Structural studies of FcεRI

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Introduction

Allergic reactions in humans derive from activation of the high-affinity IgE receptor (FcεRI). IgE molecules lacking their specific antigen (allergen) reside on mast and other cells bound to FcεRI and are oligomerized when exposed to allergen. This results in a concomitant oligomerization of receptors and thereby their activation. The binding of IgE to FcεRI involves the extracellular portion of α-chain of FcεRI, comprised of two immunoglobulin domains. Structural information on FcεRI has been published [1], but atomic coordinates have not been deposited and are not publicly available. We seek to use this and related structural information to optimize candidate medicines for the treatment of allergic disease.

Methods and Materials

The extracellular domains of the FcεRI α-chain were expressed in baculovirus-infected insect cells and purified on affinity resin. Crystals grew from hanging drops equilibrated against a reservoir containing PEG4000, NaCl, glycerol, and Tris pH 7.5 at room temperature. Some crystals were exposed to heavy atom compounds in an artificial mother liquor for use as isomorphous derivatives to aid in structure solution. For data collection, crystals were mounted with mother liquor in a small nylon loop, flash frozen in liquid nitrogen, and characterized on our home x-ray source. Crystals with the best diffraction properties (low mosaic spread, high-resolution diffraction, no ice) were shipped to the Structural Biology Center (SBC) in a liquid-nitrogen bath.

Results

A good native data set in space group C2 with cell parameters a = 95 Å, b = 70 Å, c = 49 Å, and β = 113˚ was collected extending to 2.2 Å resolution after combining data from two 180˚ sweeps on the CCD detector on beamline 19-ID. This first sweep utilized 20-second exposures of 1˚ increments, and the second sweep utilized 2-second exposures of 2˚ increments. The Rmerge was 3.8%. The heavy-atom derivatized crystals exposed at SBC diffracted poorly, with very high mosaic spreads. No useful derivative data was obtained.

Discussion

Our expectation that the journal article describing the use of data collected at the Advanced Light Source to determine the structure of the FcεRI α-chain extracellular domains provided sufficient information to allow a molecular replacement solution of our native data set has proven overly optimistic. No probe structure we have constructed based on the publication of Garman *et al* [1] has proven accurate enough to solve the structure. We have abandoned this effort in the hope that the coordinates described in the Garman paper will eventually be deposited.

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Reference

[1] S.C. Garman, J-P Kinet, and T.S. Jardetzky, *Cell* **95**, 951–961 (1998).