PROTEIN STRUCTURE REPORT

The ErbB4 extracellular region retains a tethered-like conformation in the absence of the tether

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Abstract: The epidermal growth factor receptor (EGFR) and its homologs ErbB3 and ErbB4 adopt a tethered conformation in the absence of ligand in which an extended hairpin loop from domain II contacts the juxtamembrane region of domain IV and tethers the domain I/II pair to the domain III/IV pair. By burying the hairpin loop, which is required for formation of active receptor dimers, the tether contact was thought to prevent constitutive activation of EGFR and its homologs. Amino-acid substitutions at key sites within the tether contact region fail to result in constitutively active receptors however. We report here the 2.5 Å crystal structure of the N-terminal three extracellular domains of ErbB4, which bind ligand but lack domain IV and thus the tether contact. This ErbB4 fragment nonetheless adopts a domain arrangement very similar to the arrangement adopted in the presence of the tether suggesting that regions in addition to the tether contribute to maintaining this conformation and inactivity in the absence of the tether contact. We suggest that the tether conformation may have evolved to prevent crosstalk between different EGFR homologs and thus allow diversification of EGFR and its homologs.

Keywords: ErbB4; EGFR; autoinhibition; structure

Introduction

The human epidermal growth factor receptor (EGFR) family of receptor tyrosine kinases, also known as ErbBs or HERs, consists of four members:

EGFR (ErbB1/HER1), ErbB2 (HER2), ErbB3 (HER3), and ErbB4 (HER4).^{1,2} Each ErbB is essential for normal development in the mouse,³ and abnormal activity of each ErbB has been associated with human cancer.^{4–6} EGFR and ErbB2 in particular have been associated with increased cancer severity, and monoclonal antibodies and small-molecule kinase inhibitors targeting EGFR and ErbB2 are approved cancer therapies.⁷

ErbB extracellular regions are composed of four domains arranged as a tandem array of two Large domain/Cysteine-rich (L/CR) domain pairs, termed domains I (L1), II (CR1), III (L2), and IV (CR2) beginning with the N-terminal L domain.² Ligands

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Figure 1. The tErbB4 structure. Stereo pair of an alpha carbon trace of the tErbB4 structure. Every twentieth residue is indicated with a sphere and the domain II tether hairpin, specific domains, and unobscured residue spheres are labeled.

bind to ErbB extracellular regions at binding sites composed of regions from domains I and III and stabilize a specific receptor dimer in which the intracellular kinase activity is stimulated.^{2,8,9} Crystal structures of the extracellular regions of EGFR, ErbB3, and ErbB4 revealed that in the absence of ligand a ~ 25 Å β -hairpin loop extends from domain II to contact the juxtamembrane region of domain IV.^{2,10–12} This contact constrains the extracellular region into a folded-over or “tethered” conformation in which ligand-binding sites on domains I and III are too far apart to bind ligand simultaneously. When ligand binds, the domain II/IV contact is broken and the domain I/II pair undergoes a $\sim 130^\circ$ rotation to juxtapose domains I and III and compose a complete binding site.^{2,8,9} In the ligand-bound conformation, the hairpin loop on domain II is exposed and mediates interreceptor signaling dimers. The role of the tethered conformation of EGFR, ErbB3, and ErbB4 thus appeared to be sequestering the domain II loop, now also known as the “dimerization arm,” and preventing formation of active receptor dimers in the absence of ligand.²

Mutagenesis of the tether-binding pocket on domain IV failed to result in constitutive activation of EGFR, however, and the importance of the tether for maintaining ErbBs in an inactive state was questioned.^{13,14} Subsequent SAXS studies of the EGFR extracellular region showed that mutation of the tether pocket failed to convert it from a compact conformation to the extended conformation that is observed when ligand is bound,¹⁵ suggesting that elements outside of the tether play a role in stabilizing the tethered conformation. To address this issue, we determined and report here the crystal structure of a fragment of the ErbB4 extracellular region encompassing the N-terminal three domains (tErbB4). This fragment contains all ligand-binding elements but lacks domain IV, which is necessary to

constitute the tether interaction. Consistent with the SAXS results, this structure shows that tErbB4 adopts a tethered-like domain arrangement similar to the structure of the ErbB4 extracellular region in the absence of ligand.¹⁰ A recent structure of a comparable fragment of ErbB3 also exhibits a tethered-like structure indicating this conformation is a general feature of ErbBs in the absence of the tether contact.¹⁶

Results and Discussion

A fragment of the extracellular region of human ErbB4 encompassing domains I, II, and III (tErbB4; residues 1–497) was expressed in Lec1 cells with an N-terminal human growth hormone/hexahistidine tag that was removed before crystallization,^{17,18} purified as described for a 4-domain fragment of ErbB4,¹⁰ crystallized by the hanging-drop vapor diffusion method using a 1:1 mixture of a 4 mg/mL protein solution and crystallization buffer (16% PEG 8000, 0.2M calcium acetate and 0.1M sodium cacodylate pH 6.0), and its 2.5 Å structure determined by molecular replacement using sErbB4 domains as search models (Fig. 1). Data collection and refinement statistics are shown in Table I. The structure reveals that tErbB4 retains a tethered-like domain arrangement in the absence of the domain IV tether pocket (Fig. 2). Domain III in tErbB4 is shifted relative to the domain I/II pair $\sim 28^\circ$ about an axis perpendicular to the long axis of domain II when compared with its relative orientation in the 4-domain sErbB4 structure (Fig. 2). When ligand is bound to EGFR, domain III undergoes an additional $\sim 90^\circ$ rotation about an axis parallel to the long axis of domain II relative to its conformation in unliganded receptor. The presence of a tethered-like conformation in a similarly truncated form of ErbB3¹⁶ suggests that lattice interactions are not responsible for this conformation and that structural elements in the domain II/III hinge region, which is the only

Table I. Data Collection and Refinement Statistics

Data collection	
Wavelength (Å)	1.54
Space group	P2 ₁ 2 ₁ 2 ₁
Unit cell dimensions	
<i>a</i> (Å)	42.2
<i>b</i> (Å)	85.6
<i>c</i> (Å)	152.4
Resolution (Å)	25–2.57
No. of unique reflections	17809
Completeness (%)	97.1 (97.2)
<i>I</i> / σ	17.1 (3.1)
<i>R</i> _{merge} (last shell) (%)	8.9 (47.2)
Redundancy (last shell)	5.2 (4.6)
Refinement	
<i>R</i> _{work} (%)	19.2
<i>R</i> _{free} (%)	24.3
No. of atoms	
Protein	3737
Water	86
Sugar	70
rmsd	
Bond length (Å)	0.01
Bond angle (°)	1.24
Ramachandran plot (%)	
Most favored	85.5
Additional allowed	13.5
Generously allowed	0.2
Disallowed	0.7
Average <i>B</i> -values (Å ²)	
Protein	50.1
Water	39.4

contact region between domain III and the domain I/II pair in tErbB4, stabilize the tethered conformation in the absence of the tether contact itself. This

observation may explain in part the absence of constitutive activity in EGFR variants with mutations in the tether pocket.^{13,14} Also of note is the similar arrangement of domains I, II, and III of the type I insulin-like growth factor receptor (IGF1R), which are homologous to the corresponding ErbB domains.¹⁹ A 17° rotation will superimpose domain III of IGF1R on domain III of tErbB4 following initial superposition of the domain I/II pairs, which suggests that the domain II/III hinge relationship is stabilized in different receptor classes. A partially activating mutation in the domain II/III hinge region in the *C. elegans* EGFR homolog LET-23,²⁰ which is unlikely to adopt the tethered conformation,²¹ further suggests that this hinge region conformation stabilizes an inactive conformation.

The question then arises of what, if any, functional role is played by the tether contact. A possible answer to this question is suggested by the recent crystal structures of the extracellular region of the *Drosophila* EGFR in the presence and absence of ligand.^{21,22} Only one EGFR homolog is present in the *Drosophila* genome, and these structures revealed an untethered structure for *Drosophila* EGFR in the absence of ligand.²¹ When ligand is bound a ~20° relative shift of domains I and III occurs²²; this shift is also apparent in human EGFR when ligand binds and has been characterized as a shift from “straight” to “bent” conformations of domain II.²³ The *Drosophila* EGFR structures also reveal an asymmetric receptor dimer when ligand is bound as one receptor subunit remains in the

Figure 2. Comparison of tErbB4 with liganded and unliganded ErbB structures. Orthogonal views of worm diagrams of tErbB4 (red), sErbB4 (yellow), and sEGFR when complexed with TGF α (slate blue) following superposition of the domain I/II pairs.

unliganded conformation.²¹ If the *Drosophila* EGFR activation mechanism is the precursor of the vertebrate ErbB activation mechanism, the participation of an unliganded receptor in an active signaling complex suggests that the tether may have evolved to prevent crosstalk between homologous receptors following duplication of ErbB genes. ErbBs bind different subsets of ligands, activate different collections of downstream effectors, and mediate distinct but overlapping biological effects.^{1,3,24} Such separate biological functions would have been difficult to evolve if unliganded ErbB receptors were free to participate in active signaling complexes with all other ErbBs regardless of ligand. The tether thus appears to have facilitated evolution of functional diversity among ErbBs.

PDB Coordinates

Atomic coordinates and structure factors have been deposited in the PDB and assigned accession code 3U2P.

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