Glycosylation of the Basic Fibroblast Growth Factor Receptor

THE CONTRIBUTION OF CARBOHYDRATE TO RECEPTOR FUNCTION

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We have examined the glycosylation of the basic fibroblast growth factor (bFGF) receptor to determine whether carbohydrates contribute to receptor structure and function. Using a combination of cross-linking and radioreceptor assays, we demonstrated that the two bFGF receptors in baby hamster kidney cells have protein cores of 100 and 125 kDa. They are glycosylated to high mannose forms of 115 and 140 kDa and further processed to their mature forms of 130 and 150 kDa. Because peptide:N-glycosidase F, but not endo-α-N-acetylgalactosamidase can reduce the size of the bFGF receptors, the carbohydrate residues of the receptor appear all N-linked. The inability of deglycosylated receptors to bind 125I-bFGF supports the notion that the carbohydrate residues are required for receptor function. Furthermore, the capacity of the wheat germ agglutinin lectin to inhibit 125I-bFGF binding and the biological activity of bFGF suggests that N-acetylglucosamine residues are functionally significant components of the receptor.

Basic fibroblast growth factor (bFGF) is a mitogen for several cell types derived primarily from mesoderm and neuroectoderm (reviewed in Ref. 1). Initially isolated from bovine brain and pituitary (2), it was subsequently identified in extracts of kidney, placenta, corpus luteum, adrenal gland, retina, macrophages, and various tumors (reviewed in Ref. 3). Its amino acid sequence has been demonstrated by protein sequencing (4) and its mRNA has been cloned, (5, 6). The nucleotide sequence has indicated that while bFGF is probably synthesized as a 155-amino acid protein, unlike its oncogene homologue hst (7), it does not possess a classical signal sequence. In spite of this observation, bFGF interacts with membrane-bound receptors on its target cells and has been detected in the extracellular matrix produced by endothelial cells, where it appears to bind heparan sulfate-related proteoglycans with a strong affinity (8–10).

Although the bFGF receptor has not been purified and sequenced, there is already considerable information regarding its biochemical characterization. The recent discovery of hst (7), an FGF-related protein containing a signal sequence opens the possibility that, if bFGF is an intracellular protein, the bFGF receptor and hst receptor are the same. Regardless, recent studies have established that target cells possess two binding systems for bFGF (10, 11). The low affinity binding system ($K_d = 10^{-9}$ M) is heparinase-sensitive and probably represents bFGF binding to cell-associated heparan sulfate proteoglycans (10). A high affinity system ($K_d = 10^{-11}$ M) has also been identified and is thought to represent a specific membrane receptor(s). Cross-linking of 125I-bFGF or 125I-aFGF (acidic FGF) to their receptors on baby hamster kidney (BHK) fibroblasts has been performed using various bifunctional reagents and has identified the presence of two cross-linked proteins with apparent molecular weights ranging from 125,000 to 165,000 (11–13). Similar experiments performed on Swiss 3T3 fibroblasts (12), mouse skeletal muscle myoblasts (12), or bovine epithelial lens cells (13) have identified a single receptor with an apparent molecular weight of 165,000 (3T3 and myoblasts) or 130,000 (epithelial lens cells). Differences in molecular weights have been attributed to a combination of proteolytic breakdown during the processing of tissues, interlaboratory variation, and cell specific differences.

Several well-characterized growth factor receptors, including EGF receptor (14–16), PDGF receptor (17), and insulin receptor (18), contain N-linked and/or O-linked glycosidic side chains. It is therefore possible that changes in carbohydrate residues may also contribute to differences in the bFGF receptor. Although the function of the glycosidic chains in many receptors is still unclear, it is well established that the addition of the appropriate lectins can prevent ligand binding to their respective receptors (19, 20). In this report, we investigated the possible glycosylation of the bFGF receptor(s) using a selection of lectins, glycoprotein synthesis inhibitors, and glycosidic enzymes. The results establish that 25–30 kDa of the bFGF receptors on BHK cells can be attributed to N-linked carbohydrates and that this exclusive N-linked glycosylation of the bFGF receptors appears to be essential for bFGF binding.

EXPERIMENTAL PROCEDURES

Materials. 125I-Na and [3H]thymidine were from ICN (Irvine, CA). PDGF purified from human platelets (21) was a generous gift from Dr. C-H. Heldin (Uppsala, Sweden). Recombinant human bFGF was kindly supplied by Chiron Corp. (Emeryville, CA) and was radioiodinated as previously described (22). The specific activity of 125I-bFGF was estimated at 100,000 cpm/ng and was stable upon storage at 4°C. Lectins were purchased from Pharmacia (Uppsala, Sweden), except ulex europeus agglutinin (UeA), peanut agglutinin (PNA), and

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Glycosylation of the bFGF Receptor

Phytohemagglutinin (PHA), which were obtained from IBF Biotechnics (Villeneuve-la-Garenne, France). Tunicamycin, monensin, and swainsonine were from Calbiochem (La Jolla, CA); glucosamine and other sugars were from Sigma. Endoglycosidase F/peptide:N-glycosidase F was from Calbiochem. Purified peptide:N-glycosidase F (glycosidase F) was from Genzyme Co. (Boston, MA). Neuraminidase was from ICN (Irvine, CA). The cross-linking agent disuccinimidyl suberate was purchased from Pierce Chemical Co.

Basic FGF Radioiodoreceptor Assay—The effect of lectins on the binding of radiolabeled bFGF to its receptor(s) was examined in BHK cells because of their large receptor number (10,11), estimated in our laboratory as 40,000 sites/cell.2 Cells were plated in 24-well plates and grown to subconfluence in F-12/Dulbecco's modified Eagle's medium (1:1), supplemented with 5% calf serum. The cells were washed with cold Dulbecco's modified Eagle's medium, (pH 7.5), containing 25 mM Hepes and 0.15% gelatin prior to the binding assay. Cells were incubated for 2 h at 4 or 37 °C as indicated in 0.3 ml of this buffer containing 1–2 × 106 cpm of 125I-bFGF and the indicated concentrations of lectins. At the end of the binding period, cells were washed once with PBS and twice with Hepes 20 mM, pH 7.5, containing 2 mM NaCl. These salt washes were designed to remove glycosaminoglycans that were not covalently bound to bFGF (10). The receptor-bound 125I-bFGF was then collected after solubilization of the cells in 0.5% Triton X-100 in sodium phosphate 0.1 M buffer, pH 8.1. The radioactivity present in the Triton extracts was counted in a γ-spectrometer.

Bioreassays—Mitogenic activity of bFGF was assessed in the presence of various lectins on ABAE (adult bovine aortic endothelial) cells and ACE (adrenal capillary endothelial) cells, as previously described (23). Both bFGF (2 ng/ml) and lectins were added, when indicated, to cells on day 1 and cell number was determined using a Coulter counter on day 4.

Thymidine incorporation into quiescent 3T3 fibroblasts was used to determine the effect of lectins on bFGF and PDGF mitogenic activities. The assay was performed as described previously (23). The growth factors (2 ng/ml bFGF or 10 ng/ml PDGF) were added simultaneously with the lectins to serum-starved fibroblasts and [3H]thymidine (0.5 μCi/ml) was added 20 h later for 5 h. The radioactivity incorporated into trichloroacetic acid-precipitable, NaOH-solubilized macromolecules was determined on a Beckman scintillation spectrometer.

Cross-linking of 125I-bFGF to Its Receptors(s)—The cross-linking was performed on intact BHK cells or on a crude plasma membrane preparation. Plasma membranes were prepared from 20 large plates (15-cm diameter) of subconfluent BHK cells. After washing with PBS, cells were scraped off the plates in 5 ml of Hepes 20 mM, pH 7.4, buffer containing 1.5 mg MgCl2, 1 mg EGTA, 1 mg phenylmethylsulfonfl fluoride, and 1 μg/ml leupeptin. After homogenization in a Dounce apparatus and sonication, the cell homogenate was centrifuged at 100,000 g for 30 min at 1500 °C. The supernatant was then spun 30 min at 25,000 × g; the pellet was then resuspended in 0.5 ml of Hepes 20 mM, pH 7.4, containing 1 mg phenylmethylsulfon fluoride and 1 μg/ml of leupeptin.

bFGF binding was performed in two ways prior to cross-linking with disuccinimidyl suberate. On whole cells, 125I-bFGF was bound to its receptor(s) during a 2-h incubation of BHK cells with 125I-bFGF (2 × 106 cpm/10-cm Petri dish) in the standard binding buffer described above. Alternatively, bFGF binding was performed on membranes in 250 μl of binding buffer containing 50 μl of the crude plasma membrane extract and 2 × 106 cpm 125I-bFGF. In this latter case, all washes were performed by centrifugation in an Eppendorf microcentrifuge and by resuspension of the membrane pellet. At the end of the binding period, cells or membrane pellets were washed twice with PBS, once with 2 mM NaCl in Hepes 20 mM, pH 7.4, and subsequently incubated 15 min at room temperature in PBS containing 0.25 mg disuccinimidyl suberate. The reaction was stopped by addition of 20 mM ethanolamine, pH 7.5. Cells were then scraped and homogenized in Hepes 20 mM, pH 7.5, buffer containing 1 mg phenylmethylsulfonyl fluoride and 1 μg/ml leupeptin. After centrifugation in an Eppendorf microcentrifuge, the pellet was extracted for 30 min on ice in 50 μl of the same buffer containing 1% Triton X-100. Aliquots of the supernatant were boiled, loaded on a 6% polyacrylamide, 0.1% SDS gel (24), and radioactive proteins were separated by electrophoresis and visualized by autoradiography.

Enzymatic Treatment of Cross-linked Receptor(s)—In order to visualize the bFGF receptor and remove the possibility that enzymatic treatment itself modifies bFGF binding, the receptor was labeled by cross-linking with 125I-bFGF prior to treatment with enzymes. After cross-linking of 125I-bFGF to the plasma membrane preparations as described above, the suspension was centrifuged and the pellets resuspended in the appropriate enzyme buffers. The removal of all N-linked oligosaccharide chains was carried out with peptide:N-glycosidase F after denaturation by boiling in 0.5% SDS and 80 mM dithiothreitol. Samples were then adjusted in 100 mM sodium phosphate, pH 8.6, 0.33% SDS, 2% Nonidet P-40 and incubated with 50 units/ml peptide:N-glycosidase F overnight at room temperature. Control samples did not receive the enzyme. Desialylation of the receptor was performed by boiling the labeled membranes for 2 min in 0.5% SDS containing 80 mM dithiothreitol and then incubating them for 2 h at room temperature with 20 mM Tris-maleate, pH 6.0, 53.3 mM dithiothreitol, 0.33% SDS and 1.5 units/ml neuraminidase (final concentrations). The removal of O-linked oligosaccharide chains by endo-α-O-acetylgalactosaminidase was carried out on desialylated membranes by adjusting the neuraminidase digest to 0.4% SDS, 20 mM dithiothreitol, 1% Nonidet P-40, 1 mM phenylmethylsulfonyl fluoride and adding 0.1 unit/ml endo-α-O-acetylgalactosaminidase (final concentrations). The reaction was allowed to proceed overnight at room temperature. In all instances, the electrophoretic separation of cross-linked, enzymatically treated receptors was carried out on 6% polyacrylamide, 0.1% SDS gels (24).

Molecular weight standards (Bethesda Research Laboratories, Gaithersburg, MD) included myosin (200 kDa), phosphorylase b (100 kDa), bovine serum albumin (68 kDa), and ovalbumin (45 kDa).

RESULTS

Lectins Modify bFGF Binding to its Receptor and Internalization—Several lectins with different carbohydrate-binding specificities were initially tested for their ability to modify 125I-bFGF binding. BHK cells were used for the radioreceptor assay since these have been reported to have an elevated number of high affinity receptors (≈105 binding sites/cell) (10,11). When the binding was performed at 4 °C (Fig. 1A), WGA, a lectin that binds N-acetylgalactosamine residues, was an effective inhibitor. A concentration of 10 μg/ml WGA inhibited 50% of the binding of 125I-bFGF and the inhibition was >90% with 100 μg/ml of WGA. PHA, a lectin with broader specificity, was also very effective, albeit less than WGA. Concanavalin A (ConA), which binds mannose and glucose residues) had a partial inhibitory effect, showing a 40% inhibition of 125I-bFGF binding at the highest concentration tested, 100 μg/ml. In contrast, lentil lectin (LL), a lectin that has similar specificity to ConA, had no effect on bFGF binding. Several lectins with other specificities had no significant effect on bFGF binding. As such, galactose binding by peanut lectin, fucose binding by ulex europeus lectin, N-acetylgalactosamine binding by soybean lectin or helix pomatia lectin had no effect on the ability of 125I-bFGF to bind its receptor. Similar results were observed when the radioreceptor assay was performed at 37 °C (Fig. 1B). At this temperature, bFGF is rapidly internalized after binding, and the cell-associated radioactivity represents both receptor-bound and internalized 125I-bFGF. Therefore, after 2 h at 37 °C, the cell-associated radioactivity is about four times higher than the same time at 4 °C. Within that period of time, no significant release of degraded 125I-bFGF could be observed. WGA was the most potent inhibitor of 125I-bFGF uptake (65% inhibition at 10 μg/ml and 95% inhibition at 100 μg/ml) although both PHA and ConA were potent inhibitors. LL, which was inactive at 4 °C, partly inhibited (20% inhibition) bFGF uptake at 37 °C. The WGA-mediated inhibition of 125I-bFGF uptake could be neutralized by N-acetylgalcosamine (Fig. 2) but was not affected by other sugars. This result is in agreement with the known specificity of this lectin and supports the notion that the bFGF receptor(s) contains carbohydrates, probably N-acetylgalcosamine residues, in the vicinity of the bFGF-binding region.
Glycosylation of the bFGF Receptor

Lectins Modulate bFGF-stimulated Proliferation of Endothelial Cells—The same lectins as those used in the radioreceptor assay were also tested for their capacity to inhibit both basal and bFGF-induced proliferation of endothelial cells. Both ABAE and ACE endothelial cells were examined in the presence of various concentrations of lectins. The results obtained with ABAE cells are shown in Fig. 3. Several lectins (LL, helix pomatia agglutinin (HPL), PNA, UeA and soybean agglutinin (SBA) had no effect on basal growth and only slightly reduced bFGF-stimulated cell proliferation. In contrast, WGA and PHA were potent inhibitors of both basal and bFGF-stimulated proliferation. A 50% inhibition of biological activity was obtained with 8 μg/ml WGA and 3 μg/ml PHA, respectively. It is not clear why ConA, which was partially inhibitory in the bFGF radioreceptor assay, stimulated basal growth of ABAE cells. Cell number was increased 2.5 times as compared to control when 30 μg/ml ConA were present during the 4-day incubation. Although this increase is low, it was reproducible in four different assays and was also observed with ACE cells. Little to no effect was seen in the presence of bFGF.

A more limited number of lectins was used in the ACE cell proliferation assay (Fig. 4). WGA was able to inhibit both basal and bFGF-stimulated ACE cell proliferation, whereas WGA induced inhibition of bFGF binding was calculated for each sugar, respectively.

**Fig. 1.** Effect of various lectins on the binding of 125I-bFGF to its receptor(s) and internalization in BHK cells. Concanavalin A (ConA), wheat germ agglutinin (WGA), soybean agglutinin (SBA), helix pomatia lectin (HPL), ulex europeus agglutinin (UeA), peanut agglutinin (PNA), lentil lectin (LL), and phytohemagglutinin (PHA) were added to BHK cells at the indicated concentrations and tested for their ability to compete with 1 ng of radiolabeled FGF in the binding to BHK cell bFGF receptor(s). The assay was performed at 4 °C (A) or at 37 °C (B) as described under “Experimental Procedures.”

**Fig. 2.** Suppression by monosaccharides of the WGA-induced inhibition of 125I-bFGF binding to BHK cells. Binding of 125I-bFGF to BHK cells was performed for 2 h at 4 °C as described under “Experimental Procedures,” in the absence or presence of 100 μg/ml wheat germ agglutinin (WGA) and 100 mM of the different monosaccharides. WGA-induced inhibition of bFGF binding was calculated for each sugar, respectively.

**Fig. 3.** Effect of various lectins on proliferation of ABAE cells. Wheat germ agglutinin (WGA), lentil lectin (LL), helix pomatia lectin (HPL), peanut agglutinin (PNA), phytohemagglutinin (PHA), ulex europeus agglutinin (UeA), soybean agglutinin (SBA), and concanavalin A (ConA) were added to ABAE cells in the absence or presence of 2 ng/ml bFGF. Cell number was determined 4 days later as described under “Experimental Procedures.”
Pomatia lectin binding assay. Thus, it would appear that the binding of WGA partly inhibited FGF-stimulated growth. It is interesting to note that in ACE cells, as observed previously in ABAE cells, ConA is the only lectin to behave differently in proliferation and in radioreceptor assays. The other lectins all had the same relative potencies in the proliferation assay and in the binding assay. Thus, it would appear that the binding of WGA to N-acetylglucosamine present on or located near the bFGF receptor is able to prevent both the binding of bFGF, and as such, its subsequent biological effects.

Lectins Inhibit [3H]Thymidine Incorporation into 3T3 Fibroblasts—WGA, which inhibits bFGF binding to its receptor, has also been reported to bind to the PDGF receptor (20). We thus examined the effect of different lectins on basal, bFGF-induced, and PDGF-induced mitogenesis of 3T3 fibroblasts (Fig. 5). WGA was a very potent inhibitor of thymidine incorporation into 3T3 cells. Wheat germ agglutinin (WGA), lentil lectin (LL), helix pomatia lectin (HPL), and concanavalin A (ConA) were added at a concentration of 1 µg/ml to quiescent 3T3 fibroblasts alone or in combination with 2 ng/ml bFGF or 10 ng/ml PDGF. [3H]Thymidine incorporation was determined subsequently as described under "Experimental Procedures." Results were expressed as % ± S.D. of the respective control values (without lectins). Control incorporations were 1542 ± 105 in the absence of growth factors, 14,929 ± 493 cpm in the presence of bFGF and 26,525 ± 1395 cpm in the presence of PDGF.

Endoglycosidase F/Peptide:N-glycosidase F Treatment of the FGF Receptor Inhibits bFGF Binding—Because the previous experiments suggested that the bFGF receptor contains glycosidic residues that may be important in bFGF binding, we determined the relative contribution of these carbohydrates to the binding capacity of the receptor. BHK cells were incubated with a mixture of endoglycosidase F and peptide N-glycosidase F, two enzymes which have been shown to cleave the high mannose and complex-carbohydrate chains found in glycoproteins from various cell types (25). BHK cells were incubated overnight with these enzymes in culture medium at 37 °C and the cross-linking of [125I]-bFGF to its receptor was subsequently performed as described. The cross-linked proteins were analyzed by SDS-PAGE and autoradiography (Fig. 6). Two bands of M, 170,000 and 145,000 were detected in the extracts of control cells that were incubated overnight, either at pH 7.5 or 6.5 (the optimum pH for the glycosidases). In contrast, these bands were barely detectable in cells treated overnight with the glycosidases. This effect could not be reversed by coinubation of the enzyme with an excess of protein (bovine serum albumin), thus indicating that the inability to cross-link was not a reflection of any contaminating protease activity. In contrast, when the enzymatic treatment was performed in the presence of an excess of a potential substrate such as ovalbumin, the signal was partly restored. Because the cross-linking data were obtained from cells after enzyme treatment, the results suggest that the glycolytic treatment of BHK cells suppresses its ability to bind bFGF. This effect, which is partly reversed by addition of an excess of glycoprotein, but unaffected by an excess of protein, is probably the reflection of the deglycosylation of the bFGF receptor. One cannot exclude, however, the possibility that deglycosylation of the bFGF receptor leads to its faster degradation or internalization.

Glycoprotein Synthesis Inhibitors Modify the bFGF Binding Capacity of BHK Cells—The role of the carbohydrate moieties was further investigated by examining the effect of glycopro-
Glycosylation of the bFGF Receptor

**Fig. 6.** Inhibition of bFGF cross-linking to its receptor(s) after treatment of BHK cells with endoglycosidase F/peptide:N-glycosidase F. BHK cells were incubated overnight at 37 °C in Dulbecco’s modified Eagle’s medium adjusted to pH 6.5 containing no protein (lane 1) or 6 units of endoglycosidase F/peptide:N-glycosidase F (lane 2) or 6 units of enzyme and 10 ng/ml BSA (lane 3) or 6 units of enzyme and 10 mg/ml chick ovalbumin (lane 4). The position of the M₃ standards is indicated in the left side lane (K₄ × 10⁻³).

**Fig. 7.** Effect of glycoprotein synthesis inhibitors on cross-linking of bFGF to its receptor(s) in BHK cells. BHK cells were incubated overnight at 37 °C in the absence (lane 1) or presence of 1 μg/ml swainsonine (lane 2) or 2 μg/ml tunicamycin (lane 3). Cross-linking of ¹²⁵I-bFGF to these cells was then performed as described under “Experimental Procedures.” The figure represents the autoradiogram of the cross-linked proteins separated by 6% SDS-PAGE. The position of the M₃ standards is indicated in the side lanes (K₄ × 10⁻³).

**Fig. 8.** Effect of various glycosidases on the size of the cross-linked bFGF receptor(s) in BHK cell membranes. After binding of ¹²⁵I-bFGF to BHK cell membranes, the radiolabeled growth factor was cross-linked to its receptor as described under “Experimental Procedures.” Membranes were then treated without (Ctrl) or with various glycosidases as described under “Experimental Procedures”: peptide:N-glycosidase (N-Gly); neuraminidase (Neu); neuraminidase and endo-α-N-acetylgalactosaminidase (Neu + O-gly). The figure represents the autoradiogram of the enzymatically digested cross-linked proteins separated by 6% SDS-PAGE. The right lane (SW) represents the cross-linked proteins from untreated membranes prepared from swainsonine-treated BHK cells. The position of the M₃ standards is indicated in the side lanes (K₄ × 10⁻³).

protein synthesis inhibitors on the biosynthesis of functional bFGF receptors. BHK cells were incubated overnight at 37 °C in the presence of various inhibitors of glycoprotein synthesis and ¹²⁵I-bFGF was subsequently cross-linked to its receptor(s) (Fig. 7). As expected in untreated BHK cells, two ¹²⁵I cross-linked-binding complexes with M₃ of 155 to 170 kDa and 130 to 145 kDa were detected. In extracts from tunicamycin-treated cells, the same bands were barely detectable, suggesting that carbohydrate chains that are added after mannosidase action do not modify the intracellular transport or binding capacity of the bFGF receptor. As in the case with EGF (14, 16, 26), antisera to the receptor will address this question.

The effects of swainsonine, an inhibitor of Golgi mannose-dase II (27), were tested in an effort to establish the relative importance of mannose processing in receptor function (Fig. 7). Swainsonine treatment resulted in a decreased size of the cross-linked proteins, but did not reduce the intensity of the signal, suggesting that carbohydrate chains that are added after mannosidase action do not modify the intracellular transport or binding capacity of the bFGF receptor. The estimated size of the receptor was decreased by 15-20 kDa by virtue of the failure to completely process the receptor. It is interesting to note that although swainsonine treatment inhibited carbohydrate processing, it did not modify the capacity of bFGF to bind and cross-link to the receptor.

Estimation of the Core Protein Size of the bFGF Receptor—
The presence of O-linked and/or N-linked chains was investigated by the use of specific enzymes. The results shown in Fig. 8 indicate that the size of the receptor(s) was not modified by neuraminidase and endo-α-N-acetylgalactosaminidase treatment whereas it was reduced by 25-30 kDa by removal of the N-linked sugar chains with the enzyme peptide:N-glycosidase F. Thus, the bFGF receptor(s) possesses only N-linked and no O-linked glycosidic chains. Furthermore, both bFGF receptor complexes are reduced in molecular weight by the same amount, supporting the hypothesis that they may be structurally as well as functionally related. The results presented in lane 5 of Fig. 8 show the bFGF-bFGF receptor complex cross-linked in the membranes of swainsone-treated cells. As expected from the previous study on intact cells (Fig. 7), the estimated molecular masses (140 and 115 kDa) are intermediate between those observed in control and peptide:N-glycosidase F-treated membranes, suggesting a contribution of 15-kDa high mannose carbohydrate chains.

**DISCUSSION**

The studies outlined here describe several novel structural and functional characteristics of the bFGF receptor(s). Because the purification of the bFGF receptor(s) and molecular cloning of its gene are still in progress, it is still impossible to study the biosynthesis and post-translational modifications of this receptor in a way similar to the EGF receptor (14-16), PDGF receptor (17), or insulin receptor (18). Just as two basic kinds of techniques have allowed investigators to approach the structural and functional characteristics of the EGF, PDGF, and insulin receptors, similar methods have been used for the bFGF receptor. Radioreceptor assays on various target cells (10, 11) have demonstrated that bFGF receptor for its ligand (10-14 kDa) is usually 1-2 orders of magnitude higher than that of most growth factor receptors for their respective ligands (e.g. EGF receptor, PDGF receptor, and insulin receptor). Cross-linking studies using 125I-bFGFs revealed that bFGF-responsive cells possess either two receptors of apparent Mr 125 and 145 kDa (BHK cells) or only one receptor of apparent Mr 130-165 kDa depending on the cell types, 3T3 fibroblasts (12), myoblasts (12), or epithelial lens cells (13) and laboratory. It was proposed that the larger species represents the preferential bFGF receptor and the smaller species represents the preferential acidic FGF receptor (28). On the experiments described here, radiolabeled bFGF was cross-linked to two molecular weight species of receptor.

Using a combination of cross-linking and the radioreceptor assay, we have investigated the possibility that the bFGF receptor(s) could be a glycoprotein. We established that WGA, a lectin that specifically recognizes N-acetylglucosamine residues, was the most potent inhibitor of bFGF binding at 4°C. Because the effects of WGA could be reversed by N-acetylgalactosamine, the inhibitory effects can be attributed to the lectin rather than to any possible contaminant. Moreover, because bFGF does not bind to WGA, it could be concluded that bFGF receptors probably possess some N-acetylgalactosamine-containing carbohydrate chains in the vicinity of its binding domain. It would share this property with the PDGF receptor (20), but not the EGF receptor which is better recognized by Con A (19).

BHK cells were treated with endoglycosidase F/peptide:N-glycosidase F, a mixture of glycosidases able to release any N-linked oligosaccharide from the core protein of glycoproteins (25) in order to determine the role of these glycosidic chains in mediating the biological response. Under these conditions, the attempts to cross-link 125I-bFGF to its receptor(s) were unsuccessful, thus indicating that the glycosidic chains of the receptor are necessary for bFGF binding. Because this effect could be antagonized by an excess of glycoprotein (ovalbumin) but not by an excess of unglycosylated protein (albumin), the absence of cross-linking bFGF was the reflection of a glycosidase activity and not of any possible contaminating proteolytic activity. Thus, it appears that the carbohydrate chains of the bFGF receptor are an important component of the binding domain of the receptor. Lane and co-workers (16, 26) have performed similar studies with EGF and have recently suggested that while glycosylation is a necessary intracellular step for the acquisition of EGF binding ability of A431 cells, carbohydrates are not responsible for the binding site per se. The treatment of the active high mannose form of the EGF receptor with endo-α-N-acetylgalactosaminidase H generated a fully active unglycosylated receptor (26). Further studies with anti-bFGF receptor antibodies will be required for detailed analysis of the mechanism that mediates the acquisition of the bFGF-binding activity. The results presented here suggest that the fully unglycosylated receptor is not able to bind bFGF.

It has been established in many cell models that inhibition of glycosylation with tunicamycin results in intracellular trapping, probably in the endoplasmic reticulum, of the unglycosylated proteins (27). However, it is not a general feature of membrane receptors. As an example, the cell surface expression of the human fibroblast low density lipoprotein receptor is reduced in the presence of tunicamycin (29), but the receptors still retain low density lipoprotein-binding activity (30). In contrast, the human asialoglycoprotein receptor is expressed in the presence of tunicamycin as a nonglycosylated form which is unaffected in intracellular translocation, turnover, or function (31). In the case of bFGF receptor(s), we looked at the effect of glycosylation inhibitors on the biosynthesis of bFGF receptor(s). Tunicamycin treatment resulted in an almost complete depletion of functional receptors from the cell surface, an effect that is reminiscent of the effect of tunicamycin on EGF receptor biosynthesis in A431 cells (14, 26) or Chinese hamster fibroblasts (32). Thus, it appears that tunicamycin suppresses the presence of bioactive receptors at the cell surface. The lack of availability of anti-bFGF receptor antibodies does not allow us to discriminate at the moment between the absence of receptors and the presence of non-functional receptors at the surface of tunicamycin-treated cells.

Swainsone, an inhibitor of Golgi mannosidase II, did not modify the intensity of cross-linking of 125I-bFGF receptors to the surface of BHK cells, it reduced the apparent size of these receptors. This suggests that the modifications of the carbohydrate chains that take place in the Golgi apparatus are not essential for the intracellular transport and the function of active receptors at the cell surface. It also indicates that the high mannose form of the receptor is bioactive. By virtue of the changes in molecular weights, the overall size of the carbohydrates added in the Golgi apparatus appears to be 15-20 kDa.

Endo-α-N-acetylgalactosaminidase, an enzyme that is able to remove O-linked sugars from desialylated glycoproteins, was unable to modify the size of the cross-linked bFGF receptor(s). When treated by peptide:N-glycosidase F, an enzyme that removes N-linked oligosaccharide chains at the asparagine and converts that residue into aspartic acid, a 25-30 kDa downshift in the size of the receptor(s) was observed. Thus, the bFGF receptor(s) possesses no O-linked oligosaccharide chain and the size of its N-linked chains is around 25-30 kDa. This is similar to the EGF receptor (15) whereas
the PDGF receptor (17) and low density lipoprotein-receptor (30) contain both O-linked and N-linked chains. It is thus possible to propose a schematic representation of the synthesis of the bFGF receptor in BHK cells whereby core proteins of 100 and 125 kDa are glycosylated to high mannose molecules of 115 and 140 kDa which are further processed to the mature forms of 130 and 155 kDa.

The combined studies reported here clearly establish that the bFGF receptor(s) is a glycoprotein. They allow to predict of the PDGF receptor (17) and low density lipoprotein-receptor (30) contain both O-linked and N-linked chains. It is thus possible to propose a schematic representation of the synthesis of the bFGF receptor in BHK cells whereby core proteins of 100 and 125 kDa are glycosylated to high mannose molecules of 115 and 140 kDa which are further processed to the mature forms of 130 and 155 kDa.

Certainly the availability of an anti-bFGF receptor antibody should permit more insight into the intracellular steps of bFGF receptor biosynthesis.

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