule. We collected a native data set to 2.7 Å resolution, which contains 15,631 unique reflections, based on 73,407 observations. The R_{sym} (on I) is 6.0%. The 15,631 unique reflections represent 97.2% of all possible reflections to 2.7 Å resolution. We are currently in the process of solving the structure by using a combination of heavy atom derivatives and molecular replacement.

4. Discussion

Our observation of a 1:1 interleukin-1 receptor–ligand complex is fully consistent with recent results of Greenfeder et al. who report the presence of a second subunit of the interleukin-1 receptor complex [17]. They named this protein the interleukin-1 receptor accessory protein. The authors propose on the basis of biochemical experiments that binding of agonists, but not of antagonists, brings together the interleukin-1 receptor and the accessory protein which could then lead to receptor activation.

Our crystals are grown at physiological pH and the ionic strength of the crystallization solution is not extremely high. Under similar conditions, receptor aggregation was observed in crystals of receptor–ligand complexes of the human growth hormone receptor [18] and the tumor necrosis factor receptor [19]. If the IL-1ra molecule would cause aggregation of IL-1 receptor molecules, we would expect to see this aggregation in the crystals. We therefore conclude that the IL-1 receptor forms 1:1 complexes with its natural antagonist. Failure of the IL-1 receptor/IL-1ra complex to bind the accessory protein [17] would provide a mechanism by which the antagonist blocks the receptor action.

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