Asparagine-344 Is a Key Residue for Ligand Binding in Keratinocyte Growth Factor Receptor†

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ABSTRACT: The membrane proximal, immunoglobulin- (Ig-) like domain 3 of KGFR shows significant sequence similarity to the Ig light chain variable (V) domain. According to our model, based on this similarity, the F−G loop in KGFR corresponds to the complementarity determining region (CDR) 3 of the Ig V domain. The F−G loop in the membrane proximal domain of the keratinocyte growth factor receptor has previously been shown to participate in determining the FGF ligand binding specificity of KGFR [Gray, T. E., Eisenstein, M., Shimon, T., Givol, D., & Yayon, A. (1995) *Biochemistry 34*, 10325−10333]. Here, we report the effects of additional mutations in this F−G loop. Both a single mutant KGFR Q_{348}→I and a double mutant KGFR Q_{348}→I, Q_{351}→H are found to have relatively mild effects on ligand binding, as was previously found for three other F−G loop mutant receptors. In contrast, a single mutation N_{344}→A in the F−G loop of KGFR is sufficient to abolish essentially all affinity of this receptor for its primary ligand KGF, while some affinity for aFGF is retained. Asparagine-344 is, therefore, essential for ligand binding by KGFR. We discuss the likelihood of this effect being due to global or local structural changes or to the removal of a specific interaction with the ligand, in relation to various known and model structures. Taking into account the mild effects of other mutations in the region and various other considerations, we tend to favor the idea that asparagine-344 is a key residue in determining the local conformation of the F−G loop.

The fibroblast growth factor receptors (FGFRs)[1] are membrane-spanning receptor tyrosine kinases that are involved in numerous processes during cell growth and development (Wilkie et al., 1995). Currently, the FGFR family is receiving much attention due to the recent discoveries that specific point mutations in several FGFR genes are responsible for a variety of bone growth disorders (Muenke & Schell, 1995). There are examples where such mutations to unpaired cysteine residues apparently cause the formation of stable receptor dimers (Neilson & Freisl, 1995), but alterations in affinity for receptor ligands may also play a role since in some cases the mutations do not generate free cysteine residues. There are nine FGF ligands [for review see Baird (1994)] which interact with two main types of receptor: the high-affinity FGFRs and heparan sulfate proteoglycans (HSPGs), which they bind with lower affinity. It seems that a ternary complex involving these three species is required in order to achieve efficient signaling via FGFRs (Rapraeger et al., 1991; Yayon et al., 1991).

There are four members of the FGFR family, and they display overlapping specificity for different FGFs. Acidic FGF (aFGF) is the most promiscuous FGF as it binds to all of the receptors and their various isoforms. It has been shown previously that in the case of FGFR2 and KGFR the difference in their ligand binding specificity can be attributed to changes in the membrane proximal third immunoglobulin-like (Ig-like) domain that arise due to alternative RNA splicing (Miki et al., 1992; Yayon et al., 1992). Various structural models of FGFRs have been proposed on the basis of different structural templates exhibiting the immunoglobulin fold (Bateman & Chothia, 1995; Gray et al., 1995; Pantoliano et al., 1994; Wilkie et al., 1995; Xu et al., 1992). Our structural model for the third Ig-like domain of FGFR2 is based on an immunoglobulin light chain variable domain (Gray et al., 1995). Mutagenesis experiments based on this model identified two specific regions in this domain as being involved in determining ligand binding specificity, namely, the connecting loops of β-strands D−E and β-strands F−G. The latter loop corresponds to CDR3 of Ig V domains. Another modeling study based on telokin has also implicated the F−G loop as being important in ligand binding (Bateman & Chothia, 1995).

Here, we have extended our mutational analysis of the F−G loop in domain 3 of KGFR. Previously, we found that the double mutation Y_{345}→S, Q_{348}→I in the F−G loop of KGFR alters the ligand binding specificity of KGFR, whereas the single mutation KGFR Y_{345}→S has little effect. In this

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[1] Abbreviations: FGF, fibroblast growth factor; FGFR, FGF receptor; Ig, immunoglobulin; V-domain, variable domain; aFGF, acidic FGF; bFGF, basic FGF; KGF, keratinocyte growth factor; KGFR, KGF receptor; SDS−PAGE, sodium dodecyl sulfate−polyacrylamide gel electrophoresis; Hepes, *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid; HNTG, 20 mM Hepes, pH 7.5, 150 mM NaCl, 1% Triton X-100, and 10% glycerol; PBS, phosphate-buffered saline; PDB, protein data bank; rms, root mean square; hGH, human growth hormone; hGHR, hGH receptor; CDR, complementarity determining region.

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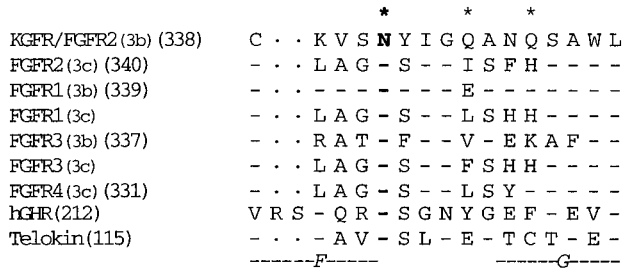

FIGURE 1: Alignment of the amino acid sequence corresponding to the F−G loops from human FGFRs 1−4 encoded by exons 3b or 3c, telokin, and hGHR. Amino acid positions in KGFR that are mutated in this study are indicated by an asterisk. Amino acids that correspond exactly to those found in KGFR are indicated by a dash (−), and gaps introduced are shown by a dot (•). The regions in telokin that form β-strands F and G are indicated by the dashed lines below the telokin sequence.

study a single mutant KGFR Q_{348}→I (previously studied only in the context of the Y_{345}→S, Q_{348}→I double mutant receptor) and a double mutant KGFR Q_{348}→I, Q_{351}→H are examined in order to further map the key ligand binding residues in the F−G loop. In addition, we noted a weak similarity between the F−G loop of the membrane proximal domain of FGFR and the F−G loop in the membrane proximal Ig-like domain of the human growth hormone receptor (hGHR). In particular, a conserved Asn in FGFRs' F−G loop may correspond to Asn_{218} in hGHR (Figure 1). We were interested, therefore, in whether an asparagine residue in the F−G loop of FGFR may play an equivalent important role to Asn_{218} that forms the only interaction between the membrane proximal domain of hGHR and the growth hormone (De Vos et al., 1992). Asparagine at position 344 in KGFR was, therefore, targeted for mutagenesis, and the effects of the mutation on ligand binding and specificity were analyzed. The implications of these results are discussed in relation to possible F−G loop structures. This study involves only the extracellular portion of the KGFR, and therefore, we investigate here only the ligand binding properties of the receptors and not receptor activation or other downstream signaling events.

EXPERIMENTAL PROCEDURES

Materials. Anti-alkaline phosphatase antibodies were from Zymed Laboratories Inc. (San Francisco, CA). Restriction and DNA modification enzymes were from New England Biolabs (Beverly, MA) or Boehringer (Mannheim, Germany). Heparin was from Hepar Industries (Franklin, OH). Protein A−Sepharose was from Repligen (Cambridge, MA). Disuccinimidyl suberate was from Pierce Chemical Co. (Rockford, IL). All other chemicals were obtained from Sigma (St. Louis, MO).

Mutagenesis. Site-directed mutagenesis was performed using the Amersham Sculptor kit on single-stranded DNA encoding the extracellular portion of mouse KGFR cloned into M13mp8. The following oligonucleotides were used in the mutagenesis reactions: 5′-AAGGTCTCCGCTTATATAGGG-3′ (KGFR N_{344}→A) and 5′-TTATATAGGGATAGCCAACCAG-3′ (KGFR Q_{348}→I). The double mutant (KGFR Q_{348}→I, Q_{351}→H) apparently arose spontaneously while generating the Q_{348}→I single mutant. The sequences of the mutants were confirmed using an ABI (Foster City, CA) automatic DNA sequencer.

Cell Transfection and Protein Expression. To facilitate production of the receptor extracellular domain fused at the C-terminus to an alkaline phosphatase enzyme tag, mutated KGFR DNA was subcloned into the APtag mammalian expression vector between the *Hin*dIII and *Bgl*II sites (Flanagan & Leder, 1990; Yayon et al., 1992). These APtag constructs (20 μg) were transiently transfected into human kidney LE293 cells by electroporation (250 V, 500 μF). Conditioned medium was collected 76 h following transfection and assayed for alkaline phosphatase activity following heat inactivation at 65 °C for 10 min (Flanagan & Leder, 1990). Conditioned medium containing the soluble receptor was stored at 4 °C.

Iodination of FGF Ligands and Chemical Cross-Linking. KGF (2 μg) was iodinated according to the chloramine T method (McConahey & Dixon, 1980). Cross-linking was performed using disuccinimidyl suberate (DSS) as described previously (Yayon et al., 1992).

Receptor Binding to FGFs Immobilized on Heparin−Sepharose Beads. Analysis of receptor specificity profiles was performed as described previously (Gray et al., 1995).

Receptor Binding to KGF Immobilized on Heparin-Coated Plates. KGF at various concentrations was incubated for 2 h at 4 °C in a 96-well microtiter plate coated with heparin (Carmeda AB, Taby, Sweden). The plate was washed three times with PBS prior to addition of conditioned medium containing soluble receptor (100 μL) normalized for alkaline phosphatase activity. After 4 h of rotation gently at room temperature, the plates were again washed three times with PBS and once with 0.5 M NaCl before addition of alkaline phosphatase assay buffer. The change in absorbance at 405 nM was monitored at various times. Absorbance measured in the absence of FGF ligand due to background chemical hydrolysis of substrate or nonspecific soluble receptor binding was subtracted from all measurements. The data were fitted as described previously (Gray et al., 1995).

Heparin Affinity Chromatography. Samples of mutant soluble receptors in conditioned medium (500 μL) were loaded onto a heparin−Sepharose HiTrap column (Pharmacia, Uppsala, Sweden), equilibrated with 50 mM phosphate, pH 7.4. The receptor was then eluted with a 0−1.0 M NaCl gradient in the same buffer; 500 μL fractions were collected and assayed for alkaline phosphatase activity.

Sequence Alignment. Alignments of the membrane proximal domain of KGFR with the PDB (Abola et al., 1987; Bernstein et al., 1977) were generated using the alignment programs FASTA and BLAST which are part of the Wisconsin Package, Genetics Computer Group, Madison, WI.

RESULTS

Expression of Soluble Receptors. The expression of the wild-type and mutant secreted soluble receptors was confirmed by western blot analysis using antibodies specific to the alkaline phosphatase enzyme tag (Figure 2). All the mutant proteins migrate on SDS−PAGE with a similar mobility, which corresponds to a molecular mass higher than calculated, due to posttranslational modifications. There is no evidence for degradation of any of the soluble receptors or to alteration in their affinity for heparin as judged by their elution profile in a salt gradient from a heparin−Sepharose column (data not shown). The samples used for western blot analysis were normalized for alkaline phosphatase activity prior to loading on the gel. All the receptors appear therefore to be present in similar amounts (Figure 2).

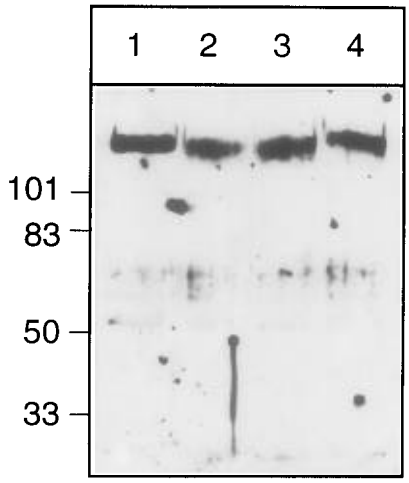

FIGURE 2: Western blot of soluble receptors used in this study, probed with antibodies specific to the alkaline phosphatase enzyme tag. Soluble receptor samples normalized for alkaline phosphatase activity were immobilized on protein A beads coupled to anti-alkaline phosphatase antibodies prior to separation on a 6% denaturing polyacrylamide gel. Lanes 1, 2, 3, and 4 correspond to wild-type KGFR, KGFR N_{344}→A, KGFR Q_{348}→I, and KGFR Q_{348}→I, Q_{351}→H, respectively.

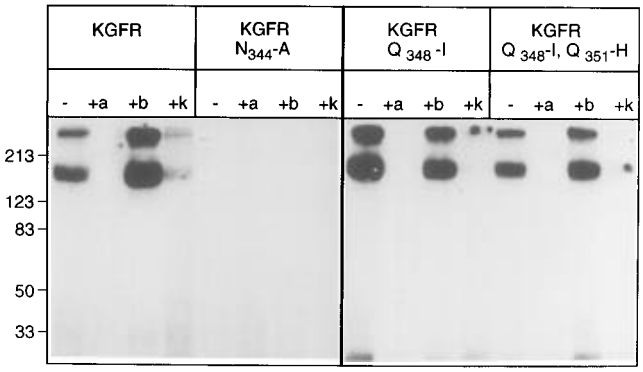

FIGURE 3: Cross-linking of ^{125}I-KGF to immobilized receptors in the presence or absence of a 100-fold excess of unlabeled ligands: acidic FGF (a), basic FGF (b), and keratinocyte growth factor (k). Samples were separated on a 6% denaturing polyacrylamide gel prior to autoradiography.

Cross-Linking of Iodinated KGF to Soluble Receptors. ^{125}I-KGF was cross-linked to the soluble receptors (Figure 3) in the presence or absence of a 100-fold excess of unlabeled ligands (aFGF, bFGF, and KGF). The overall cross-linking pattern was found to be similar in the case of the wild-type receptor and the mutant receptors KGFR Q_{348}→I and KGFR Q_{348}→I, Q_{351}→H. ^{125}I-KGF is displaced from KGFR Q_{348}→I and KGFR Q_{348}→I, Q_{351}→H by an excess of either aFGF or KGF. Basic FGF, on the other hand, is unable to compete efficiently with ^{125}I-KGF for binding to KGFR Q_{348}→I and KGFR Q_{348}→I, Q_{351}→H (Figure 3). In the case of the wild-type receptor the relative intensity of the cross-linked product is somewhat greater in the presence of bFGF than in the absence of competitor. The underlying reason for this is not understood, but a similar observation has been made previously (Ornitz et al., 1992). Taken together, these results indicate that KGF and aFGF but not bFGF are specifically bound with high affinity by the two mutants KGFR Q_{348}→I and KGFR Q_{348}→I, Q_{351}→H in a manner similar to that of the parental receptor. Only the KGFR N_{344}→A mutant failed to cross-link efficiently to ^{125}I-KGF, and no cross-linking products can be seen even in the absence of an excess of unlabeled competitor. The mutant KGFR N_{344}→A is, therefore, unable to specifically bind KGF with high affinity, as observed by this assay.

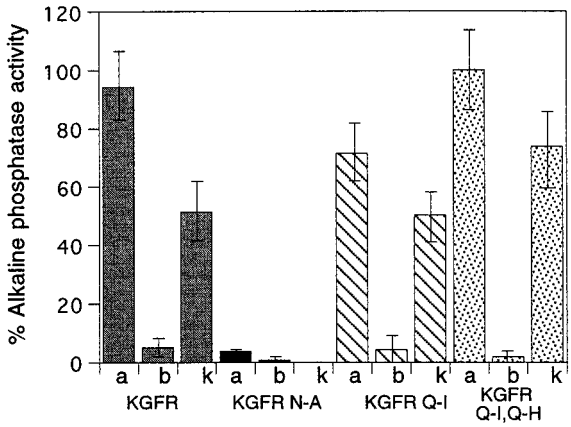

FIGURE 4: Ligand binding profiles of wild-type soluble KGFR and the mutated receptors. FGF ligands were immobilized on heparin-Sepharose beads prior to incubation with the soluble receptors normalized for alkaline phosphatase activity. After extensive washing the alkaline phosphatase activity associated with the beads was determined. Acidic FGF (a), basic FGF (b), and keratinocyte growth factor (k).

Analysis of the Ligand Binding Characteristics of Soluble Mutant Receptors. Binding analysis of the mutant receptors was performed with FGFs immobilized on heparin−Sepharose beads (Figure 4). This analysis confirms the cross-linking results (Figure 3); the ligand binding profile for the mutants KGFR Q_{348}→I and KGFR Q_{348}→I, Q_{351}→H is very similar to that for the wild-type KGFR. These mutations do not alter significantly the ligand binding specificity of KGFR. The mutant KGFR N_{344}→A, on the other hand, abolishes measurable binding for KGF, and the weak binding to bFGF exhibited by the parental receptor is also reduced. In addition, affinity for aFGF is drastically reduced (24-fold) (Figure 4), but it is noteworthy that some residual affinity for aFGF is still detectable in this assay (4% compared to that found for wild-type KGFR).

Direct Binding of KGF to KGFR and the KGFR Mutants. The results obtained from direct binding experiments of KGF to KGFR and the KGFR N_{344}→A mutant are shown in Figure 5A and of KGF to KGFR Q_{348}→I and KGFR Q_{348}→I, Q_{351}→H in Figure 5B. Affinity of the wild-type receptor for KGF is approximately 5 nM as measured by this assay. Binding of KGF to the KGFR N_{344}→A mutant does not display a saturatable binding curve, implying that the affinity is too low to be measured (>500 nM). The binding curves for KGFR Q_{348}→I and KGFR Q_{348}→I, Q_{351}→H (Figure 5B) indicate that both of these receptors exhibit high-affinity binding for KGF with a dissociation constant of approximately 10 nM.

Model Building Study. In order to obtain further insight into the role of Asn_{344} in the structure of KGFR domain 3, we reviewed our model taking into account the telokin-based homology as well as other alignments against sequences in the protein data base. Good sequence alignment between the KGFR domain 3 and either telokin or MOPC21 L chain (entry 1IGC in the PDB) is obtained for two segments of domain 3 comprising (i) β-strand B, the B−C loop, and β-strand C and (ii) the E−F loop, β-strand F, the F−G loop, and β-strand G. Superposition of the framework of MOPC21

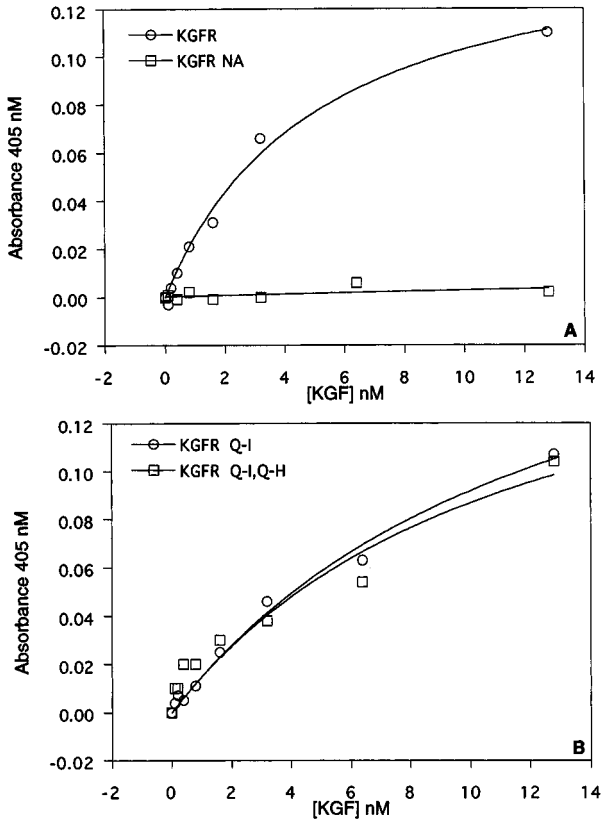

FIGURE 5: Direct binding of soluble receptors to KGF immobilized on heparin-coated 96-well microtiter plates. (A) Open circles represent wild-type receptor and open squares represent the mutant KGFR N_{344}→A. (B) Open circles represent KGFR Q_{348}→I and open squares represent the mutant KGFR Q_{348}→I, Q_{351}→H.

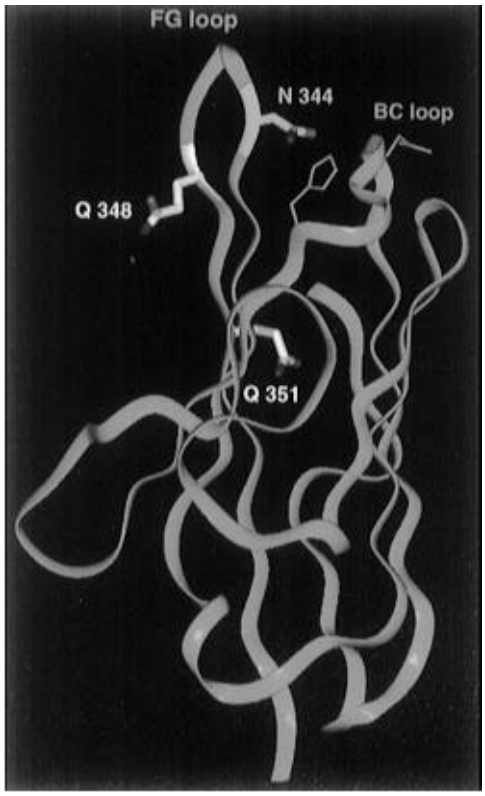

FIGURE 6: Ribbon diagram depicting the C_α backbone of the model structure for KGFR domain 3. The model structure is based on a V light chain domain, and the F−G loop was modeled on the basis of telokin. Amino acids mutated in this study at positions 344 (yellow) and 348 and 351 (white) are shown with their side chains. The side chains of residues in the B−C loop which may interact with Asn_{344} are shown in blue.

on telokin (43 C_α atoms in β-strands A′, B, C, D, E, and F) gives an rms deviation of 0.76 A; thus the two structures are very similar and can serve equally well as a template for model building of these fragments. Notably, however, the intervening sequence between these fragments is 12 amino acids shorter in telokin than in FGFR, whereas it is only one amino acid shorter in MOPC21 than in the FGFR sequence. This segment in the V light chain contains Arg_{61} that forms a highly conserved salt bridge with Asp_{82} whereas in our model the homologous salt bridge is formed between Lys_{313} and Asp_{334}, which are both conserved in FGFRs. We, therefore, conclude that the Ig light chain variable domain remains the better template structure for the overall three-dimensional model for the membrane proximal domain of FGFR. The structure of the F−G loop, however, was modeled as in telokin because FGFR and telokin both contain the NXXG sequence motif whereas in MOPC21 it is NXXP.

In our original model, based entirely on a V light chain domain (Gray et al., 1995), Asn_{344}, Tyr_{345}, and Gln_{348} in the F−G loop are exposed and can interact with ligands. In the present model where the F−G loop is modeled on telokin (Figure 6) Asn_{344} is partially exposed whereas Gln_{348} becomes part of β-strand G. Notably, Asn_{344} can interact with the backbone of Asp_{283} and the side chain of the highly conserved His_{287} in the B−C loop, hence stabilizing the conformation of both loops.

DISCUSSION

Ligand binding by FGFRs occurs mainly *via* the second and third extracellular Ig-like domains. The major KGF ligand binding site resides in the membrane proximal domain of FGFRs (Gray et al., 1995; Miki et al., 1992; Wang et al., 1995; Yayon et al., 1992). Owing to the absence of structural data from NMR or X-ray crystallography regarding FGFRs, the membrane proximal domain of FGFR2 and KGFR was modeled on the basis of an Ig light chain variable domain (Gray et al., 1995). Other FGFR models have been proposed on the basis of variable and constant Ig-like domains (Pantoliano et al., 1994; Xu et al., 1992) and more recently on the basis of telokin (Bateman & Chothia, 1995).

Our model (Gray et al., 1995) as well as that of Bateman and Chothia (1995) suggests that in Ig-like domain 3 the F−G loop composes part of the ligand binding site. To test this hypothesis, we targeted this loop of KGFR for various mutations. The F−G loop in our model comprises the following amino acids, S-N-Y-I-G-Q-A-N-Q_{351}, and in the model of Bateman and Chothia (1995) it is shortened by three residues at the C-terminal side. We have shown that the double mutation Y_{345}→S, Q_{348}→I (a change from the KGFR to FGFR2 sequence) resulted in a regain of bFGF binding by the mutant (Gray et al., 1995). On the other hand, the single mutation Q_{348}→I and the double mutation Q_{348}→I, Q_{351}→H did not affect significantly the ligand binding properties of the receptor in qualitative assays (Figures 3 and 4), and both of these receptors display only slightly reduced affinity for KGF (10 nM) compared with the wild-type receptor (5 nM) as judged by a direct binding assay (Figure 5). In contrast, the single point mutation N_{344}→A dramatically reduced the binding affinity toward both KGF and aFGF. Such a marked change of the binding properties

by a single amino acid substitution may be explained if this residue (Asn_{344}) either is making a major contact with the ligand or is a key residue in shaping the F−G loop and the correct folding of the domain. As shown in Figure 1, Asn_{344} appears in a similar position in the F−G loops of Ig domain 3 of all FGFRs as well as in telokin. Since various FGFRs bind different FGFs, it is difficult to see how Asn_{344} can be a major specificity determining residue for different FGFs by FGFR. The high degree of conservation of Asn_{344} in FGFRs prompted us to look at other proteins and receptor tyrosine kinases that contain Ig domains at their ligand binding site for the presence of Asn at the same position (four residues C-terminal to the second Cys of the Ig domain). Indeed, Asn is present at this position in domain 2 of all FGFRs. In receptor tyrosine kinases with five Ig domains (Kit, CSF-1 receptor, and PDGF receptor) (Claesson-Welsh et al., 1988) there is an Asn at this position in the membrane proximal domain implicated in ligand binding (domain 5). In Kit and CSF-1 receptor Asn also occupies this position in Ig domains 3 and 4, whereas in PDGF receptor it is present also in Ig domain 1.

Our model was built on the basis of the Ig light chain V region. We therefore also checked the amino acid occupying the fourth position from Cys_{88} in 1234 V domain light chain sequences (Kabat, 1991). This position (no. 92) shows a very high variability index of 96 (following Kabat's nomenclature); nevertheless, 15% of the sequences have Asn at position 92 and 15.6% contain Asp which may fulfill similar structural requirements in the F−G loop. This high frequency is even greater if one compares specific subgroups of light chains. For example, mouse K light chain group V has 40% Asn at position 92 (98 of 243 sequences), and human K light chain subgroup I has 33% Asn at this position (20 of 60 sequences). The human λ light chain subgroup I has 87% (21 of 24 sequences) containing Asp at position 92, and the chicken γ chain subgroup has 94% Asp at this position. Thus, in several V light chain subgroups, Asn_{92} (or Asp_{92}), which is part of the complementary determining region 3 (CDR3), is highly conserved and forms the antigen binding site. For example, according to the X-ray structure of the complex between HyHEL-10 and lysozyme, Asn_{92} of the V light chain is in contact with the antigen. It is also interesting to note that in hGHR Asn_{218} in the F−G loop of domain 2 makes a direct contact with the growth hormone ligand. In this latter case the Asn is six residues C-terminal to the second Cys (Figure 1). These data support the possibility that Asn_{344}, in spite of being conserved in many FGFRs, may play a role as a ligand binding residue.

An alternative explanation, however, for the effect of the $N_{344}\rightarrow A$ mutation on ligand binding by KGFR is that Asn_{344} is essential for the structural conformation of the F−G loop. This notion is consistent with the recent model of the FGFR1 membrane proximal domain based on the telokin structure where the Asn_{121} in telokin and Asn_{344} in KGFR are described as key structural residues with only limited accessibility to solvent (Bateman & Chothia, 1995). An additional possible explanation for the effect of the mutation $N_{344}\rightarrow A$ on ligand binding may be related to interaction between the F−G loop and other regions of domain 3, in particular, the B−C loop. As discussed above Asn_{344} may interact with residues in the B−C loop and contribute to the stabilization of the domain. It is also possible that this mutation affects interdomain interaction. In that case the correct structure of the F−G loop may affect the interaction between domain 2 and domain 3 of FGFR which is critical for high-affinity ligand binding (Zimmer et al., 1993).

Our results on the severe and moderate effects of point mutations in the F−G loop of KGFR are different from recent results (Wang et al., 1995) where a 139 amino acid fragment composed of domain 2 and a short segment of 30 residues at the NH_2 terminus of domain 3 are apparently sufficient for aFGF, bFGF, and KGF binding. In addition, it is suggested that the presence of the sequence encoded by exon 3b abrogates the restriction on binding of KGF and lowers affinity for bFGF (Wang et al., 1995). It is difficult to reconcile our results with this view since, in the case of KGFR $N_{344}\rightarrow A$, the mutation is outside the borders of their active fragment. How then is binding by the intact extracellular domain of KGFR abolished? It is also difficult to reconcile our results with the notion that the binding sites for aFGF and KGF reside within the individual Ig domains 2 and 3, respectively (Cheon et al., 1994), since a single point mutation in domain 3 markedly reduced the binding for both aFGF and KGF. We have shown previously that the ligand binding and specificity of FGFRs involve multiple receptor elements which are located in both Ig-like domains 2 and 3 of FGF receptors (Zimmer et al., 1993).

The molecular mechanism by which heparan sulfate proteoglycans (HSPGs) induce the formation of the ternary signaling complex is still unclear. The dramatic effect of the KGFR $N_{344}\rightarrow A$ mutant on ligand binding is unlikely to be due to an effect on heparin binding of the receptor since the binding site for heparin has been shown to reside in domain 2 of FGFR (Kan et al., 1993; Pantoliano et al., 1994) and we observe no change for any of the mutants in their elution profile from a heparin−Sepharose column.

In summary, this study has identified Asn_{344} in KGFR as a crucial residue for receptor function in terms of ligand binding, probably by determining the local fold of the F−G loop. Two models for the membrane proximal domain, one based on telokin (Bateman & Chothia, 1995) and our own based on an Ig light chain variable domain (Gray et al., 1995), offer plausible explanations for the result, but given the low overall sequence identity to any known structure, the true conformation of this domain and its interaction with ligand remain to be determined.

It is of great interest that many of the genetic mutations in human FGFR2 that cause various disorders of bone development are clustered in the region corresponding to the F−G loop of Ig domain 3 and in its vicinity [reviewed by Wilkie et al. (1995a)]. For example, mutations of Tyr_{340}, Cys_{342}, Ala_{344}, and Ser_{347} appear in Crouzon syndrome, Pfeiffer syndrome, and Jackson−Weiss syndrome. Some of these mutations result in the formation of unpaired Cys residues and may lead to receptor dimerization. However, the mutation $Ala_{344}\rightarrow Gly$ (corresponding to Val_{342} in KGFR) in Jackson−Weiss syndrome and the mutation $Ser_{347}\rightarrow Cys$ in Crouzon syndrome are in the F−G loop on either side of Asn_{346} and may have an effect similar to that of the Asn_{344} in the KGFR mutation described here. The effect of these mutations on ligand binding or dimerization of the receptor may provide a clue to the cause of these disorders.

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