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Acidic and Basic Fibroblast Growth Factor Bind With Differing Affinity to the Same Heparan Sulfate Proteoglycan on BALB/c 3T3 Cells: Implications for Potentiation of Growth Factor Action by Heparin

Kathryn J. Brown, Ian A. Hendry, and Christopher R. Parish

Divisions of Cell Biology (K.J.B., C.R.P.) and Neuroscience (I.A.H.), John Curtin School of Medical Research, Australian National University, Canberra ACT 2601, Australia

Abstract Heparan sulfate proteoglycans on the cell surface act as low affinity binding sites for acidic and basic fibroblast growth factor (FGF) [Moscatelli (1987): J Cell Physiol 131:123–130] and play an important role in the interaction of FGF with the FGF receptor (FGFR). In this study, several aspects of the interaction of FGFs with cell surface heparan sulfate proteoglycans were examined. Reciprocal cross blocking studies demonstrated that acidic FGF (aFGF) and basic FGF (bFGF) bind to identical or closely associated heparan sulfate motifs on BALB/c 3T3 cell surface heparan sulfate proteoglycans. However, the binding affinity of the two growth factors for these heparan sulfate proteoglycans differs considerably, competition binding data indicating that aFGF has a 4.7-fold lower affinity than bFGF for 3T3 heparan sulfate proteoglycan. Subsequent studies of dissociation kinetics demonstrated that bFGF dissociates from the FGFR at least 10-fold slower than aFGF, whereas, following removal of cell surface heparan sulfate proteoglycans by heparinase treatment, the dissociation rate of both FGFs is similar and rapid. These results support the concept that cell surface heparan sulfate proteoglycans stabilize the interaction of FGF with FGFR, possibly by the formation of a ternary complex. © 1995 Wiley-Liss, Inc.

Key words: heparan sulfate proteoglycans, fibroblast growth factor receptor (FGFR), dissociation kinetics, ternary complex

Acidic and basic fibroblast growth factor (FGF) bind to two classes of binding sites on the cell surface, the FGF receptor (FGFR), which is regarded as a "high affinity" binding site on the basis of low dissociation constant (Kd) values (Kd = 10^{-9}–10^{-12}M) [Neufeld and Gospodarowicz, 1985; Moenner et al., 1986; Olwin and Hauschka, 1986], and to heparan sulfate proteoglycans (HSPGs), regarded as "low affinity" binding sites on the basis of relatively high Kd values (Kd = 10^{-8}–10^{-9}M) [Moscatelli, 1987]. HSPGs on the cell surface generally outnumber FGFRs by 1–3 orders of magnitude [Moscatelli, 1987; Burgess and Maciag, 1989]. Furthermore, it is generally accepted that the presence of either HSPG or heparin is required for the binding of FGFs to the "high affinity" receptor. This study aimed to clarify several aspects of the interaction of FGFs with HSPGs.

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Address reprint requests to Kathryn Brown, Division of Cell Biology, The John Curtin School of Medical Research, Australian National University, Canberra, ACT 2601, Australia.

Initially, we examined whether both acidic and basic FGF recognize similar or different motifs within the heparan sulfate (HS) chains of cell surface HSPGs and determined their relative binding affinities for these HS motifs.

We also examined the importance of HSPG in stabilizing the interaction of FGFs with their "high affinity" receptor. Two models have been proposed to explain how HSPGs promote the binding of FGFs to their receptors. In one model it is proposed that HSPGs induce a conformational change in FGF which enables the growth factor to bind with high affinity to its receptor [Yayon et al., 1991]. In a second model, Nugent and Edelman [1992] have proposed that formation of a "ternary complex" is required which does not involve a conformational change in the molecule, but requires the simultaneous binding of FGF to FGFR and HSPG for stable binding to occur. We have undertaken a comparative study, using both acidic FGF (aFGF) and basic FGF (basic FGF), to determine the relative importance of HSPG in stabilizing the FGF/FGFR

complex. In particular, the role of the "ternary complex" model of FGF binding was examined for both growth factors.

Finally, based on these observations an attempt has been made to explain why heparin potentiates aFGF action but has little or no potentiating effect on bFGF function. Of particular interest was whether differences in the affinity of the two FGFs for cell surface HSPGs can explain why acidic but not basic FGF is potentiated by heparin.

MATERIALS AND METHODS

Growth Factors

Human recombinant acidic and basic FGF were obtained from Bio Source International (Camarillo, CA) and Pepro Tech Inc. (Rocky Hill, NJ). Acidic and basic FGFs were reconstituted at 200 μg/ml in phosphate buffered saline (PBS)/0.1% (3-[(3-Cholamidopropyl)-dimethylammonio]-1-propanesulfonate (Sigma Chemical Co., St. Louis, MO) to prevent adherence to the walls of the tubes and aliquots stored in polyethylene tubes (Kartell, Milan, Italy) at −70°C. Aliquots were not frozen more than once and were not used any longer than 2 weeks after thawing. ^{125}I-aFGF (1,234 Ci/mmol) and ^{125}I-basic FGF (920 Ci/mmol) were obtained from Amersham International plc (Amersham, UK). ^{125}I-aFGF was resuspended in distilled water to a final concentration of 600 ng/ml and aliquots frozen. ^{125}I-bFGF was resuspended in distilled water to give a final concentration of 200 ng/ml and aliquots frozen.

Polysaccharides

Heparin (bovine lung), HS (bovine kidney), HS-fast moving fraction (bovine intestinal mucosa), HS (bovine intestinal mucosa), hyaluronic acid (human umbilical cord), chondroitin-4-sulfate (whale cartilage), chondroitin-6-sulfate (whale cartilage), dermatan sulfate (porcine skin), and keratan sulfate (bovine cornea) were all obtained from Sigma. Chondroitin-4,6-disulfate (whale cartilage; Sigma) was prepared in this laboratory. Heparan sulfate (porcine mucosal, 32 kDa) was a generous gift from Organon International bv (Oss, Netherlands). Based on SDS-PAGE these glycosaminoglycans (GAGs) gave single, toluidine blue staining, bands with negligible protein contaminants.

Cell Culturing

Mouse BALB/c 3T3 cells were maintained in Dulbecco's modified Eagle's medium (DMEM; Gibco, Gaithersburg, MD) supplemented with $NaHCO_3$, 1% L-glutamine, 20 mM HEPES, pH 7.4, and 10% fetal calf serum (FCS; Commonwealth Serum Laboratories, Melbourne, Australia) at 37°C (5% CO_2 incubator) in 80 cm^2 tissue culture flasks (Nunc, Roskilde, Denmark). Cell monolayers were released for subculturing with 0.1% trypsin (Cytosystems, Sydney, Australia) and 0.1% ethylene diamine tetracetic acid in PBS when cells were subconfluent (every 3 days) and cells were then resuspended at 1.5×10^4 cells/ml in culture medium. Confluent monolayers used in mitogenic and binding assays were prepared by seeding 3×10^3 cells/well in 96-well plates (Nunc) in the same medium as used for subculturing, and incubating at 37°C for 4 to 5 days. For mitogenic assays, confluent monolayers were serum starved (DMEM/1% L-glutamine/20 mM HEPES pH 7.4) for 48 h.

Binding of Radiolabeled Acidic and Basic FGF to BALB/c 3T3 Cells

^{125}I-FGF binding was conducted with confluent BALB/c 3T3 cells. Prior to the initiation of the ^{125}I-FGF binding, the monolayers were washed once with 200 μl/well of ice cold binding buffer (DMEM/1% L-glutamine/0.1% BSA/20 mM HEPES, pH 7.4) and then incubated at 4°C for 10 min to precool the monolayers.

Dissociation rate constants for ^{125}I-aFGF and ^{125}I-bFGF were determined using the method of Nugent and Edelman [1992] with minor modifications, as follows. As described by Nugent and Edelman [1992], unlabeled FGF was included in the dissociation medium to ensure that released ^{125}I-FGF would not rebind to unoccupied receptors; however, a higher concentration of bFGF than aFGF was used because of bFGF's stronger tendency to reassociate with HSPG during the final washing process. When determining dissociation rate constants in the absence of cell surface HSPGs, cells were initially treated with 2–4 units/well of heparinase 1 (EC 4.2.2.7; Sigma) for 30–60 min. Such treatment generally resulted in the removal of 70–90% HSPGs as determined by comparing the number of cpm eluting from the cell surface of treated cells with the number of cpm eluted by the same buffer (2M NaCl in binding buffer) in 10 s from un-

treated cells. Heparinase treatment had no effect on cell viability.

To examine the effect of heparin on the binding of ^{125}I-FGFs to heparinase treated cells, serial dilutions of heparin in binding buffer were added to an equal volume of either ^{125}I-aFGF or ^{125}I-bFGF (final concentration 20 ng/ml) and incubated on ice with regular mixing for 30–60 min. Nonspecific binding of ^{125}I-aFGF and ^{125}I-bFGF in the presence and absence of heparin was determined by including an excess of unlabeled aFGF or bFGF (final concentration 50 μg/ml) in the incubation mixtures. Heparin-FGF mixtures were added to heparinase treated BALB/c 3T3 cells (100 μl/well), incubated for 4 h on ice, and, after washing once with ice cold medium, ^{125}I-aFGF or ^{125}I-bFGF bound to residual HSPG or FGFR released by exposing the cells to 2M NaCl and pH 4.0 solutions, respectively, as described by Nugent and Edelman [1992].

Rose Bengal Cell Adhesion Assay

A modified version of the cell-adhesion assay developed by Ishihara et al. [1992] was used to investigate the interaction of BALB/c 3T3 cell surface HSPG with FGFs immobilized on plastic. Ninety-six well round bottom polyvinylchloride (PVC) Microtiter plates (Dynatech Laboratories, Chantilly, VA) were coated with 50 μl/well of either aFGF or bFGF (at the concentrations indicated) overnight at 4°C. Wells were aspirated, the plate submerged twice in a PBS bath, and nonspecific binding sites blocked by incubation with Hanks Balanced Salt Solution 0.1% BSA, pH 7.0 (HBSS), at 37°C for 1 h. BALB/c 3T3 cells were suspended in HBSS at a cell density of 2.5×10^6 cells/ml, 0.1 ml applied to each coated well, and incubated at 37°C for 1 h. The plate was flicked to remove unbound cells and 100 μl/well of 0.25% Rose Bengal dye (Koch-Light Laboratories Ltd, Colnbrook Berks, England) in PBS added for 3 min at room temperature. Rose Bengal stains the nuclei and cytoplasm of both live and dead cells [O'Neill and Parish, 1983]. The unadsorbed dye was removed by flicking the plate and submerging it twice in two separate PBS baths. The plate was allowed to drain before adding 200 μl/well of 50% ethanol in PBS. Each well received a constant amount of mixing with a multichannel pipette to allow liberation of the dye from the cells. Nonspecific binding of the dye to FGF-coated and uncoated wells in the absence of cells was also determined and subtracted from experimental points. The relative number of cells in each well was quantified by determination of each well's optical density ($\lambda_1 = 540$ nm, $\lambda_2 = 650$ nm) using a Thermomax microplate reader (Molecular Devices, Menlo Park, CA).

To determine whether acidic and basic FGF adhere to the PVC plate to an equal extent, quadruplicate wells were coated with either 50 μl/well of ^{125}I-bFGF or ^{125}I-aFGF, both at 313 ng/ml, and left overnight at 4°C. Unbound FGF was removed, 100 μl/well of HBSS then added, and the plate incubated for 1 h at 37°C. Supernatants were collected and individual wells detached from the plate by a hot wire. The amount of labeled FGF in each wash and that which remained bound to the wells was determined by a gamma counter.

The ability of various GAGs (100 μg/ml) to inhibit the binding of cell-surface HSPGs to immobilized aFGF and bFGF was also tested. Fifty μl/well of GAG was incubated with FGF-coated wells for 1 h at 4°C before 50 μl of cells (5×10^6 cells/ml) were added to each well and incubated at 37°C for 1 h. To titrate the inhibitory activity of some GAGs or FGFs, doubling dilutions of each inhibitor were prepared in HBSS and 50 μl/well of each concentration aliquoted in triplicate into FGF coated wells and the same procedure followed as described above. Control wells received medium without inhibitor.

RESULTS

Binding of Acidic and Basic FGF to Cell Surface HSPGs

In order to examine the interaction of acidic and basic FGF with BALB/c 3T3 cell surface HSPGs, an assay based on that of Ishihara et al. [1992] was developed to determine whether acidic and basic FGF interact with the same HSPGs, and, if so, whether they differ in their affinity for these HSPGs. The assay utilized the ability of the FGFs to adhere to 96-well PVC plates and of cells to bind to the FGF-coated wells via their cell surface HSPGs. Binding of cells to plates coated with aFGF was found to occur most rapidly at 37°C, a little slower at room temperature, and not at all at 4°C (data not shown). This temperature effect is probably due to a rapid redistribution of ligands on the cell surface at 37°C which would allow more HSPGs to come into contact with the factor on the plate, and thereby result in multivalent bind-

ing. The optimum cell concentration for 3T3 cells binding to the FGF-coated plates was 2.5 × 10^5 cells/well (data not shown). The heparan sulfate dependence of the binding assay was validated by the demonstration that treatment of cells with heparinase prior to their addition to the plate, reduced cell binding to aFGF- and bFGF-coated plates by 73–94%. The specificity of the heparinase was demonstrated by the ability of heparin to partially inhibit the effect of the enzyme (data not shown).

The optimal FGF concentration to be used for coating the wells was also determined (Fig. 1). It was found that for optimum cell binding, an approximately 8-fold higher concentration of aFGF than bFGF was required for coating. This difference was not due to differences in the ability of acidic and basic FGF to adhere to plastic, as studies with ^{125}I-labeled FGFs showed that both growth factors bound equally well to the plates, i.e., approximately 15% of added radiolabeled FGF at 0.3 μg/ml. Acidic and basic FGF were generally used at concentrations of 2.5 μg/ml and 0.313 μg/ml, respectively, to coat plates in subsequent assays.

Subsequent studies demonstrated that binding of BALB/c 3T3 cells to the immobilized FGFs was heparin inhibitable, although of 11 GAGs tested, only heparin, fast moving HS, and bovine intestinal HS were inhibitory, indicating that they share with 3T3 cell surface HSPGs, the structure required for FGF binding. The eight noninhibitory GAGs were hyaluronic acid, chondroitin-4-sulfate, chondroitin-6-sulfate, chondroitin-4,6-disulfate, dermatan sulfate, keratan sulfate, bovine kidney HS, and porcine mucosal HS. The complete inhibition curves for heparin, fast moving HS, and bovine intestinal HS are depicted in Figure 2A for aFGF, and Figure 2B for bFGF. For both growth factors, heparin was the most potent inhibitor. Fast moving HS was, on a weight basis, 480-fold less effective at inhibiting aFGF and 600-fold less effective at inhibiting bFGF binding than hepa-

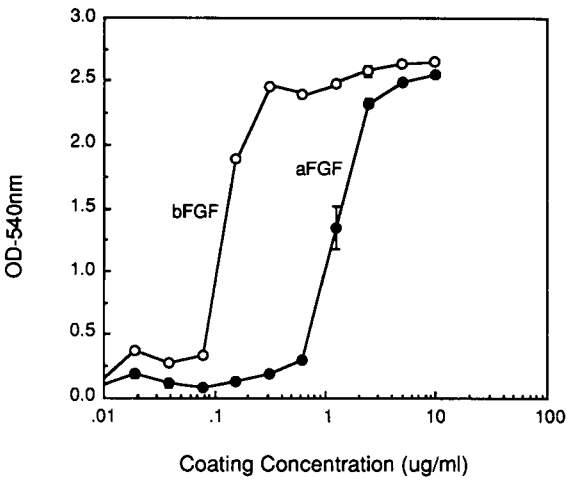

Fig. 1. Determination of optimum coating concentration of FGF required for the binding of BALB/c 3T3 cells (2.5 × 10^5 cells/well) to PVC plates. Wells of a plate were coated with a range of concentrations of either aFGF or bFGF in triplicate. Cell binding was performed for 60 min at 37°C and the number of cells bound/well quantified by Rose Bengal staining. Values represent means ± SEM (n = 3) of one representative experiment.

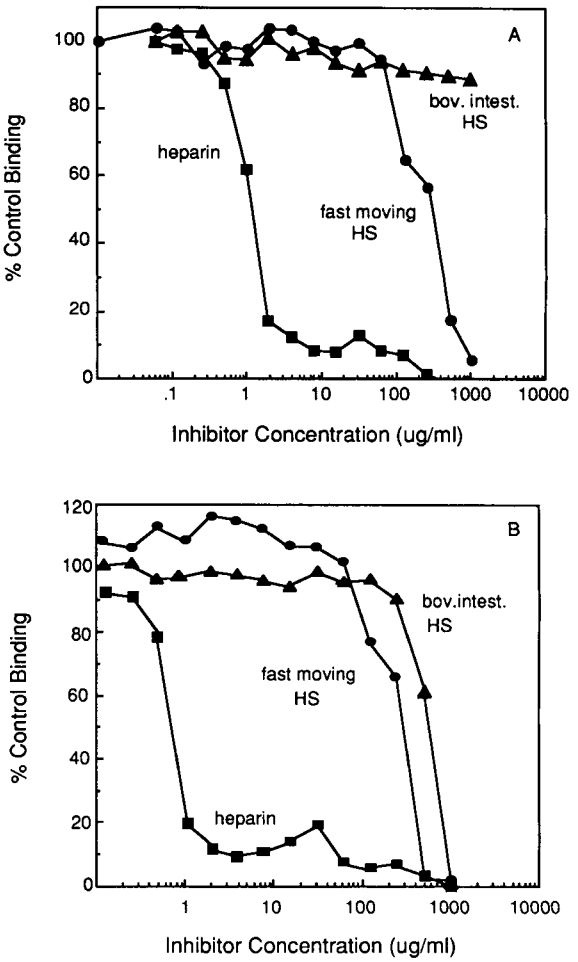

Fig. 2. Comparison of the ability of heparin (■), fast moving heparan sulfate (●), and bovine intestinal heparan sulfate (▲) to inhibit the binding of BALB/c 3T3 cells to PVC wells coated with (A) aFGF (2.5 μg/ml) and (B) bFGF (0.625 μg/ml). Heparin and heparan sulfates were incubated with plate-bound FGF for 1 h on ice before adding cells (2.5 × 10^5 cells/well) for 1 h at 37°C. 100% binding represents that occurring in the absence of inhibitor. Each value represents the mean of triplicate treatments. Standard errors of the means were <10%.

rin. Bovine intestinal HS, at high concentrations, also inhibited bFGF binding but this inhibition was not observed with aFGF. This difference suggests that there may be some subtle differences in the HS motifs recognized by acidic and basic FGF.

Figure 3A shows that both soluble aFGF and bFGF can totally block the binding of immobilized aFGF to HSPGs on BALB/c 3T3 cells. Similarly, both FGFs totally inhibited immobilized basic fibroblast growth factor from binding to 3T3 cells (Fig. 3B). Thus, acidic and basic FGF cross react with the same species of cell surface HSPG on 3T3 cells. However, in both assays aFGF was a less effective inhibitor, the difference being 2.5-fold with immobilized bFGF (Fig. 3B) and 7.8-fold with immobilized aFGF (Fig. 3A) in the experiments shown. Subsequent replicate inhibition assays gave similar results irrespective of the immobilized FGF used, the pooled data showing that aFGF was a 4.7 ± 1.0 (SEM, n = 4) -fold less effective competitor than basic fibroblast growth factor. This result suggests that bFGF has a higher affinity for 3T3 cell surface HSPGs than does aFGF.

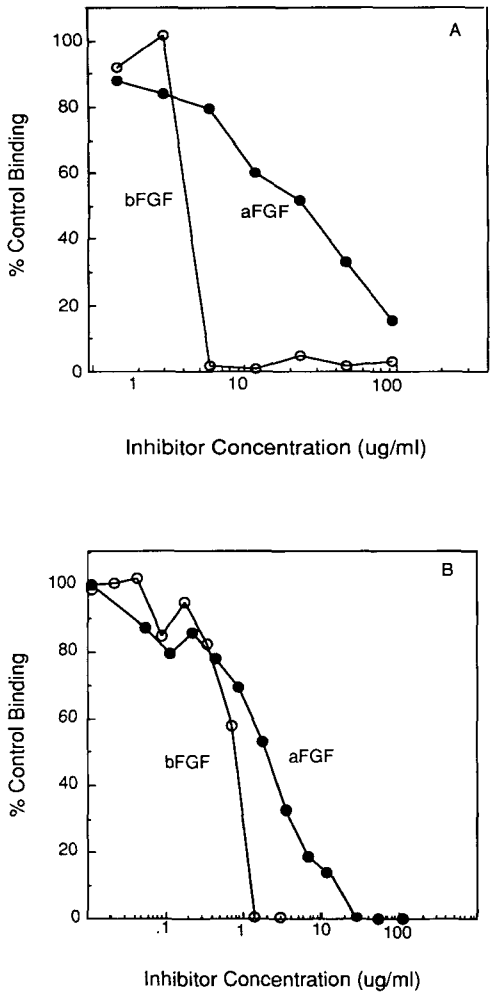

Fig. 3. Ability of soluble aFGF and bFGF to inhibit the binding of BALB/c 3T3 cells to immobilized (**A**) aFGF and (**B**) bFGF. Cells were incubated with a range of FGF concentrations for 2 h on ice before being added to bFGF-coated wells for a further 1 h incubation at 37°C. Each value represents the mean of triplicate treatments. 100% binding is the number of cells bound to FGF in the absence of FGF inhibitor. Standard errors of means were <5%.

Role of Cell Surface HSPGs in Binding of FGFs to FGFR

In order to determine whether aFGF's ability to be potentiated by exogenously added heparin is in any way dependent on its affinity for cell surface HSPG and its ability to form ternary complexes with them and the FGFR, studies were performed which compared the dissociation rates of aFGF and bFGF from FGFR and HSPG on BALB/c 3T3 cells. As found by Nugent and Edelman [1992], the rates of dissociation of bFGF from the FGFR and HSPG differed significantly (Fig. 4B). There was a biphasic release of bFGF from FGFR. Initially, approximately 20% of bound bFGF was released relatively rapidly from FGFR. This was followed by a second phase of dissociation during which the majority (80%) of FGFR-bound-bFGF dissociated extremely slowly, with the time required for half of the bound bFGF to be released ($t_{1/2}$) from the FGFR being > 250 min, compared with approximately 9 min for the HSPG. In contrast, aFGF dissociated from the FGFR and HSPG at similar rates, $t_{1/2}$ being determined as 25 min and 16 min, respectively (Fig. 4A). Thus, aFGF dissociates from the FGFR considerably faster than does bFGF, and this difference is reflected in their $t_{1/2}$ values, being 25 min for aFGF and > 250 min for basic fibroblast growth factor. In contrast, the two growth factors dissociated from HSPG relatively rapidly and at similar rates, the $t_{1/2}$ values for aFGF and bFGF being 16 min and 9 min, respectively.

In order to examine the relative importance of cell surface HSPGs in the binding of acidic and basic FGF to the FGFR, cells were treated with heparinase to remove cell surface HSPGs and the dissociation of aFGF and bFGF from the FGFR examined (Fig. 5). As observed by Nugent and Edelman [1992], in the absence of HSPG, bFGF dissociates >20-fold faster from the FGFR, the time required for half of the bound

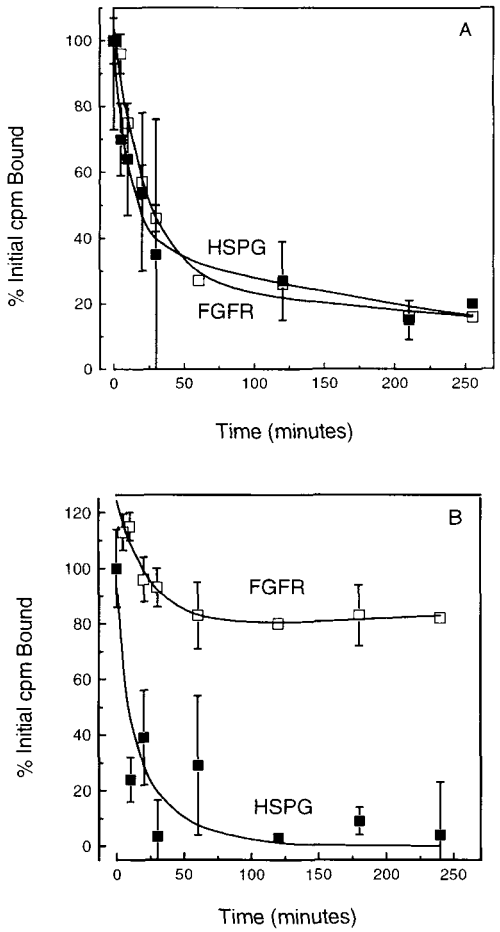

Fig. 4. Dissociation of (**A**) aFGF and (**B**) bFGF from FGFR (□) and HSPG (■) on intact BALB/c 3T3 cells. Dissociation was performed in the presence of an excess of unlabeled aFGF (1 μg/ml) or bFGF (7 μg/ml) to minimize rebinding of released ^{125}I-FGF. Each data point represents the mean ± SEM of triplicate treatments. Initial binding (100%) to FGFR was 893 ± 38 cpm and 4701 ± 166 cpm for bFGF and aFGF, respectively. Initial binding (100%) to HSPG was 994 ± 120 cpm and 434 ± 60 cpm for aFGF (10 ng/ml) and bFGF (2 ng/ml), respectively. FGF bound to HSPG was released by exposing the cells to 2M NaCl in binding buffer for 10 s and FGF bound to FGFR was released after incubation of the monolayers in low pH buffer (2M NaCl, 20 mM sodium acetate, pH 4) for 5 min followed by a brief wash in the same buffer.

bFGF to be released from FGFR being 12 min as compared with >250 min on untreated cells (Fig. 4B). In fact, bFGF dissociated from FGFR at a rate similar to that at which bFGF dissociated from HSPG on untreated cells. In the absence of cell surface HSPGs, aFGF dissociated from FGFR with a $t_{1/2}$ of approximately 7 min (Fig. 5). Thus aFGF dissociated from FGFR in the absence of HSPG at a rate almost 3.5-fold faster than in their presence. Therefore, in the absence of HSPG the dissociation rates of aFGF and bFGF from FGFR differed by a factor of only 1.7, whereas in their presence, they differed by a factor of >10. These results suggest that the difference in the dissociation rates of aFGF and bFGF from FGFR on untreated 3T3 cells is very likely due to a difference in their ability to interact with cell surface HSPGs.

Heparin was shown to enhance the binding of both aFGF (Fig. 6A) and bFGF (Fig. 6B) to the FGFR on heparinase treated cells. Heparin significantly potentiated the binding of aFGF to FGFR at concentrations ranging from 0.001 μg/ml to 100 μg/ml but had an inhibitory effect on aFGF binding at 1 mg/ml. Enhancement of bFGF binding by heparin was less marked than with aFGF and occurred over a narrower concentration range (0.1–1 μg/ml), although again at 1 mg/ml, heparin was highly inhibitory. This observation that heparin does not enhance the binding of bFGF to its receptor to the same extent as it does aFGF is in agreement with the observation that heparin does not generally potentiate bFGF-induced mitogenesis in vitro to the same degree as it does aFGF. This further supports the idea that potentiation of aFGF activity by heparin is related to its ability to enhance aFGF binding to its receptor.

DISCUSSION

The three main findings in this study are, firstly, that acidic and basic FGF interact with

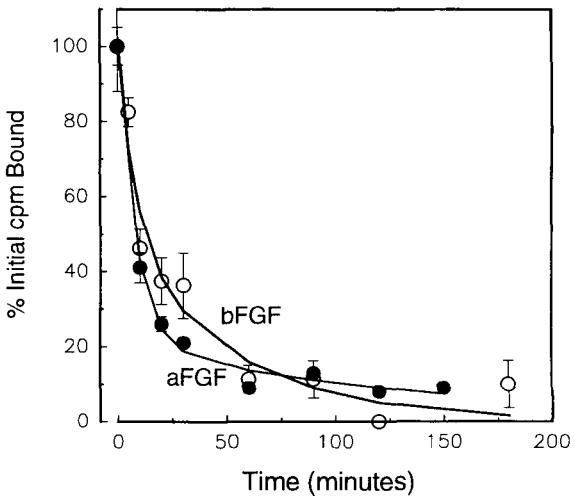

Fig. 5. Dissociation of aFGF (●) and bFGF (○) from FGFR on heparinase treated BALB/c 3T3 cells. Dissociation was performed in the presence of an excess of unlabeled aFGF (1 μg/ml) or bFGF (7 μg/ml) to minimize rebinding of released ^{125}I-FGF. Each data point represents the mean ± SEM of triplicate treatments. Initial binding (100%) to FGFR was 1170 ± 129 cpm and 1120 ± 14 cpm for bFGF and aFGF, respectively.

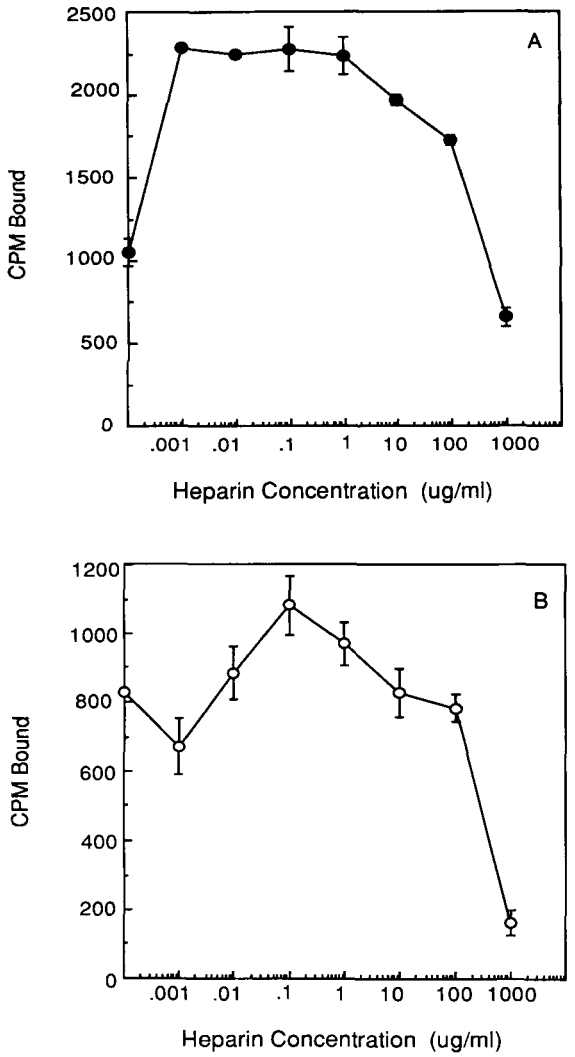

Fig. 6. Effect of different concentrations of heparin on (A) aFGF (20 ng/ml) and (B) bFGF (20 ng/ml) binding to FGFR on heparinase-treated cells. Values represent means ± SEM of quadruplicate values for aFGF and triplicate values for bFGF. Similar results were obtained on 3 separate occasions. Nonspecific binding was <20% of cpm bound.

the same HSPGs on the BALB/c 3T3 cell surface; secondly, that bFGF binds these HSPGs with a substantially higher affinity than does aFGF; and thirdly, that cell surface HSPGs stabilize the interaction of bFGF with the FGFR much more effectively than they do aFGF.

The ability of acidic and basic FGF to interact with the same HSPGs was demonstrated by receptor blocking studies where soluble aFGF and bFGF totally blocked binding of immobilized aFGF and bFGF to cell surface HSPGs on 3T3 cells (Fig. 3A,B). Although these results demonstrate that acidic and basic FGF bind to the same species of HSPG on the 3T3 cell surface, they do not enable us to determine whether both FGFs bind to exactly the same sequence of saccharides on the HS chains. In the light of recent findings by Nurcombe et al. [1993], who have demonstrated that acidic and basic FGF are able to recognize differentially glycosylated HSPGs with differing affinity, it would appear more likely that acidic and basic FGF bind to slightly different sequences which are either adjacent or overlapping. The differential inhibitory effect of bovine intestinal HS on acidic and basic FGF binding supports this point (Fig. 2A,B). Although chemical analysis of the saccharide sequences to which acidic and basic FGF bind have not to date demonstrated any significant differences in the sequences recognized, it seems likely that subtle differences in the sequences recognized will eventually be discovered. Basic FGF has been shown to bind sequences in which the predominant disaccharide is IdoA(2-OSO_3)α1,4GlcNSO_3 [Turnbull et al., 1992; Habuchi et al., 1992], while aFGF is thought to bind sequences in which the predominant disaccharides are IdoA(2-OSO_3)α1, 4GlcNSO_3(6-OSO_3) [Barzu et al., 1989; Mach et al., 1993].

The binding inhibition studies also indicate that bFGF has a 4.7-fold higher binding affinity for 3T3 cell surface HSPG than aFGF (Fig. 3A,B). In support of this conclusion was the observation that the PVC plates had to be coated with approximately an 8-fold higher concentration of aFGF than bFGF to facilitate HSPG-mediated binding of 3T3 cells (Fig. 1), despite the fact that radiolabeled FGFs adhered to the plates equally well. These studies were performed at 37°C but additional blocking studies performed at 4°C with a heparan sulfate binding protein, histidine-rich glycoprotein which binds to the same heparan sulfate motifs as the FGFs, have also shown that there is a 4–5-fold difference in the affinity of acidic and basic FGF for cell surface HSPGs [Brown and Parish, in press]. Although it is generally accepted that aFGF has a lower affinity for heparin than does bFGF, their Kds being determined as 91 nM and 2.2 nM, respectively [Lee and Lander, 1991], the relative affinities of acidic and basic FGF for cell surface HSPGs are less well defined, their Kds being reported to lie within the range of 2–10 nM [Moscatelli, 1987]. However, lower salt concentrations have been used to elute aFGF (0.75 M) from cell surface HSPG than are used to elute bFGF (>2.0 M), suggesting that aFGF

exhibits a lower affinity for HSPG than bFGF [Olwin and Rapraeger, 1992]. On the other hand, Nurcombe et al. [1993] have detected a HSPG during embryonic development, which has a higher affinity for acidic than basic FGF. Presumably such a HSPG is not expressed on BALB/c 3T3 cells.

The kinetic studies which compared the rates of dissociation of acidic and basic FGF from FGFR in the presence and absence of endogenous HSPG highlight the difference in affinities of acidic and basic FGF for HSPGs and the importance of HSPG in stabilizing the interaction of FGF with the FGFR. Basic FGF was shown to dissociate from the FGFR >20-fold slower in the presence of HSPGs than in their absence, $t_{1/2}$'s being >250 min and 12 min, respectively (Figs. 4B, 5). These results are in agreement with those of Nugent and Edelman [1992], who found bFGF to dissociate from FGFR at a 16-fold faster rate in the absence of HSPGs. As Nugent and Edelman [1992] have suggested, HSPGs may prolong the period that bFGF is bound to the FGFR, thereby enabling receptor activation which might not occur in the absence of HSPGs. In contrast to bFGF, aFGF was found to dissociate relatively rapidly from FGFR both in the presence and absence of HSPG, $t_{1/2}$ being determined as 25 min and 7 min, respectively (Figs. 4A, 5). Thus, these results demonstrate that aFGF dissociates from FGFR in the presence of HSPG at least 10-fold faster than does bFGF. Since this study has shown that bFGF has a considerably higher affinity for 3T3 cell surface HSPG than aFGF, these results also suggest that aFGF is not able to utilize 3T3 cell surface HSPGs to form ternary complexes as effectively as does bFGF.

Previous studies have shown that heparin potentiates aFGF-induced mitogenesis to a significantly greater extent than it does bFGF-induced mitogenesis [Thornton et al., 1983; Schreiber et al., 1985; Lobb et al., 1986; Orlidge and D'Amore, 1986]. A similar effect was observed with BALB/c 3T3 cells in this study (data not shown). A possible explanation for this potentiation difference may be that aFGF's low affinity for endogenous HSPG on the 3T3 cell surface is not sufficient to ensure maximal binding of aFGF to its receptor. Acidic FGF very likely has a higher affinity for heparin and is able to utilize exogenously added heparin more effectively than endogenous HSPG to promote high affinity binding to the FGFR. In contrast, bFGF has a sufficiently high affinity for cell surface HSPG to enable it to utilize them in high affinity interactions with the FGFR without any requirement for exogenously added heparin. This may explain why heparin increased the net amount of aFGF bound to the FGFR on heparinase treated cells (Fig. 6A) to a greater degree than it did bFGF binding to similarly treated cells. It seems likely that bFGF was able to utilize any HSPGs remaining on the cell surface after heparanase treatment more effectively than did aFGF and therefore the increase in aFGF binding seen in the presence of heparin was relatively greater than that seen with bFGF and heparin.

The two most likely possibilities as to how heparin/HSPG enhance the binding of FGF to the FGFR are that heparin/HSPG induce a conformational change in the growth factor thus enabling it to bind to the FGFR [Schreiber et al., 1985; Kaplow et al., 1990; Yayon et al., 1991] or that HSPGs are required to stabilize the binding of FGF to its receptor and form a "ternary complex" with them [Nugent and Edelman, 1992]. In support of the "ternary complex" model, our data strongly suggest that the difference in the dissociation rate of acidic and basic FGF from the FGFR is primarily due to their differences in affinity for cell surface HSPGs. In order for heparin to act as a substitute for HSPG in the formation of a ternary complex, it would have to bind heparin receptors located adjacent to the FGFR or to a heparin-binding domain on the FGFR itself [Kan et al., 1993]. Indeed, potentiation of aFGF-induced mitogenesis by heparin may be the result of the formation of more ternary complexes between aFGF, heparin and FGFR than aFGF would form with FGFR and HSPG.

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