Molecular Structure of the EGF Receptor Kinase Domain with Erlotinib (OSI-774, CP-358,774, Tarceva™) Determined by X-ray Crystallography

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Introduction

The epidermal growth factor receptor (EGFR) is frequently activated in a number of human solid tumors. erlotinib (TARCEVA™, OSI-774, CP-358,774) is a 4-anilinoquinazoline derivative that inhibits EGFR by competing with ATP for binding to the EGFR tyrosine kinase domain. Clinical studies demonstrate that erlotinib has single agent biological activity in non-small cell lung, ovarian, and head and neck cancers. Despite the fact that EGFR is a target for a number of therapeutic interventions, surprisingly little structural information is available for the receptor. Here we describe for the first time, the interactions between erlotinib (or any ATP mimetic) and the kinase domain of EGFR.

EGFR and its close relatives HER2, HER3, and HER4 comprise a family, which, under the influence of a small family of cognate ligands, oligomerize into dimeric or higher order combinations and thereby produce a growth and/or proliferative signal. The signal is communicated to second messengers via trans tyrosine phosphorylation that create docking sites for downstream substrates. The EGFR family is unique among receptor tyrosine kinases in that the receptor kinase domain is an active catalyst for phospho-transfer without first being phosphorylated itself. Additionally, a tripeptide sequence immediately following the formal end of the kinase domain has been associated with a special conformational influence on receptor activity.

Material and Methods

The human EGFR kinase domain was expressed in baculovirus infected insect cells and purified to homogeneity. This construct includes a poly-Histidine tag, the tyrosine kinase domain, and is extended to incorporate a putative dimerization motif. Crystals were grown after removal of the histidine tag in hanging drops in 1.1 M tartrate at pH 6.5 and belong to cubic space group I23 with a = 147.8Å. X-ray diffraction data (2.6 Å) collected at beamline 19-ID at the Structural Biology Center of the Advance Photon Source at Argonne National Laboratory are being used to complete refinement from the current agreement factors (R and Rfree) of 25.6% and 28.9%.

Results

The EGFR kinase domain adopts a bilobate tertiary structure akin to those of the many previously reported protein kinase domains. Erlotinib, an ATP-competitive inhibitor, is found bound in the ATP binding cleft between the β-strand rich N-terminal lobe and the larger, mostly α-helical C-terminal lobe. Erlotinib adopts a conformation allowing an H-bond between a quinazoline nitrogen atom the main chain nitrogen of kinase residue methionine 769, and with the plane of the substituent aniline moiety making a 45˚ with the quinazoline plane. Solubilizing ether linkages at the opposite end of the quinazoline extend into solvent and are poorly ordered. Overall, this arrangement is similar to those found for other 4-anilinoquinazoline inhibitors bound to other kinase domains.

The activation loop (A-loop) of EGFR is found in a conformation which is associated with the active state of other protein kinases, for instance the kinase domain of the insulin receptor (IR). Many of the beneficial energetic interactions found for the active conformation of the IR kinase have close analogues in the EGFR structure, but the tyrosine in the A-loop which is a closely homologous to those in other kinases is not phosphorylated. Once this fact is appreciated, there are few unanticipated structural features.

The kinase domain crystallized here extends approximately 50 amino acids beyond the formal end of the kinase domain. The early part of this extension includes the sequence Leucine-Valine-Isoleucine (“LVI”), with which a special long-range conformational effect has been associated. We find the Leucine of LVI inserted in a small hydrophobic pit and as a result well-ordered. The succeeding peptide chain extends away from the rest of the protein, becomes difficult or impossible to trace, and then becomes well-ordered when associated with an adjacent kinase molecule in the crystal lattice.

Discussion

Our findings allow recognition of those features of erlotinib that provide it

s relative specificity for EGFR. A detailed analysis of the molecular interactions between erlotinib and EGFR may provide sufficient insight to allow improved inhibitors of EGFR and other family members. The fact that we find the EGFR A-loop is an “active” conformation despite the

lack of a phospho-tyrosine is consistent with prior characterizations that catalytic activity of this kinase domain does not require it. Among kinase structures reported so far, “active” A-loops share much closer structural conformity than do the “non-active” forms. As a result, although we can parse the energetic contributions to the “active” A-loop conformation in EGFR, our comprehension of a putative alternate “inactive” conformation is restricted to its higher energy. At the very least, such a state (or states) exists as a minor contributor to a equilibrium dominated by the conformation observed here. The available literature on the LVI tripeptide had left the possibility that it played either a “docking” role or a long-range conformational role. The well-ordered LVI tripeptide is found to be buried and so not available for a direct docking interaction. The succeeding polypeptide traces a relatively unusual course with respect to the C-terminal extensions of other kinases. However, our findings are insufficient to demonstrate a specific long-range conformational influence of the LVI, not least because our construct lacks the docking sites for second messengers that, within oligomerized receptor complexes, are trans-phosphorylated. Nonetheless, the fact that LVI is obscured by its interactions with the body of the protein combined with the good shape and charge complimentarity of the interface between the final part of our construct and a neighboring kinase molecule offer some support for a long range conformational influence upon mutation of the LVI tripeptide.

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