A 40-kDa Epidermal Growth Factor/Transforming Growth Factor α-binding Domain Produced by Limited Proteolysis of the Extracellular Domain of the Epidermal Growth Factor Receptor*

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Elucidation of the three-dimensional structure of the complex of the epidermal growth factor (EGF) and its receptor is essential for understanding the molecular mechanisms of the EGF-receptor interaction and EGF-induced receptor-receptor interaction. NMR is useful to investigate interactions in solution between macromolecules at atomic resolution, but has a limitation in molecular masses of target proteins: less than 300 residues. We have prepared a fragment with apparent molecular mass of 40 kDa in SDS gels from the soluble extracellular domain of the EGF receptor (sEGFR, 619 residues) by sequential limited proteolysis with proteinase K and bromelain. This fragment is a monomeric structural domain consisting of 202 amino acid residues (Cys302-Arg603) and 18-kDa sugar chains, and binds EGF and transforming growth factor-α (TGFα).

This 40-kDa domain has a dissociation constant of about 1 μM for human TGFα, which is similar to that of the parental sEGFR. sEGFR oligomerizes in response to EGF and TGFα, while the 40-kDa domain does not, suggesting that the sequences other than this domain is required for receptor oligomerization. The 40-kDa ligand-binding domain described in this report is suitable for analysis by various physico-chemical approaches such as NMR.

The human epidermal growth factor receptor (EGFR) is a 170-kDa glycoprotein that mediates the biological effects of polypeptide growth factors such as epidermal growth factor (EGF) and transforming growth factor-α (TGFα) (1, 2). The EGFR consists of two major functional domains, the extracellular domain and the cytoplasmic domain, linked by a single transmembrane region (3). The binding of EGF and TGFα to the extracellular domain of EGFR induces conformational changes in the extracellular domain (4) and results in rapid oligomerization of the receptors (5-9). In the oligomerized state, the tyrosine kinase in the cytoplasmic domain phosphorylates tyrosines in the C-terminal tail region of the adjacent receptors through an intermolecular mechanism (10). Proteins containing Src homology region 2 (SH2) bind EGFR through interaction between the SH2 domain and the phosphorylated C-terminal tail of EGFR. Subsequently, these substrate proteins become phosphorylated (11).

The extracellular domain of EGFR is divided into four subdomains: I, II, III, and IV, from N to C terminus on the basis of sequence homology (12). Domain III (residues 310-474) has been shown to be a major ligand-binding region of the EGFR receptor. Clear experimental evidence has been presented using a chimeric chicken EGFR that contained the human sequence of domain III (residues 300-484) in place of the corresponding chicken sequence (13). It has also been shown that the entire sequence of domain III is required for full binding capacity using similar chimeric approach (14). Wu et al. (15, 16) showed that a covalent cross-linking agent DSS cross-links the amino group of Asn1 of mouse EGF to that of Lys302 of sEGFR, and the monoclonal antibodies that are competitive with EGF recognize sEGFR residues 351-364, suggesting that the N-terminal half of the domain III sequence directly contacts with EGF/TGFα.

For elucidating the molecular mechanism of EGF/TGFα-receptor interactions and mechanism of receptor oligomerization, structural analysis of EGF/TGFα and EGFR is essential. X-ray crystallography and NMR provide three-dimensional structures of macromolecules at atomic resolution. Although crystallization of human EGFR was reported (17), no x-ray analyses of EGF and TGFα have been reported yet. The NMR structures of EGFs from human and mouse, and TGFα from human have been described (18-22). X-ray and NMR analyses of the intact receptor molecule are difficult, since EGFR is a heavily glycosylated membrane protein with a high molecular mass. The use of a truncated receptor consisting of the extracellular domain will be helpful for the structural analyses. A soluble receptor (sEGFR) is secreted by A431 human tumor cells (23-25) or can be prepared by the expression of cDNA encoding the extracellular domain in baculovirus/insect cell expression system (26, 27) and in Chinese hamster ovary (CHO) cell expression system (28). Crystallization of the EGF-sEGFR complex was reported (29). The
dissociation constant $K_d$ of these soluble fragments to EGF is similar to the $K_d$ of the detergent-solubilized full-length receptors (26–30). Hurwitz et al. (27) and Lax et al. (28) showed that sEGFR forms oligomers in response to EGF by cross-linking. By contrast, Greenfield et al. (26) and Günther et al. (29) reported that sEGFR did not oligomerize using density gradient ultracentrifugation. This discrepancy may be due to differences in the sEGFR concentrations used.

The protein structure determination by NMR and distance geometry calculation is now established (31–33). However, at present, the NMR method is restricted to proteins which contain less than 300 residues even if we use three- and four-dimensional heteronuclear NMR (33). Thus the large molecular mass of sEGFR (~100 kDa) prevents detailed NMR analysis of the protein structure. O’Keefe et al. (34) observed several proteolytic fragments (25–48 kDa) of the EGF receptor that retained the ability to bind EGF. However, they used crude EGF receptor preparations that had been affinity-labeled with $^{125}$I-EGF, and identified these fragments by SDS-PAGE and autoradiography after limited proteolysis. That is, they did not actually isolate these proteolytic fragments. In the present study we have carried out experiments of limited proteolysis of sEGFR to isolate small domains with EGF/TOGF-binding capacity. We obtained a fragment with apparent molecular mass of 40 kDa containing 202 amino acid residues with full ligand-binding capacity. This fragment contains the entire sequence of domain III.

**EXPERIMENTAL PROCEDURES**

**Materials**—The sources of materials were as follows: electrophoresis gels and a silver-staining kit from Pharmacia LKB Biotechnology Inc., Affi-Prep 10 from Bio-Rad, dimethylsuberimidate (DMS) and disuccinimidyl suberate (DSS) from Nacalai Tesque, Japan, bis(sulfosuccinimidyl) suberate (BS3) from Pierce Chemical Co., Fun-gizone from Gibco, Dulbecco’s modified Eagle’s medium/F-12 (1:1) culture medium and dialized fetal bovine serum from Hazleton.

**Enzymes**—Sialidase (Arthrobacter ureafaciens), endoglycosidase F/N-glycosidase F, proteinase K, and bromelain were purchased from Boehringer Mannheim. Lysyl endopeptidase (Achromobacter protease I) was from Wako Pure Chemical Industries, Japan.

**EGF and TOGF**—Mouse EGF from syngeneic glands of adult male mice was purified as described (35). Recombinant human EGF was a generous gift from Wakanaga Pharmaceutical Co., Japan. Recombinant human TGFα was produced in Escherichia coli and was used for further experiments. The fraction containing the 40-kDa fragment was used for further experiments. The fraction containing the 48-kDa fragment was concentrated with a Centricon-30 (Amicon), and the buffer was changed to 50 mM Hepes, pH 7.4.

**Analytical Methods**—Proteins were analyzed with a PhastSystem (Pharmacia) on 7.5, 12.5, and 20% sodium dodecyl sulfate-polyacrylamide gels (SDS-PAGE), and on pH 3–9 isoelectric focusing polyacrylamide gels. Cystines in proteins were reduced by dithiothreitol prior to SDS-PAGE. Proteins in gels were detected by either Coomassie or silver staining. The concentration of proteins was determined on the bases of the following adsorption coefficients: mouse and human EGFs, $A_{280}$ (1 mg/ml, 1 cm) = 3.1; human TGFα, 0.36; sEGFR, 0.74 (4). Amino acid composition was determined with a model 420A derivatizer/analyzer (Applied Biosystems). Glycopeptides were denatured by heating in the presence of 0.25% SDS and 0.5% 2-mercaptoethanol and then diglycosylated with endoglycosidase F/N-glycosidase F. An excess of the peptide fragment overnight at room temperature in 50 mM Hepes buffer, pH 7.4, containing 50 mM EDTA and 2% N-octyl-β-D-glucoside. The molecular masses of the generated peptides without carbohydrate were estimated by SDS-PAGE.

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In this paper, “the 40-kDa fragment” means a 40-kDa proteolytic fragment generated by sequential sialidase/proteinase K/bromelain digestion, and “the 48-kDa fragment” means a 48-kDa proteolytic fragment generated by sequential sialidase/proteinase K digestion. The solution of the sialidase/proteinase K-generated product was dialyzed against 50 mM Hepes, pH 7.4, and was used for further experiments. The fraction containing the 48-kDa fragment was concentrated with a Centricon-30 (Amicon), and the buffer was changed to 50 mM Hepes, pH 7.4.

**Bromelain Digestion and Purification of the 40-kDa Fragment**—To find smaller fragments, we subjected the proteinase K-generated 48-kDa fragment to the second digestion with trypsin, lysyl endopeptidase, endoglycosidase H (Roche), papain, bromelain, α-chymotrypsin, subtilisin, and proteinase K. We selected proteinase K to generate a fragment of sEGFR. sEGFR (5.2 mg) in 4.4 ml of 50 mM Hepes, pH 7.4, was treated with 0.16 unit of sialidase at 37 °C for 1 h. After the incubation EDTA was added to the reaction mixture at a final concentration of 1 mM. The solution of sialidase/proteinase K-generated product was digested with 0.5% trypsin overnight at 37 °C for 24 h in a sealed tube. sEGFR was converted to 60–70-kDa intermediate fragment(s), and finally to a 48-kDa fragment, which was accumulated after 8 h. The digestion was stopped by phenylmethylsulfonyl fluoride at a final concentration of 1 mM. The resulting solution excluded from the analysis by SDS-PAGE. The solution of the sialidase/proteinase K-generated protein was dialyzed against 50 mM Mes buffer, pH 6.0, and then to a MonoS HR5/5 column (5 mm × 5 cm, Pharmacia) equilibrated with the same buffer. Several peaks appeared as a linear gradient of NaCl was run from 0 to 0.4 M over a period of 30 min at a flow rate of 1.0 ml/min. N-terminal sequencing of these peaks revealed that the material was a mixture of several 48-kDa species with N-terminal (and probably C-terminal) heterogeneity. This is reasonable considering the broad specificity of proteinase K. Fortunately, the fragment in one peak had a single N-terminal sequence. The fraction containing the 48-kDa fragment was concentrated with a Centricon-30 (Amicon), and the buffer was changed to 50 mM Hepes, pH 7.4.
solution, EDTA, cysteine, and NaCl were added at final concentrations of 1 mM, 5 mM, and 1 mM, respectively. The 48-kDa fragment was digested at 37 °C for 24 h with bromelin (10:1, w/w). The digestion was stopped by chilling on ice. The bromelin-generated products were separated on a G3000SWx̅̅ gel filtration column because the conversion of 48-kDa fragment to 40-kDa fragment was incomplete (<50% yield). The fractions containing the 40-kDa fragment were concentrated with a Centricon-10 (Amicon). The purified 40-kDa fragment eluted as a single peak when analyzed by the cation-exchange chromatography.

Binding of the Proteolytic Fragments to Immobilized EGF—Mouse EGF was coupled to Affi-Prep10 in 20 mM Hepes buffer, pH 7.4. One milligram of mouse EGF was bound per milliliter of Affi-Prep 10 resin. The succinimidyl group that did not react was blocked with ethanolamine. Mouse EGF was better than human EGF as an immobilized ligand, because mouse EGF contains only one N-terminal amino group, which can be blocked without loss of receptor binding (36). sEGFR (5 μg/μl) or the proteolytic products (5 μg/μl) were mixed with 5 μl of resin carrying immobilized EGF in 0.5-ml tubes. The tubes were turned gently end-over-end at 4 °C for 1 h. The resin was washed twice with 0.5 ml of 20 mM Hepes buffer, pH 7.4, containing 1 mM NaCl, and then once with 0.5 ml of the Hepes buffer without NaCl. The sEGFR fragments bound to the resin were analyzed by SDS-PAGE and silver staining. Resin without immobilized EGF was used to check nonspecific binding.

Determination of the Dissociation Constants of TGFO to the Proteolytic Fragments—Fluorescence was recorded with a Shimadzu RF-5000 fluorescence spectrophotometer. Fluorescence of Trp residues was excited at 295 nm with a bandwidth of 3 nm and measured at 345 nm with a bandwidth of 20 nm. The temperature of the sample solution (2.0 ml) was controlled at 37 ± 0.1 °C with a circulating water bath (Haake D8/L). The buffer was 50 mM sodium phosphate buffer, pH 7.0, containing 100 mM NaCl. Corrections for volume changes (<3%), photochemical decomposition (approximately −0.15%/min irradiation), and the intrinsic Tyr fluorescence of hTGFO were made. The concentrations of sEGFR or its fragments (5 ng/μl) used were lower than the estimated Kd value of ligand-induced oligomerization (10 μM, see “Discussion”), and so no correction was made for oligomerization. No inner filter effects were corrected because of the low absorbance (<0.05) at the excitation wavelength.

A titration curve monitoring Trp fluorescence of sEGFR and its proteolytic fragments allows the determination of the dissociation constants (Kd) to human TGFO. The ratio θ = ΔF/ΔFmax is related to the total concentration of hTGFO, [hTGFO]r, by the following equation,

\[
\frac{1}{1 - \theta} = \frac{1}{K_d} \left( \frac{[hTGFO]_r}{\theta} - p[fragment]_r \right)
\]

(Eq. 1)

where ΔF is the change in Trp fluorescence of the receptor fragments due to interaction with hTGFO. ΔFmax is the maximum fluorescence change when all binding sites are saturated with hTGFO, p is the number of sites for hTGFO, and [fragment]r is the total concentration of the receptor fragments (37). Here p is equal to 1, since the stoichiometry of ligand binding to the EGF receptor is 1:1 (38).

Covalent Cross-linking Experiments—Purified sEGFR (5 μM) or the proteolytic fragments (5 μM) was incubated with human EGF (20 μM) or human TGFO (20 μM) or insulin (20 μM) in 50 mM Tris-HCl, pH 9.0, containing 100 mM NaCl for 30 min at room temperature. A covalent cross-linking agent DMS was added at a final concentration of 10 mM, and the reaction mixture was left for 30 more min. In the cases of DSS or BS3-cross-linkers, 50 mM Hepes buffer, pH 7.4, was used. The final concentrations of DSS and BS3 were 0.5 and 1 mM, respectively. DSS was dissolved in Me2SO, and so the reaction mixture contained 10% Me2SO. BS3 is a water soluble analog of DSS. The cross-linking products were analyzed by SDS-PAGE and silver staining.

RESULTS

We prepared fragments with apparent molecular sizes of 48 and 40 kDa in SDS gels from soluble EGFR by limited proteolysis with proteinase K and bromelin as described under “Experimental Procedures.” SDS-PAGE analysis of the purified 48- and 40-kDa fragments is shown in Fig. 1A. The molecular masses of the protein portion of these fragments were estimated after deglycosylation (Fig. 1B). Endoglycosidase F/N-glycosidase F treatments of these fragments gave several bands corresponding to various extent of deglycosylation. The minimum molecular masses were considered as the molecular masses of the proteins portions: 29 kDa for the 48-kDa fragment and 22 kDa for the 40-kDa fragment (Fig. 1B).

The C-terminal tail of the 48-kDa fragment was predicted to end at residue 580 ± 5 as revealed by analysis of the molecular mass of the protein portion and its amino acid composition. Precise determination of the C-terminal residue of the 40-kDa fragment is important for future experiments such as NMR analysis and gene manipulation. Our strategy was to completely sequence a short (<15 residues) peptide containing the C-terminal tail. The 48-kDa fragment and the 40-kDa fragment were cleaved completely with lysyl endopeptidase after reduction and S-carboxamidemethylation, and the resultant peptides were separated on a reversed-phase column. Since the N-terminal positions of these two fragments differ only by 1 residue, a new peak which appeared in the chromatogram of the 40-kDa fragment digest (data not shown) was likely a peptide containing the C terminus of the 40-kDa fragment. This peptide was recovered and further digested with endoproteinase Asp-N, and the resultant peptides were separated on the reversed-phase column. We sequenced these two peptides completely; one peptide was ATGGVCHALCSPEGCWGPEPX-end and the other peptide was DCVSCR-end. Thus the 40-kDa fragment terminates at Arg382. The molecular mass (22 kDa) of the protein portion and the amino acid composition of this fragment confirmed this result.

The molecular masses of the 48- and 40-kDa fragments under native conditions estimated by gel filtration chromatography were 58 and 39 kDa, respectively, indicating that...
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these fragments are monomeric proteins under native conditions.

The two fragments generated with lysyl endopeptidase from the 48-kDa fragment were separated on a reversed-phase column, and subjected to N-terminal sequencing. The 24-kDa fragment starts with Lys304 and the 32-kDa fragment with Gln40R. The molecular masses of the protein portion were estimated by SDS-PAGE after deglycosylation: 11 kDa and 19 kDa for the 24-kDa and 32-kDa fragment, respectively. Thus the 24-kDa fragment probably ends with Lys407 and the 32-kDa fragment ends in residues 570-580. The analysis on a gel filtration column under native conditions showed that the lysyl endopeptidase products consisted of a single component of molecular mass 56 kDa, indicating that the two fragments formed a complex. Fragmentation by limited proteolysis is summarized in Fig. 2.

Binding of the Fragments to Immobilized EGF—Binding to immobilized EGF provides a simple and rapid assay to examine the EGF-binding capacity of the EGFR fragments. Fig. 3 shows that the purified 48- and 40-kDa fragments as well as sEGFR are able to bind to immobilized mouse EGF (lanes 2, 4, and 6). Nonspecific binding to the resin was negligible (lanes 1, 3, and 5). The nicked complex generated by lysyl endopeptidase digestion also has EGF-binding capacity (data not shown).

Dissociation Constants of hTGFα to the Fragments—sEGFR has 6 Trp residues, and both of the 48-kDa and 40-kDa fragments contain 3 Trp residues, whereas no Trp residues are found in hTGFα. Trp fluorescence of sEGFR and the fragments increased as the concentration of hTGFα increased (Fig. 4). ΔF_max values are small (+3-6%), but sufficient to determine the dissociation constants. The titrations were analyzed according to Equation 1. The calculated dissociation constants were 0.50 PM for sEGFR, 0.95 PM for the 48-kDa fragment, and 1.3 WM for the 40-kDa fragment. Unfortunately, EGF has 2 Trp residues, which prevents us from determining...
the dissociation constants for EGF and the fragments.

Cross-linking in the Presence of EGF—Cross-linking experiments demonstrated that sEGFR forms oligomers upon binding of EGF (27, 28). Hence we investigated whether EGF induced oligomerization of the fragments. EGF and the fragments were incubated together and subsequently treated with the covalent cross-linking agent, DMS. The products of this reaction were analyzed by SDS-PAGE and silver staining. Fig. 5 shows that neither the 48-kDa fragment nor the 40-kDa fragment were able to aggregate in the presence of EGF or TGFα. Under the same conditions, sEGFR oligomerized in response to EGF and TGFα (data not shown). For the 48-kDa fragment, cross-linking with DMS generated a small amount of nonspecific cross-linked aggregation even without any ligands (Fig. 5A, arrow). The amount of this nonspecific cross-linked material was not changed upon addition of EGF or TGFα. Under the same conditions, sEGFR oligomerized in response to EGF and TGFα (data not shown). For the 48-kDa fragment, cross-linking with DMS generated a small amount of nonspecific cross-linked aggregation even without any ligands (Fig. 5A, arrow). The amount of this nonspecific cross-linked material was not changed upon addition of EGF or TGFα. Under the same conditions, sEGFR oligomerized in response to EGF and TGFα (data not shown).

**DISCUSSION**

The use of the soluble EGFR solves several problems that we encounter when using the intact EGF receptor molecules. First, sEGFR can be prepared in milligram quantity. In fact we obtained at least 1 g of sEGFR using a hollow fiber bioreactor. Second, sEGFR does not require detergents for solubilization. Finally, the molecular mass is reduced from 170 to about 100 kDa. However, sEGFR is still too large for physico-chemical characterization such as NMR. Since domain III of EGFR is shown to be a major ligand-binding region from several lines of evidence (13–16), the expression of cDNA encoding the domain III sequence alone seemed a rational experiment. However, the expression of residues 319–472 in E. coli resulted in formation of inclusion bodies.⁸ One point to be clarified is whether the domain III exists as a structural domain in the sEGFR structure. Previous experiments did not provide any answer to this question.

We have carried out limited proteolysis of the soluble EGF receptor to obtain fragments that retain EGF/TGFα binding capacity (Table I). This was achieved by a sequential digestion with sialidase, proteinase K, and bromelain (Fig. 2). The requirement of high doses of the proteases (5–10% of the weight of sEGFR) and long incubation periods indicate unusual stability for sEGFR. The 40-kDa fragment starts with Cys⁴⁹⁶ and ends with Arg⁵⁰⁰, and thus contains 202 amino acid residues. This sequence contains four potential N-glycosylation sites at Asn⁴⁰⁸, Asn⁴⁷⁷, Asn⁴⁸⁰, and Asn⁴⁹⁰, and carbohydrate chains contribute about half (18 kDa) of the apparent molecular mass. Fluorescence experiments showed that this fragment binds to human TGFα with affinity similar to binding of sEGFR (Fig. 4 and Table I). This result suggests that this fragment has a major contribution toward the binding of EGF/TGFα.

It appears that the 40-kDa fragment is a structural domain in the sEGFR structure, because various proteases with different specificities generates similar fragments (data not shown). The fact that the 40-kDa fragment is a stable monomer judging from the apparent molecular mass (39 kDa) on a gel filtration column provides additional support for this notion. Hence we call the 40-kDa fragment “40-kDa ligand-binding domain.” This ligand-binding domain contains the entire domain III sequence and 5 and 20% of the residues of domain II and IV sequences, respectively. Thus the boundary between domains II and III defined by the sequence homology almost exactly corresponds to the N-terminal position of the ligand-binding domain. By contrast the boundary between domain III and IV does not correspond to the C-terminal position; the ligand-binding domain must extend to the C terminus about 30 residues. Note that both cleavage sites fall in the cysteine-rich regions. Extra short peptides could attach to the ligand-binding domain through disulfide bonds; because these short peptides are lost during SDS-PAGE and the pretreatment for N-terminal sequencing, these extra peptides were undetectable. However, good coincidence of the molec-

**TABLE I**

**Properties of the proteolytic fragments of the soluble EGFR**

Molecular masses were estimated by SDS-PAGE. Dissociation constants were determined by fluorescence spectroscopy. Ligand-induced oligomerization was detected by SDS-PAGE after cross-linking with DMS.

| Fragment          | Molecular mass (kDa) | Glycoprotein | Protein* | Carbohydrate* | lig- | $K_{d}$ | hTGFα | Ligand-induced oligomerization |
|-------------------|---------------------|--------------|----------|---------------|------|--------|-------|-----------------------------|
| sEGFR             | 95                  | 69 (68)      | 26       | 0.50          | +    |
| 48-kDa fragment   | 48                  | 29 (31)      | 19       | 0.95          | -    |
| 40-kDa fragment   | 40                  | 22 (22)      | 18       | 1.3           | -    |

* After deglycosylation. Calculated molecular mass from the sequence in parentheses.

* Molecular mass of carbohydrate is calculated as the difference of the molecular masses before and after deglycosylation.

* On a homogenous 20% gel (PhastGel, Pharmacia). Previously reported value was 105 kDa (28).

⁸ I. Lax and J. Schlessinger, unpublished results.
ular mass estimated by SDS-PAGE (40 kDa) with that by gel filtration under native conditions (39 kDa) suggests that the final preparation of the 40-kDa ligand-binding domain does not contain these short peptides. The fact that no extra bands of low molecular masses were seen on the SDS-PAGE of the purified fragments also supports this conclusion (Fig. 1A).

Lysyl endopeptidase cleaves the 48-kDa fragment at the center of the domain III sequence. The resultant two halves of the domain III sequence remain complexed under native conditions. This suggests that the ligand-binding domain of the domain I11 sequence contains 1 conserved cysteine residue, Cys99 and Cys446, which might form an intradomain disulfide bond (12). However, the two fragments were separated on a reversed-phase column at an acidic pH, indicating that no intradomain disulfide bond is formed.

The soluble extracellular domain of EGFR has the intrinsic capacity to undergo EGF-dependent oligomerization, which is thought to be important in receptor activation and subsequent signal transduction (5-9). Covalent cross-linking experiments demonstrate the EGFR oligomerization in response to EGF (27, 28), whereas density gradient ultracentrifugation experiments do not (4, 29). The used concentrations of sEGFR and EGF were quite different; 5-30 pM in the former and 0.1-1 pM in the latter experiments. Hence the dissociation constant of the receptor oligomer is perhaps of the order of 10 μM. Of course, the cross-linking experiments may give artifactual results. But the oligomers can be detected without cross-linking by electron microscopy (28) and by NMR.4 It is interesting to know whether the 40-kDa ligand-binding domain undergoes oligomerization after ligand addition. Covalent cross-linking experiments indicated that the proteolytic fragments obtained in this study did not form oligomers in response to EGF or TGFα (Fig. 5). Thus regions other than the domain III sequence may be involved in receptor oligomerization. Alternatively, the domain III could be directly involved in receptor-receptor interaction regulated by other regions.

In this study, we obtained the 40-kDa structural domain by limited proteolysis of sEGFR with full EGF-binding capacity. The size of the protein portion (202 residues) is within reach of the structure determination by NMR with isoep labeling technique. We hope we can also determine the structure of the complex of the domain and EGF/TGFα.

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