Biochemical Pharmacology, Vol. 45, No. 11, pp. 2348–2351, 1993.
Printed in Great Britain.

0006-2952/93 $6.00 + 0.00

Inhibition of binding of basic fibroblast growth factor to low and high affinity receptors by carrageenans

(*Received* 21 *January* 1993; *accepted* 10 *March* 1993)

Abstract—The effect of carrageenans, a family of polysulphated polysaccharides, on the binding of basic fibroblast growth factor (bFGF) to low affinity (heparin-like) receptors and high affinity receptors on baby hamster kidney cells was investigated. κ-, ι- and λ-carrageenans all inhibited binding of bFGF to both types of receptors with ι-carrageenan being the most potent inhibitor (IC_{50} values of approx. 0.7 and 4 μg/mL for inhibition of binding to low and high affinity receptors respectively). Heparin reduced the inhibition of bFGF binding to high affinity receptors caused by ι-carrageenan. Heparin and ι-carrageenan were comparable in their activities at displacing pre-bound bFGF from both low affinity receptors and high affinity receptors. These results indicate that ι-carrageenan binds to the heparin-binding domain on bFGF and that this may be sufficient to reduce the ability of bFGF to bind to high affinity receptors.

The fibroblast growth factor (FGF*) family represents a multipotential group of growth factors with mitogenic activity against a broad range of tissues [1]. FGFs are implicated in the aetiology of some cancers. For example, anti-sense oligomers to basic fibroblast growth factor (bFGF) block the growth of malignant glioblastoma cells [2]. bFGF is also both a mitogen for capillary endothelial cells [3] and a potent angiogenic factor. FGF antagonists therefore have potential for the treatment of cancer either by directly inhibiting FGF-stimulated growth of tumour cells, or by inhibiting neo-vascularization—a process essential for the continued growth of tumours [4].

FGFs characteristically associate with the polysulphated polysaccharide heparin (reviewed in Ref. 5), and this observation has been exploited in order to develop polysulphated carbohydrates as FGF antagonists. For example, pentosan polysulphate binds Kaposi's sarcoma-derived FGF and inhibits the mitogenic activity of this growth factor [6]. Heparin itself stabilizes acidic FGF (aFGF) and potentiates the mitogenic activity of this growth factor [7, 8]. In some, but not all, cases heparin can have a similar effect on bFGF [9, 10]. Some polysulphated compounds, such as dextran sulphates, also enhance, rather than inhibit, the mitogenic effects of FGFs [9].

Recently, work in this laboratory has identified carrageenans as a new group of polysulphated polysaccharides which inhibit the binding of some growth factors, including bFGF [11]. The ability of carrageenans to inhibit binding of bFGF to high affinity receptors will be a necessary pre-requisite for the development of these agents as anti-proliferative agents since it is the high affinity receptors which mediate the mitogenic signal [12]. Baby hamster kidney (BHK) cells express both heparin-like low affinity bFGF receptors and high affinity bFGF receptors [13] and the domains on bFGF which interact wth these receptor types have now been partially characterized [14]. We have used BHK cells in order to investigate which binding domains on bFGF interact with carrageenans and are responsible for the inhibition of binding of bFGF to bFGF receptors.

Materials and Methods

Materials. Carrageenans κ (type III), λ (type IV) and ι (type V) (Sigma Chemical Co., Poole, U.K.) were freshly made up prior to each experiment at 2 mg/mL in 25 mM Hepes, pH 7.4 heated to 60°. Heparin (H 8514 from Sigma) was made up at 10 mg/mL in 25 mM Hepes, pH 7.4 and stored at −20°. [^{125}I]bFGF (Amersham International plc, Aylesbury, U.K.) and cold bFGF (Bachem, Saffron Walden, U.K.) were stored in aliquots at −20°.

Cells. BHK clone 13 was purchased from the European Collection of Animal Cell Cultures (Porton Down, Salisbury, U.K.) and maintained in minimum essential

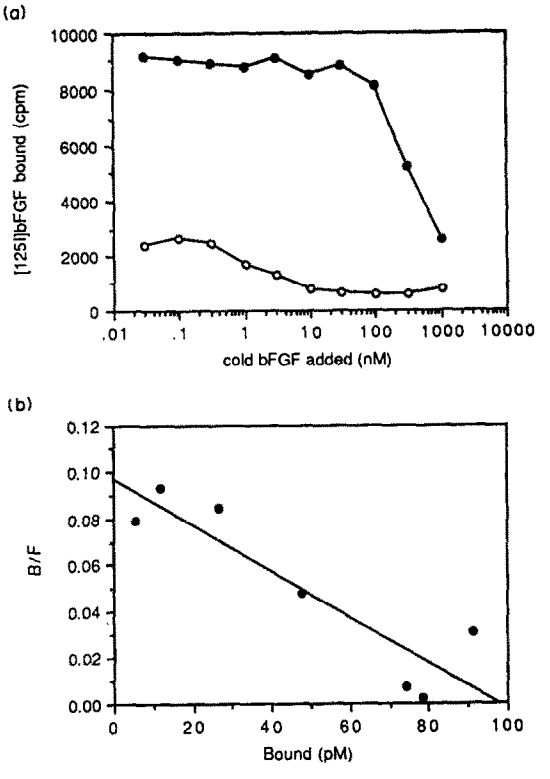

Fig. 1. (a) Competition between cold bFGF and [^{125}I]bFGF for binding to NaCl-extractable (●) and Triton X-100-extractable (○) binding sites. (b) Scatchard analysis of the competition experiment for the Triton X-100-extractable binding sites.

* Abbreviations: BHK, baby hamster kidney; aFGF, acidic fibroblast growth factor; bFGF, basic fibroblast growth factor; FGF, fibroblast growth factor family.

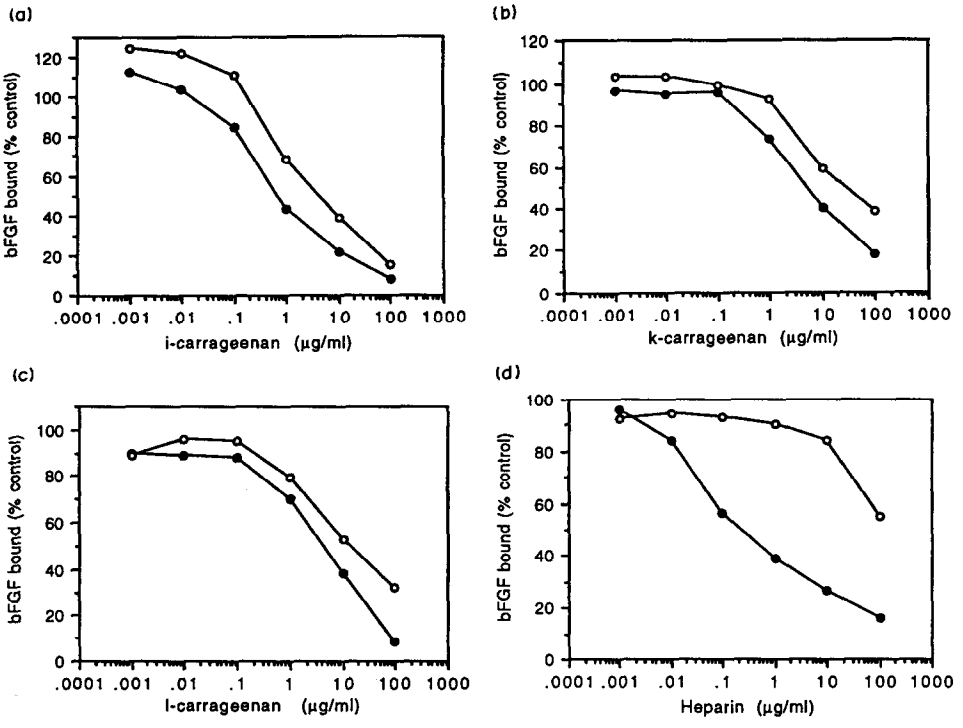

Fig. 2. Inhibition of binding of bFGF to low affinity receptors (●) and high affinity receptors (○) by (a) ι-carrageenan (b) κ-carrageenan (c) λ-carrageenan and (d) heparin.

medium (MEM)/10%, foetal calf serum (FCS)/5% tryptose phosphate broth containing antibiotics (100 U/mL penicillin and 100 μg/mL streptomycin).

bFGF binding assay. Binding of bFGF to low and high affinity receptors on BHK cells was determined by a slight modification of the method of Moscatelli [13]. BHK cells were plated out at 10^5 cells/well (1 mL/well) in 24 well plates and left 2 days until >90% confluent. The cells were incubated with Dulbecco's modified Eagle's medium (DMEM) containing 0.15% gelatin at 37° for 2 hr and then washed twice with ice-cold phosphate-buffered saline (PBS). Binding experiments were carried out in binding buffer (bicarbonate-free DMEM/0.15% gelatin/25 mM Hepes, pH 7.4) containing 0.1 nM [^{125}I]bFGF at 4° for 3 hr on a rocking shaker. Preliminary experiments showed that maximum binding occurred after 3 hr and hence a steady state was achieved. Following binding, cells were washed three times with ice-cold PBS (1 mL per wash). Binding to low and high affinity receptors was determined by measuring [^{125}I]bFGF released by two washes with 2 M NaCl in 25 mM Hepes, pH 7.4 (0.75 mL per wash) (low affinity receptors) followed by a 10 min extraction with 0.5% Triton X-100 in 0.1 M sodium phosphate, pH 8.1 (0.2 mL) (high affinity receptors). All experiments were carried out with duplicate determinations and repeated at least three times.

Scatchard analysis. Scatchard analysis was performed using LIGAND [15] as modified for microcomputer by McPherson [16].

Results

Binding sites on BHK cells for bFGF which could be washed off with 2 M NaCl and sites which could be extracted with Triton X-100 were characterized by competition experiments between unlabelled bFGF and 0.1 nM [^{125}I]bFGF. Concentrations of unlabelled bFGF in excess of 100 nM were required to compete with [^{125}I]bFGF

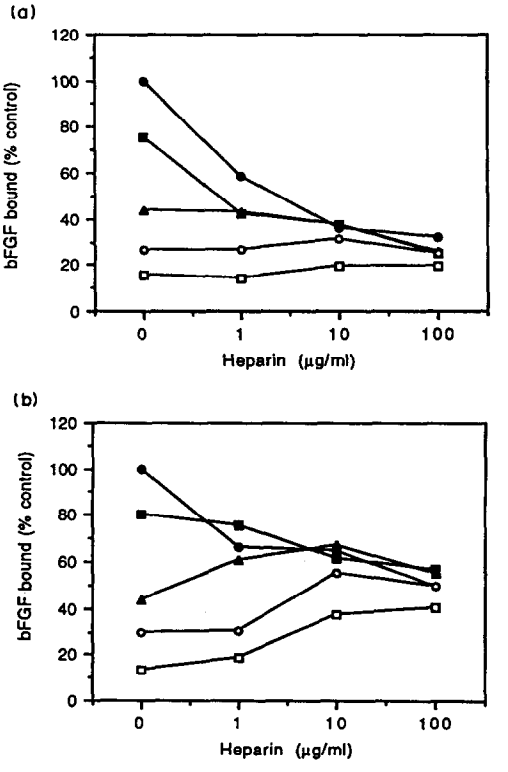

Fig. 3. Inhibition of bFGF binding to (a) low affinity receptors and (b) high affinity receptors in the presence of heparin and ι-carrageenan. Various concentrations of heparin alone (●) or mixed with ι-carrageenan [0.1 μg/mL (■), 1 μg/mL (▲), 10 μg/mL (○), 100 μg/mL (□)] were added to cells and binding determined 3 hr later.

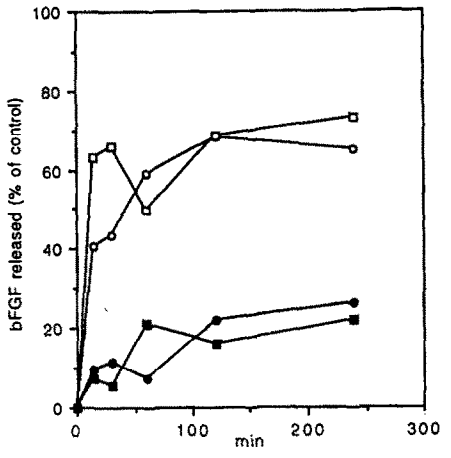

Fig. 4. Displacement of pre-bound bFGF from low affinity receptors (open symbols) and high affinity receptors (closed symbols) by ι-carrageenan (○, ●) and heparin (□, ■). [^{125}I]bFGF (0.1 nM) was incubated with BHK cells for 4 hr at 4°. ι-Carrageenan or heparin was added to give a final concentration of 100 μg/mL and bFGF bound to low affinity receptors and high affinity receptors was determined at intervals. Values are expressed relative to control wells which did not receive ι-carrageenan or heparin and were extracted at the same times. Over the course of the experiment, the amount of bFGF measured in control wells did not vary by more than 20% from values determined after the 4-hr pre-incubation.

washed off with 2 mM NaCl whereas concentrations of unlabelled bFGF in excess of 0.3 nM competed with [^{125}I]-bFGF extractable with Triton X-100 (Fig. 1a). These data confirm earlier reports [13] that 2 M NaCl and Triton X-100 can be used to distinguish between low and high affinity bFGF receptors on BHK cells. Scatchard analysis of binding to the Triton-extractable sites gave a K_d value of 0.9 nM (Fig. 1b).

All of the carrageenans tested inhibited binding of 0.1 nM bFGF to both low and high affinity receptors (Fig. 2a–c). ι-Carrageenan was the most potent antagonist in agreement with earlier results where low and high affinity receptors were not distinguished [11]. By contrast, heparin inhibited binding of bFGF to low affinity receptors to a greater extent than to high affinity receptors (Fig. 2d). Pentosan polysulphate was also more potent at inhibiting binding of bFGF to low affinity receptors compared to inhibition of binding to high affinity receptors [IC_{50} values 13 ± 3 μg/mL and 77 ± 30 μg/mL (N = 3), respectively]. Concentrations of bFGF between 0.1 and 1.0 nM did not significantly influence the potency of inhibition of binding of bFGF by carrageenans (data not shown).

Since ι-carrageenan is a more potent antagonist of high affinity binding than heparin, we investigated the inhibitory effect of ι-carrageenan in the presence of heparin. When 100 μg/mL ι-carrageenan was incubated with increasing concentrations of heparin, binding of bFGF to high affinity receptors was reduced in a dose-dependent manner to levels of inhibition approaching those caused by heparin alone (Fig. 3b). This suggests that heparin and ι-carrageenan are competing for the same binding sites on bFGF and that the inhibition of binding of bFGF to the high affinity sites by ι-carrageenan is mediated, at least in part, through heparin-binding sites on bFGF. There was only a slight reversal by heparin of inhibition of binding of bFGF to the low affinity receptors by ι-carrageenan (Fig. 3a). This would be expected of two compounds which have a similar potency as inhibitors of low affinity binding (see Fig. 2a and d).

The interaction between ι-carrageenan and bFGF was further studied by comparing the abilities of heparin and ι-carrageenan to displace pre-bound bFGF from high and low affinity sites. Both ι-carrageenan and heparin caused the loss of over 50% of bFGF pre-bound to low affinity receptors within one hour (Fig. 4). By contrast, ι-carrageenan and heparin caused significantly lower, but comparable, loss of bFGF from high affinity receptors (Fig. 4).

Discussion

Therapies which target growth factors are promising new areas for the development of new anti-cancer agents. The results of phase II trials of suramin have now been reported [17, 18], and pentosan polysulphate has undergone preliminary trials [19]. The present study demonstrates that carrageenans are able to inhibit binding of bFGF to both low and high affinity receptors on BHK cells. Since the biological response of cells to bFGF is mediated through high affinity receptors [12], carrageenans have potential anti-proliferative and therapeutic use. Recently, we have found that ι-carrageenan is a potent inhibitor of the proliferation of the bFGF-dependent cell line FBHE (Hoffman and Donaldson, unpublished observations).

In theory, carrageenans could block binding of bFGF to low and high affinity receptors by acting independently at both sites. However, several lines of evidence from the present work support the hypothesis that ι-carrageenan is binding primarily to the heparin-binding site on bFGF. Firstly, heparin competed with ι-carrageenan for binding to bFGF and this reduced the ability of ι-carrageenan to inhibit high affinity binding of bFGF. Secondly, ι-carrageenan was no better at displacing bFGF from high affinity receptors than heparin.

The precise mechanism whereby binding of ι-carrageenan to the heparin-binding site on bFGF could reduce binding of bFGF to high affinity receptors remains to be clarified. One possibility is that ι-carrageenan, which has a molecular mass of 140 kda (Sigma, personal communication), simply masks the high affinity binding site on bFGF (molecular mass 18 kda). An alternative explanation is that binding of ι-carrageenan to bFGF prevents bFGF adopting the correct conformation to allow binding to the high affinity receptor. Heparin is known to induce a conformational change in bFGF [20], and binding to heparin-like low affinity receptors is necessary before bFGF can bind to high affinity receptors [21, 22].

Although ι-carrageenan is a potent antagonist of bFGF binding to high affinity receptors, this agent has several undesirable properties. These include its high molecular mass, which may reduce drug delivery, and also the known immunosuppressive effects of carrageenans [23]. Whether or not these properties of ι-carrageenan will restrict its *in vivo* utility as an anti-tumour agent is currently under investigation.

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