Structure of the EGF Receptor Kinase Domain[1]

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INTRODUCTION

Growth factor interactions with cell surface receptors influence proliferation, survival, differentiation, and metabolism[2]. The loss of control over these vital cellular processes is a hallmark of oncogenesis[3]. Growth factors bind to a cognate membrane-bound receptor system and mediate changes in the intracellular portion of the receptor, often through the formation of dimers or oligomers of receptors that initiate signal transduction cascades. The epidermal growth factor receptor (EGFR, also ErbB1 or HER1) and its ligands epidermal growth factor (EGF) and transforming growth factor-α (TGF-α) are among the earliest characterized members of the growth factor/receptor tyrosine kinase (RTK) family. Normal signaling in the EGFR system involves ligand-induced, homo-oligomerization or hetero-oligomerization with the closely related RTKs ErbB2 (HER2), ErbB3(HER3) and/or ErbB4 (HER4)[4]. Autophosphorylation of key tyrosine residues within the carboxy-terminal portion of the receptor provides sites for direct interaction with SH2-containing proteins leading to subsequent signal transduction events.

In the non-signaling state, most RTKs possess low basal kinase activity that increases substantially upon growth factor binding. This results from receptor oligomerization and subsequent transphosphorylation of tyrosine residues within a partner kinase domain. Specifically, initial phosphotyrosine (p-Tyr) modification of the "activation loop" (A-loop) generates optimal catalytic activity and subsequent rapid phosphorylation at substrate docking sites elsewhere on the receptor intracellular domain (ICD). The EGFR, ErbB2, and ErbB4 receptors are the only known RTKs that do not require this initial phosphorylation of kinase domain residues for full catalytic competency. This unique feature may partially explain why EGFR family members are frequently involved in cellular transformation.

Despite extensive study of the EGFR family, only very recently have crystal structures been determined for the extracellular region of any member[5-8]. X-ray crystal structures of kinase domains of several RTKs have been reported, although, unlike EGFRK, all of these kinases require phosphorylation for full activity. Here we present the crystallographic analysis of the EGFR kinase alone and in complex with the inhibitor erlotinib.

METHODS

DNA encoding EGFR residues 672 to 998 was amplified from full length EGFR cDNA[9] by PCR. Further PCR was performed on the EGFRK-pET-28b plasmid to acquire the histidine tag and thrombin site using an N-terminal primer. Expression was performed in High Five™ cells using virus produced in *Spodoptera frugiperda* insect cells (SF9). Purified yield was approximately 1-2 mg per liter.

Small crystals of EGFRK formed over 1 day in hanging drops when protein was mixed with the reservoir buffer (1.0M Na/K tartrate, 0.1 M MES pH 7.0) in a 1:1 ratio. Crystals of EGFRK complexed with erlotinib were obtained by soaking crystals of apo-EGFRK in a solution containing 1.1M Na/K tartrate, 0.1 M MES pH 7.0, 3 μM erlotinib, for 3 weeks. Diffraction data were collected at beamline 19-ID of the Structural Biology Center (Advanced Photon Source,

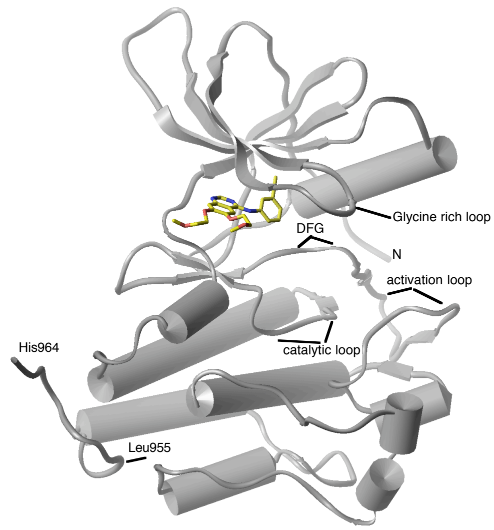

Figure 1. The EGFRK structure with key features indicated. Erlotinib is found in the cleft between amino-terminal and carboxy-terminal lobes. The C-terminal ordered region including residues Leu977 to Pro995 is not included here.

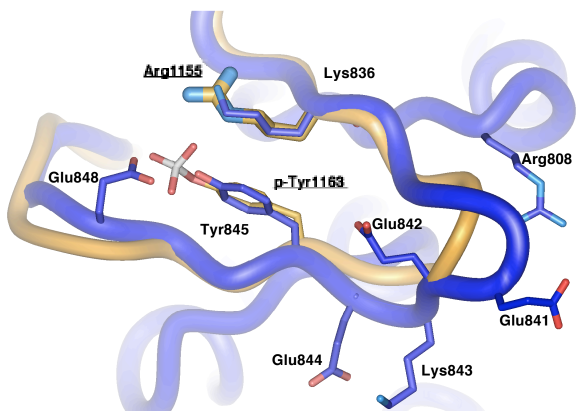

Figure 2. Activation loops. The close structural correspondence between the EGFRK A-loop (blue) and the A-loop from the phosphorylated form of the insulin receptor kinase (gold). The hydrophobic interaction between Lys836 and Tyr845 almost exactly reprises that between Arg1155 and Tyr1163 of p-IRK (underlined). The presence of four glutamate residues in this part of EGFRK has been suggested as a cause for its intrinsic catalytic activity.

Argonne National Laboratory), and at beamline 5.0.1 of the Berkeley Center for Structural Biology (Advanced Light Source, Lawrence Berkeley National Laboratory) extending to 2.6 Å for both apo-EGFRK and EGFRK/erlotinib crystals, respectively.

RESULTS

The EGFR kinase domain (EGFRK) adopts the bilobate fold characteristic of all previously reported protein kinase domains (Fig 1). The N-terminal lobe (N-lobe) is formed from mostly β-strands and one α-helix (αC), while the larger C-terminal lobe (C-lobe) is mostly α-helical. The two lobes are separated by a cleft like those in which ATP, ATP analogues and ATP-competitive inhibitors have been found to bind. Important elements of the catalytic machinery bordering the cleft on the N-lobe include the glycine-rich nucleotide phosphate binding loop (Gly695 to Gly700), while the C-lobe contributes the DFG motif (Asp831 to Gly833) , the presumptive catalytic (general base) Asp813, the catalytic loop (Arg812 to Asn818), and the A-loop (Asp831 to Val852).

In most protein kinases, the activation loop assumes its catalytically competent conformation only if it first becomes phosphorylated on a Tyr or Thr. For these kinases, the unphosphorylated activation loop is positioned many Ångstroms from the active conformation, and may include a direct inhibitory element. The A-loop in apo-EGFRK (and EGFRK/erlotinib) differs significantly from other apo-, unphosphorylated A-loop structures. Earlier work has shown that Tyr845 of the EGFRK A-loop, at a position that is phosphorylated in other RTKs, can be replaced by Phe without loss of function[10]. Consistent with this, we see that the A-loop of EGFRK adopts an "active" conformation, similar to the phosphorylated A-loop of p-IRK (Fig. 2). Tyr845 aligns well structurally with p-Tyr1163 of p-IRK and makes van der Waals contact with the aliphatic part of neighboring Lys836, a residue which occupies the space of Arg1155 in the p-IRK structure. An H-bond between side chains of Tyr845 and Glu848 mimics that between p-Tyr1163 and the main chain nitrogen of Gly1166 in p-IRK. The relationship between Tyr845 and Arg812 (preceding the catalytic Asp813) is the same as between the analogous residues in p-IRK and other tyrosine kinases. This relationship is central to arranging the catalytic machinery and substrate for phospho-transfer.

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