Structural studies of p185/HER2

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Introduction

The human epidermal growth factor receptor (EGFR) family comprises four receptor tyrosine kinases: EGFR, HER2, HER3, and HER4. These receptors are expressed on a wide variety of tissue types and have been implicated in many human cancers, including breast, gastric, colon, and prostate [1, 2]. Overexpression of HER2 is an important diagnostic indicator for poor prognosis [1]. Recombinant humanized antibodies that recognize the HER2 extracellular domain (ECD), such as Mab4D5 (Herceptin™), are proving effective in combination therapy regimens for HER2 overexpressing breast tumors, which represent the most aggressive form of the disease [3, 4].

Signaling via the EGFR family involves the combinatorial dimerization of the four kinases [5, 6]. This dimerization is mediated by at least eight different hormones, including EGF and the heregulins [5]. So far, no known ligand binds specifically to HER2. However, heregulin binding and tyrosine phosphorylation by HER3 or HER4 is enhanced by the presence of HER2, suggesting that for these receptors the physiologically relevant species is a HER3:HER2 or HER4:HER2 heterodimer [7, 8].

Little is known about the structures of the EGFR family members. The full-length receptors are 1210–1342 amino acids in length, with the ~650 residue ECDs extensively glycosylated. The ECDs are highly homologous (44%–59% identity) and contain four subdomains: an L1 domain, a cysteine-rich domain (CRD1), a second L2 domain, and a final CRD2. The L domains and CRDs are homologous to the first two subdomains, respectively, of the type-one insulin-like growth factor receptor (IGFR) [9]. The L1 domain of the IGFR forms a right-handed β-helix structure, while the CRD is composed of disulfide-linked modules reminiscent of the tumor necrosis factor receptor superfamily [10].

To begin to understand the complicated signaling mechanisms exhibited by these receptors, we have undertaken the structure determination of the ECD of HER2 in complex with Fab 4D5, which is the corresponding Fab of Herceptin™.

Results and Discussion

The ECD of HER2 was produced as a secreted, monomeric protein in Chinese hamster ovary cells and purified by immunoaffinity chromatography from the concentrated cell-culture fluid. The HER2 ECD was crystallized in complex with recombinant humanized Fab4D5 [11]. The complex crystallizes readily in space group $P2_12_12_1$, and we have collected multiple data sets at the Structural Biology Center beamline 19-ID.

The crystals diffract to 2.6 Å resolution, with unit cell axes a = 63 Å, b = 115Å, and c =206 Å.

Data were reduced with the HKL package [12] (Table 1).

Table 1: Native Data Statistics

Resolution (Å)	99-2.60	2.69-2.60
Rmerge	0.050	0.269
Chi^2	0.785	0.679
I/s(I) (%<2)	15.8	46.5
Redundancy (%>4)	67.5	41.8
Completeness (%)	99.3	95.5

Attempts at molecular replacement using the coordinates of either the IGFR [9] or Fab4D5 [11] have failed. This is not surprising, given the multidomain structure of both of these search models. Even for Fab4D5, significant differences (especially in the elbow angle) are likely to exist between the free and complexed forms of the molecule. A search for heavy-atom derivatives is currently under way.

Acknowledgments

This work was supported by Genentech, Inc. Use of the Advanced Photon Source was supported by the U.S. Department of Energy, Basic Energy Sciences, Office of Science, under Contract No. W-31-109-Eng-38.

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