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In vitro inhibition of the actions of basic FGF by a novel 16 amino acid peptide

Irena Cosic,[1] Ann E. Drummond, John R. Underwood and Milton T.W. Hearn

Department of Biochemistry and Centre for Bioprocess Technology, Monash University, Clayton, Victoria, Australia, 3168, Australia;[1] current address: Department of Electrical and Computer Systems Engineering, Monash University, P.O. Box 197, Caufield East, Victoria, 3145, Australia

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Abstract

A composite procedure involving molecular modelling and a property-pattern algorithm, the Resonant Recognition Model (RRM), has been applied to structure-function studies with basic fibroblast growth factor (bFGF). Property-pattern characteristics for biological activity and receptor recognition for a group of FGF-related proteins were defined and then used to aid the design of a set of peptides which can act as bFGF antagonists. Molecular modelling techniques were then employed to identify the peptide within this set with the greatest conformational similarity to the putative receptor domain of bFGF. This 16 amino acid residue peptide (16mer), which exhibits no sequence homology to bFGF, antagonised the stimulatory effect of bFGF on fibroblast [^{3}H]thymidine incorporation and cell proliferation, but exerted no effect itself in these *in vitro* bioassays. (Mol Cell Biochem **130:** 1–9, 1994)

Key words: fibroblast growth factor, resonant recognition model, algorithm, fibroblasts, peptide

Introduction

The proliferative and angiogenic potential of bFGF and other members of the heparin binding family of polypeptide growth factors have been well documented in recent years [1–7]. FGF-related growth factors have been postulated to play significant roles during embryogenesis [8], vascularization of the ovary and related reproductive tissues [9, 10], in pathological states such as wound healing, tissue repair [11] and the development and expansion of some solid tumours [12–15]. To date the treatment of malignant tissues has been based largely on conventional regimens of surgery and chemo- or radio-therapy. Alternative therapeutic modes for the regulation of FGF-dependent solid tumour growth and tumour dependent angiogenesis could arise with the availability of highly specific peptide analogues capable of interacting with FGF receptors. The therapeutic potential of bFGF (as well as acidic FGF [aFGF]) analogues with agonist properties for use in wound healing, bone regeneration and in duodenal ulcer therapy has already attracted considerable attention [16].

In recent studies from this laboratory [17–19], a composite property-pattern search algorithm, known as the Resonant Recognition Model (RRM), has been used in conjunction with molecular modelling procedures, to

Address for offprints: M.T.W. Hearn, Department of Biochemistry, Monash University, Wellington Rd, Clayton, Victoria, 3168, Australia

evaluate the relationship between the biological properties of polypeptides and proteins and their structural motifs, dictated by the conformational clustering of specific amino acid residues and their associated physicochemical descriptors, including the electron-ion interaction potential (EIIP) characteristics. In the present investigations characteristic properties of FGFs associated with mitogenic activity and receptor binding behaviour were defined in order to aid the design of a set of peptide analogues. A specific 16 amino acid peptide analogue, designed to incorporate the essential characteristics for bFGF receptor binding, has been evaluated and data on the activity of this peptide and its ability to antagonise bFGF action *in vitro* are reported herein.

Materials and methods

Peptide design

The property-pattern algorithmic procedures [17–19, 21, 22] are based on the representation of the protein primary structure as a numerical series by assigning a numerical value of a physicochemical parameter to each amino acid. The parameter employed in these studies, i.e. electron-ion interaction potential, EIIP [20, 23], describes a physicochemical property of the amino acids within the protein sequence which highly correlates with biological function. The obtained numerical series can then be converted, via discrete Fourier transformation, into a discrete spectrum which carries the same information content about the arrangement of the amino acids in the sequence as the original numerical sequence [24]. Comparative analyses of several hundreds of proteins and their biological function have shown that: (a) each functional group of proteins exhibits at least one characteristic frequency in their Fourier spectra; and (b) proteins and their receptors have the same frequency characteristic but opposite phases [19]. Accordingly, similar methods of analysis were applied to a group of 4 bFGF (human, bovine, rat and frog), 3 aFGF (human, bovine and rat) and 2 bFGF receptors (flg and bek), the amino acid sequences of which are available from the NBRF data base. A common frequency characteristic at $f_1 = 0.4412$, was identified for the examined bFGFs and their receptors using multiple cross spectral analysis methods as described in detail elsewhere [17–19]. An inverse Fourier transformation was employed to design *de novo,* peptides of different length and sequence which exhibit the same prominent frequency characteristic as bFGF.

Molecular modelling

Molecular mechanics and dynamics techniques were used to predict energy minimised structures of the designed peptides and the bFGF fragment (bFGF[97-120]). For these investigations, the Insight and Discover (Biosym, San Diego, CA, USA) software packages were utilised. Steepest descent minimisation and dynamics [25] were implemented to assess the conformational states of the designed peptides.

Peptide synthesis

Peptides were synthesised using Fmoc protected amino acids and Wang resin. The peptides were purified by reverse phase HPLC with 0.1% TFA-water-acetonitrile (0–50%) gradients and gave amino acid composition values, FAB-MS and N-terminal sequence data consistent with the amino acid sequence.

[^{3}H]Thymidine incorporation assay

An established protocol [26] was utilised for monitoring [^{3}H]thymidine uptake by Balb/c 3T3 fibroblasts (CSL, Melbourne, Australia). Briefly, fibroblasts were grown to confluence in DMEM (Flow/ICN, Sydney, Australia) containing 10% foetal calf serum (FCS) (P.A. Biological Co., Sydney, Australia). The day prior to the addition of test substances the media were removed and replaced with DMEM containing 0.5% FCS. Samples were tested at multiple doses and 50 µl aliquots were added to culture wells. After a 20 hr incubation with test substances, [^{3}H]thymidine (1 : 15 dilution, specific activity 6.7 Ci/mmol, NEN, Boston, MA, U.S.A.) was added to the wells and the cells were pulsed with tracer for 2 hr. The cells were then washed, trichloroacetic acid (TCA) (Ajax Chemicals, Sydney, Australia) precipitated, solubilised with 0.3 M NAOH (Ajax Chemicals) and neutralised with 0.3 M HCL (Ajax Chemicals). Aliquots were prepared for liquid scintillation counting (Wallac).

Cell proliferation assay

The Promega Cell Titer 96 Nonradioactive Cell Proliferation Assay (Promega Corporation, Madison, WI, U.S.A.) was used to monitor cell numbers *in vitro.* Balb/c 3T3 fibroblasts (1×10^4/well) were plated in 96 well tissue

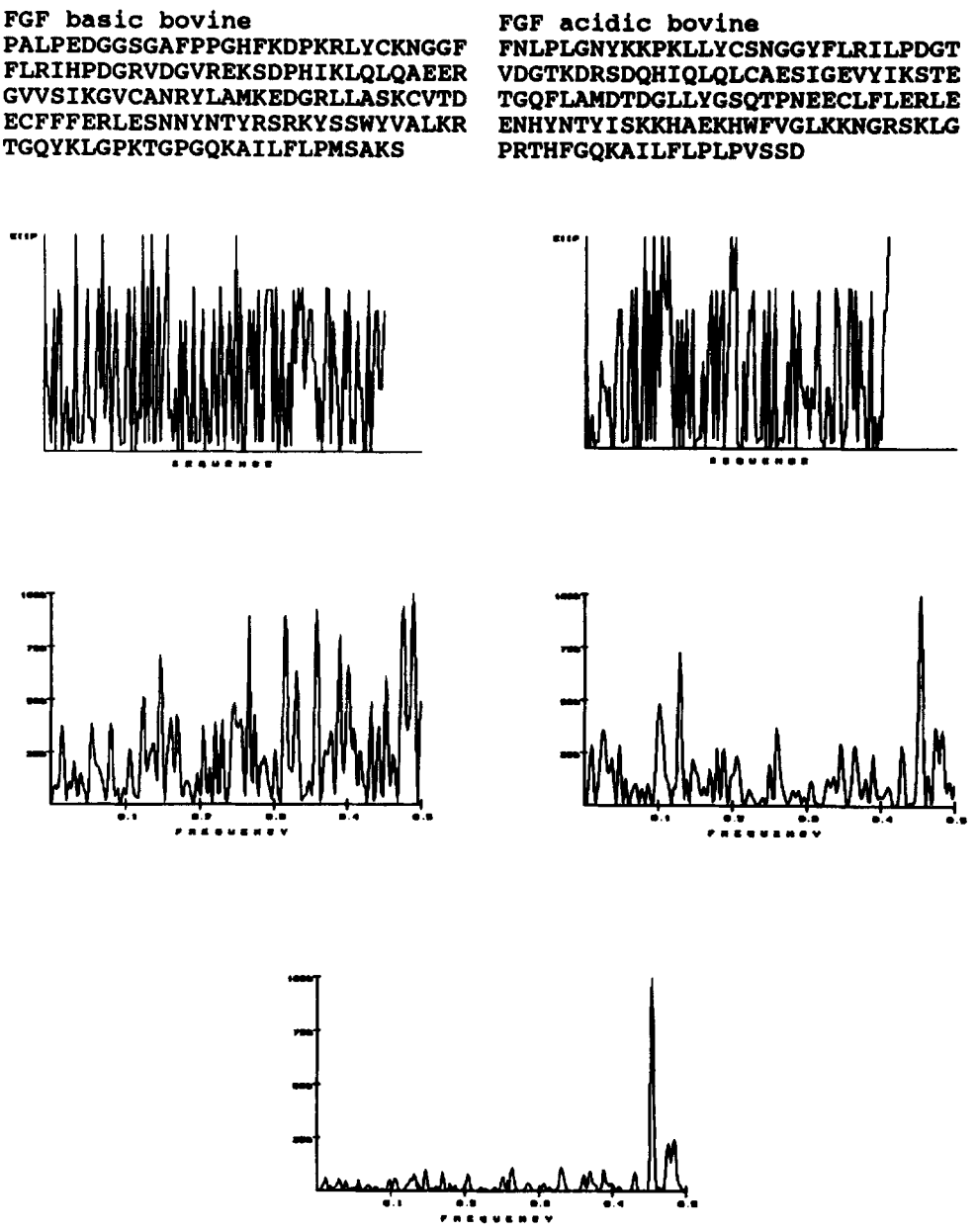
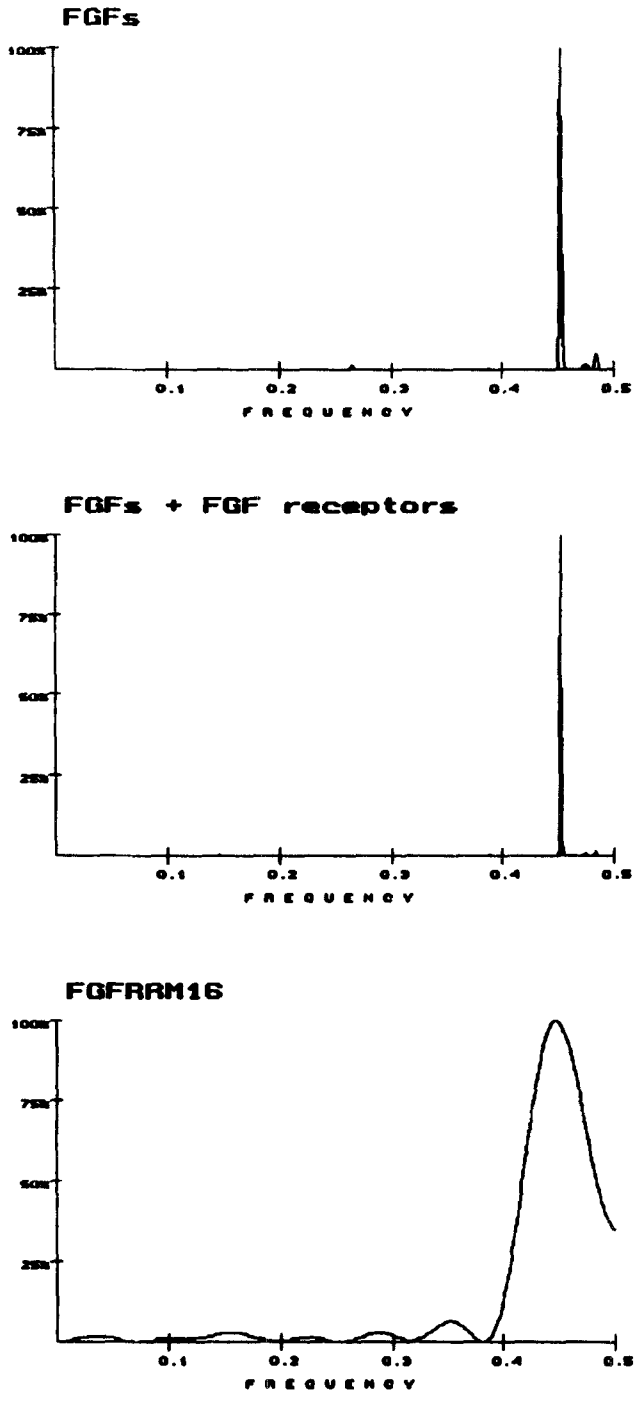


Fig. 1. A schematic representation of the algorithmic procedure applied to the basic and acidic bovine FGFs.
(a) bovine basic and acidic FGF sequences.
(b) numerical series obtained by assigning EIIP values to each amino acid in the sequence.
(c) corresponding Fourier spectra.
(d) cross-spectral function of bovine basic and acidic FGFs. The prominent peak at frequency $f_i = 0.4412$ denotes a common frequency characteristic for these two FGF sequences.

culture plates and grown to confluence in DMEM containing 10% FCS. The media were removed and replaced with DMEM containing 0.5% FCS the day prior to the addition of test samples. Test substances were added in 25 µl aliquots at multipe doses and cultures were incubated for 48 hr. Dye (15 µl/100 µl well volume) was added to the culture wells and incubated for a further 4 hr at 37° C. Living cells convert a tetrazolium component of the dye to an insoluble blue formazan product. Solubilisation solution (100 µl/115 µl well volume) dissolved the formazan crystals and the absorbance of the resultant colored solution was determined by an Elisa plate reader (Bio Rad, N.S.W., Australia) set to a wavelength of 595 nm.



Results

The procedure for defining the frequency characteristics of the FGF-related proteins by the property-pattern search algorithim, is presented schematically in Fig. 1. The acidic and basic bovine FGF amino acid sequences (Fig. 1a) were converted to their corresponding numerical series (Fig. 1b) using EIIP values corresponding to

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Fig. 2. (a) Cross-spectral function of 4 basic FGFs (human, bovine, rat and frog) and 3 acidic FGFs (human, bovine and rat). The prominent peak at frequency $f_1 = 0.4412$ with S/N = 120, denotes the common frequency characteristic for these FGF sequences. (b) Cross-spectral function of 7 previously analysed FGFs and 2 FGF receptors (bek and flg). The same common frequency characteristic is observed at $f_1 = 0.4412$, with enhanced amplitude and higher S/N ratio of 215. (c) Single spectrum of the 16mer peptide designed *de novo* using the inverse algorithmic procedures.

each amino acid within the sequence. These numerical series were then converted to the corresponding Fourier spectra by discrete Fourier transformation procedures (Fig. 1c). Digital spectra of basic and acidic FGF were then compared by cross-spectral analysis with peaks in the resulting spectral function (Fig. 1d), denoting common frequency characteristics for both proteins. It is noteworthy that the analogous cross-spectral function of the 3 acidic and 4 basic FGFs (Fig. 2a) reveals one prominent peak at the frequency $f_1 = 0.4412 \pm 0.005$ (S/N = 120). In order to determine whether this frequency characteristic is relevant to FGF receptor recognition, the corresponding frequency characteristic of the two FGF receptors, flg and bek, was established. The resulting multiple cross-spectral function presented in Fig. 2b, shows that the amplitude of the characteristic frequency f_1 is enhanced (S/N = 215) while the other frequency components were diminished. From these data it can be concluded that frequency f_1 is relevant for recognition between FGFs and their receptors. The other frequency characteristic at $f_2 = 0.258 \pm 0.002$ was found in previous studies [21] to be associated with the mitogenic response of competence growth factors, including FGFs.

Using the peptide design module of the property-pattern search algorithim, a number of peptides exhibiting the frequency characteristic $f_1 = 0.4412$, were designed with lengths between 10 to 24 amino acids. The procedure for designing the peptides includes: (a) derivation of numerical sequences for peptides of different lengths (10 to 24 points), which have only f_1 in their spectra and phase equal to the phase for bovine bFGF and (b) determination of the amino acid which corresponds to each element of this new numerical sequence from tabulated values of EIIP [22]. Energy minimisation techniques were then applied to the bFGF[97-120] fragment, which is believed to be involved in the receptor binding domain [27] and to the designed peptides with the aim of identifying stable peptide structures. The derived structures of these designed peptides were then compared with the corresponding sequence region within the crys-

tal structures of the bFGF variants [28]. Although no sequence homology exists between the designed peptides and the bFGF[97-120] fragment, significant similarities in the conformation of the peptides backbone were detected. On the basis of root mean square (RMS) deviations between the predicted backbone structures, a 16 amino acid residue peptide (16mer), with the amino acid sequence MWYRPDLDERKQQKRE, was found to have the most similar backbone structure, RMS = 1.79 Å, to the bFGF[97-120] fragment (Table 1). The superimposed three dimensional structures of the bFGF[97-120] fragment and the 16mer peptide are presented in Fig. 3. This 16mer and other peptides were prepared by solid phase peptide synthesis, purified by reverse phase HPLC, analytically characterised by amino acid composition, N-terminal sequence analysis and FAB mass spectrometry and their function evaluated in terms of two *in vitro* cell responses.

As expected, bFGF stimulated [^{3}H]thymidine uptake and proliferation of fibroblasts in a dose-dependent manner (Fig. 4). The half maximal responsiveness to bFGF varied from 5 to 25 ng/ml and was dependent on the number of culture passages the cells had experienced and the age of the cell line. The results shown in Fig. 4, represent a typical dose-response curve for cell proliferation by bFGF in the range 0–50 ng/ml with cells of greater passage number. Since the 16mer peptide was designed to occupy the receptor binding site, the corresponding bFGF[97-120] fragment, with receptor binding activity, was used as a control peptide. As previously reported [27], bFGF[97-120] exhibited a small mitogenic potential, stimulating a 2-fold increase in [^{3}H]thymidine incorporation by cultured fibroblasts of lower passage number, at a dose of 14.4 nmol (Fig. 5). In the presence of a stimulatory dose of bFGF (5 ng/ml), which represents a 35-fold stimulation over background, the bFGF[97-120] fragment antagonised the action of bFGF, resulting in a significant inhibition of bFGF-mediated [^{3}H]thymidine uptake by fibroblasts at doses of 14.4–28.8 nmol. The 16mer peptide itself exerted no effect on [^{3}H]thymidine uptake (Fig. 5) or the proliferation of fibroblasts in culture (Fig. 6). However, in the presence of stimulatory doses of 5 ng and 25 ng/ml bFGF, this peptide antagonised the actions of bFGF, inhibiting [^{3}H]thymidine incorporation (Fig. 5) and cell proliferation (Fig. 6) at doses of 5 and 40 nmol and 10 and 40 nmol respectively, in a significant and reproducible manner. Interestingly, at intervening doses of the 16mer peptide, the inhibition of [^{3}H]thymidine incorporation was attenuated.

Table 1. Root mean square (RMS) deviations of designed peptides relative to the backbone structure of the [97-120] sequence region within the crystallographic structure of the bFGF variant [Cys^{76}-Ser^{70}, Cys^{90}-Ser^{90}]

Peptide	Sequence	RMS (Å)
11mer	LDERKQQKRED	3.73
16mer	MWYRPDLDERKQQKRE	1.79
23mer	MWYRPDLDERKQQKREDIDPRYW	2.90
bFGF[97-120]	RLESNNYNTYRSRKYSSWYVALKR	2.35

The backbone structures of individual peptides were determined using molecular mechanics and dynamics techniques. 64 atoms were superimposed on the backbone structure of the active site.

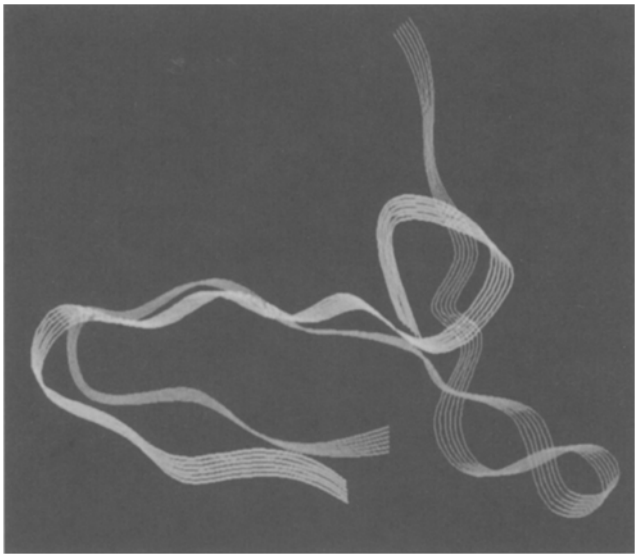
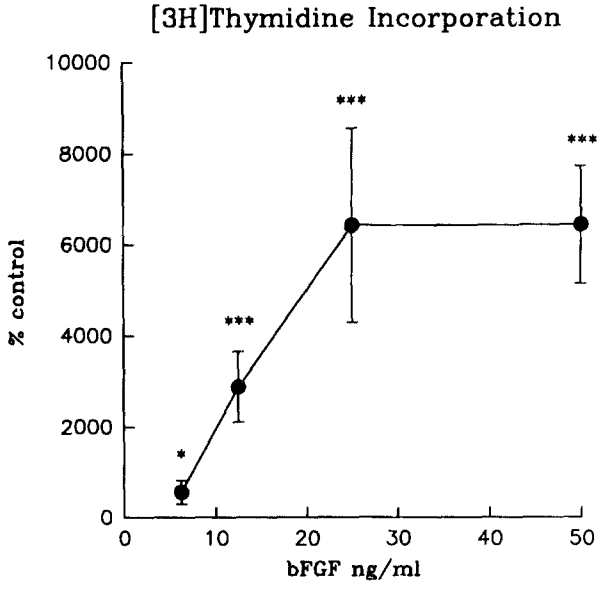


Fig. 3. Comparison of the predicted energy minimised conformation of the *de novo* designed 16mer peptide and the bFGF[97-120] fragment within the crystallographic structure of the bFGF variant (cysteine residues at 70 and 88 were replaced with serine residues). The 16mer peptide is represented as the 'in front' structure and the bFGF[97-120] fragment as the 'behind' structure. Sixty-four atoms were superimposed on the backbone structure of the active site (RMS = 1.79 Å).

Discussion

These studies were undertaken in order to test the capacity of a composite property-pattern algorithm incorporating molecular modelling procedures, to design bFGF antagonist peptides based on specific property-pattern characteristics for biological activity and receptor recognition, previously derived for a group of FGF-related proteins. In the case of the FGFs, it was possible to distinguish receptor recognition from growth pro-



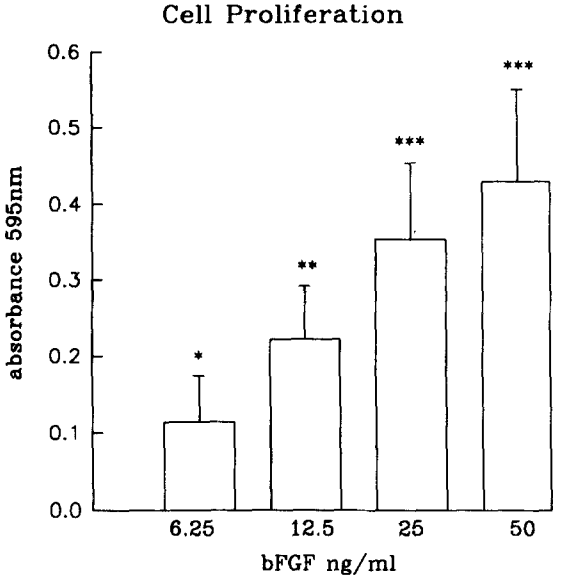
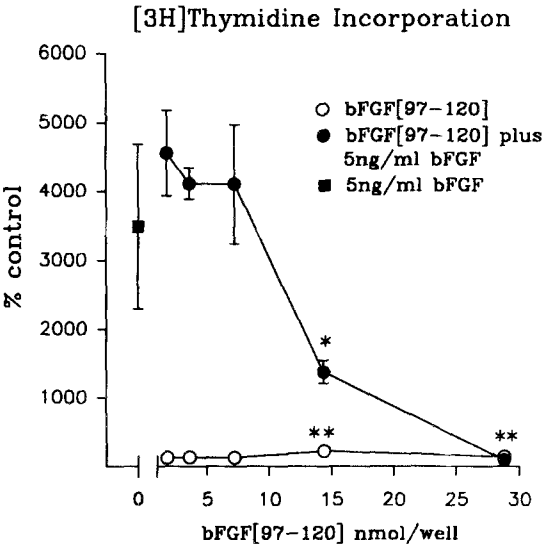


Fig. 4. The effects of bFGF on 3T3 fibroblast [^{3}H]thymidine incorporation and proliferation *in vitro*. Aliquots of bFGF (50 µl [n = 4], or 25 µl [n = 8], for uptake or cell proliferation, respectively), were added to culture wells and incubated as described in the Materials and methods. The 100% incorporation value is defined as the [^{3}H]thymidine incorporated by cells with media alone. For example, 200% incorporation represents a 2-fold increase and 1000% incorporation represents a 10-fold increase above control levels. The absorbance values of control wells (for the proliferation assay) have been subtracted. Mean ± SD, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared to control wells.



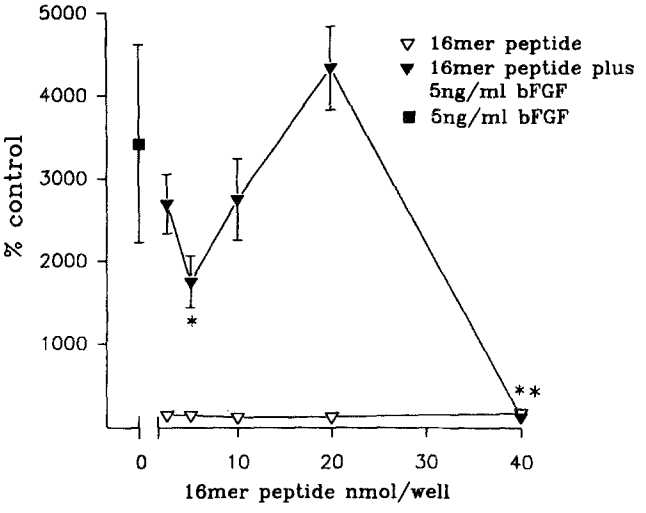


Fig. 5. The effects of the synthetic 16mer peptide and bFGF[97-120] on fibroblast [^{3}H]thymidine incorporation. Peptides were added to cell cultures in the presence or absence of bFGF (5 ng/ml) and incubated as described in the Materials and methods. Mean ± SD, n = 4–8 replicates, * $p < 0.05$, ** $p < 0.01$ compared to the appropriate control wells. In some cases standard deviations were too small to be represented in the figure.

moting activity in terms of different frequency characteristics. Consequently, peptides designed to have receptor binding characteristics but not growth promoting activity incorporate the frequency characteristic at f_1 = 0.4412, with the frequency characteristic at f_2 = 0.258 omitted. Thus, the 16mer peptide has been designed to competitively inhibit the binding of bFGF to its receptor and therefore acts as a bFGF antagonist.

Since bFGF is known to exert stimulatory effects on DNA synthesis and initiate proliferation of 3T3 fibroblasts [29] *in vitro,* these cultures were used for testing the bioactivity of these bFGF-related peptides. These observations were confirmed (Fig. 4) in both [^{3}H]thymi-

dine incorporation and cell proliferation bioassays with a dose-dependent stimulation by bFGF of [^{3}H]thymidine uptake and proliferation of fibroblasts in culture. The highest doses of the control peptide bFGF[97-120], inhibited the proliferation of bFGF stimulated 3T3 fibroblasts and may be attributed to competitive receptor occupancy. Similar results were observed with another control peptide, bFGF[1-24] (data not shown), also known to induce a weak proliferative response on 3T3 fibroblasts.

The biphasic nature of the response elicited by the 16mer peptide on [^{3}H]thymidine uptake and proliferation of fibroblasts in culture, is not a new phenomenon, since similar effects on [^{3}H]thymidine incorporation and DNA synthesis have been reported for other growth factors, in particular IGF-1, insulin, EGF and TGF-β [30–33]. The mechanism underlying this biphasic response remains to be elucidated, but may be related to the receptor-peptide interaction. Although it has been established that heparin-like molecules interact with bFGF to prevent inactivation by heat, extremes of pH and protease digestion [34, 35], as well as facilitating the binding of bFGF to high affinity receptors [36, 37], it is unlikely that the 16mer peptide actually interferes with these low affinity binding interactions. An alternative possibility related to high affinity receptor aggregation or recognition of specific receptor isoforms, could account for this biphasic phenomenon noted with many growth factors. Clearly, further studies including ligand cross-linking experiments are required to characterise the precise mode of interaction between the 16mer peptide and the FGF receptors. Cell surface heparan sulfate proteoglycans, functioning as low affinity receptors, have been found to facilitate the binding of bFGF to specific high affinity receptors, e.g., flg and bek [36]. It can be postulated that a conformational change occurs upon the binding of bFGF to heparin-like molecules which enables bFGF to bind to these high affinity receptors. Interestingly, recent reports indicate that cell proliferation is particularly dependent on the presence of these coupled bFGF-heparin-like molecules and that activation of the mitogenic response cannot be achieved by bFGF alone [36, 38]. The biphasic response demonstrated in these studies may be due to this interplay of multiple receptors and transducing systems. In all cell types studied to date, there are considerably fewer high affinity receptors compared with low affinity receptors, as is evident for 3T3 fibroblasts (2×10^4 versus 1×10^6, respectively [39]). Clearly, at low doses, the 16mer peptide will compete with bFGF for binding to the appropriate receptors, thus reducing the proliferative effect of bFGF. From the data it is evident that the highest concentrations of the 16mer peptide saturate the available receptor sites, significantly reducing the binding of bFGF and thus dramatically attenuating the proliferative response. Further studies are required to establish whether the 16mer peptide will bind to both low and high affinity bFGF receptors and to determine the affinities with which the 16mer peptide binds to each receptor type. These data may provide an explanation for the recovery of [^{3}H]thymidine incorporation by fibroblasts which occurs in the presence of a stimulatory dose of bFGF and concentrations of 16mer peptide between 5–40 nmol.

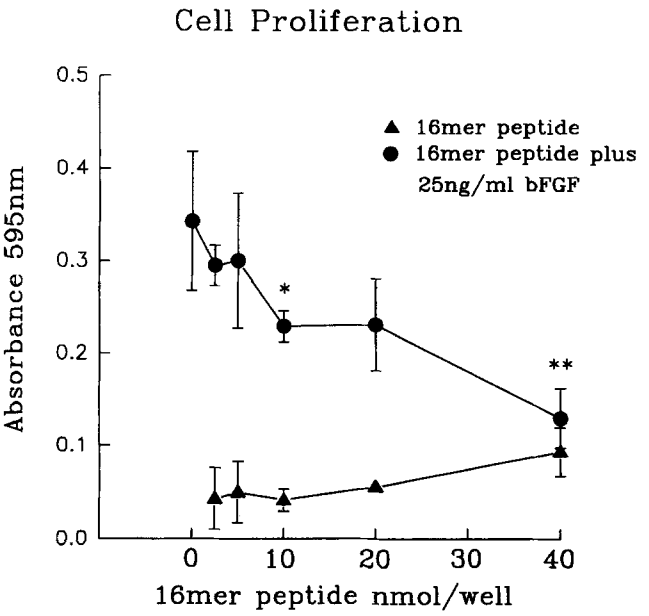


Fig. 6. The effect of the synthetic 16mer peptide on the proliferation of fibroblasts in culture, in the presence or absence of bFGF (25 ng/ml). Peptide and/or bFGF were added to cell cultures as described in Materials and methods. The absorbance values for control wells (cells incubated with media only) have been subtracted. Mean ± SD, n v 8 replicates, * $p < 0.05$, ** $p < 0.01$, compared to control wells.

These studies document the biological properties of a small peptide analogue related to bFGF. This peptide was designed according to specific property-pattern characteristics to block (antagonise) bFGF binding to its corresponding receptors. As evident from the results, the composite algorithmic approach incorporating molecular modelling procedures permits peptides to be designed with conformational structures similar to those found for the polypeptide-protein of interest. The effects of this synthetic 16mer peptide on other bFGF responsive cell lines, including aortic endothelial cells are currently in progress. This approach may prove of gen-

eral utility in the design and development of new peptide agents for therapeutic purposes.

Acknowledgements

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References

1. Sato Y, Rifkin DB: Autocrine activities of basic fibroblast growth factor: Regulation of endothelial cell movement, plasminogen activator synthesis and DNA synthesis. J Cell Biol 107: 1199–1205, 1988
2. Gospodarowicz D: Fibroblast and epidermal growth factors: Their uses *in vivo* and *in vitro* in studies on cell functions and cell transplantation. Mol Cell Biochem 25: 79–110, 1979
3. Montesano R, Vassali JD, Baird A, Guillemin R, Orci L: Basic fibroblast growth factor induces angiogenesis *in vitro*. Proc Natl Acad Sci (USA) 83: 7297–7301, 1986
4. Gospodarowicz D, Moran JS: Mitogenic effect of fibroblast growth factor on early passage cultures of human and murine fibroblasts. J Cell Biol 66: 451–456, 1975
5. Delli-Bovi P, Curatola AM, Kern FG, Greco A, Ittmann M, Basilico C: An oncogene isolated by transfection of Kaposi's sarcoma DNA encodes a growth factor that is a member of the FGF family. Cell 50: 729–737, 1987
6. Dickson C, Peters G: Potential oncogene product related to growth factors. Nature (Lond.) 326: 833, 1987
7. Delli-Bovi P, Curatola AM, Newman KM, Sato Y, Moscatelli D, Hewick RM, Rifkin DB, Basilico C: Processing, secretion and biological properties of a novel growth factor of the fibroblast growth factor family with oncogenic potential. Mol Cell Biol 8: 2933–2941, 1988
8. Slack JMW, Darlington BG, Heath JK, Godsave SF: Heparin-binding growth factors as agents of mesoderm induction in early *Xenopus* embryo. Nature (Lond.) 326: 197–200, 1987
9. Neufield G, Ferrara N, Mitchell R, Schweigerer L, Gospodarowicz D: Bovine granulosa cells produce basic fibroblast growth factor. Endocrinology 121: 597–603, 1987
10. Gospodarowicz D, Cheng J, Lui GM, Bohlen P: Corpus luteum angiogenic factor is related to fibroblast growth factor. Endocrinology 117: 201–213, 1985
11. Baird A, Mormede P, Bohlen P: Immunoreactive fibroblast growth factor in cells of peritoneal exudate suggests its identity with macrophage-derived growth factor. Biochem Biophys Res Commun 126: 358–364, 1985
12. Lobb R, Sasse J, Shing Y, D'Amore P, Fett J, Sullivan R, Jacobs J, Klagsbrun M: Purification and characterisation of heparin-binding endothelial cell growth factors. J Biol Chem 261: 1924–1928, 1986
13. Gimbrone Jr MA, Cotran RS, Leapman SB, Folkman J: Tumour dormancy *in vivo* by prevention of neovascularisation. J Exp Med 136: 261–276, 1972
14. Folkman J, Cotran RS: Relation of vascular proliferation to tumour growth. Int Rev Exp Path 16: 207–248, 1976
15. Folkman J, Klagsbrun M: Angiogenesis factors. Science 235: 442–447, 1987
16. Folkman J: In: R Steiner, PB Weisz, R Langer (eds.) Angiogenesis Key Principles: science, technology, medicine. Birkhauser Verlag Busch., 1992, pp 4–13
17. Cosic I, Hodder A, Aguilar M, Hearn MTW: Resonant recognition model and protein topography: Model studies with myoglobin, hemoglobin and lysozyme. Eur J Biochem 198: 113–119, 1990
18. Cosic I, Hearn MTW: Studies on protein DNA interactions using the resonant recognition model: Application to repressors and transforming proteins. Eur J Biochem 205: 613–619, 1992
19. Cosic I: Resonant recognition model of protein-protein and protein DNA interaction. In: D Wise (ed.) Bioinstrumentation and Biosensors. Marcel Dekker Inc., New York, 1990, pp 475–510
20. Heine V, Koen M, Uir D: Theory of Pseudopotentials. MIR Moscow, 1973
21. Cosic I, Pavlovic M: Informational spectrum method applied to growth factors. Proc Med Biol Eng Puntex, Barcelona, 309–312, 1986
22. Cosic I, Pavlovic M, Vojisavljevic V: Prediction of 'hot spots' in interleukin 2 based on informational spectrum characteristics of growth regulating factors. Biochimie 71: 333–342, 1989
23. Veljkovic V, Slavic I: General model of pseudopotentials. Physical Rev Lett 29: 105–108, 1972
24. Rabiner L, Gold B: Theory and Application of Digital Signal Processing. Prentice-Hall, New York, 1975
25. Karplus M, McCammon JA: Dynamics of proteins: Elements and function. Ann Rev Biochem 53: 263–300, 1983
26. Risbridger GP, Drummond AE, Kerr JB, de Kretser DM: Effect of cryptorchidism on steroidogenic and mitogenic activities in rat testicular interstitial fluid. J Reprod Fert 87: 617–624, 1987
27. Baird A, Schubert D, Ling N, Guillemin R: Receptor and heparin-binding domains of basic fibroblast growth factor. Proc Natl Acad Sci (USA) 85: 2324–2328, 1988
28. Zhu X, Komiya H, Chirino A, Faham S, Fox GM, Arakawa T, Hsu BT, Rees DC: Three dimensional structures of acidic and basic fibroblast growth factors. Science 251: 90–93, 1991
29. Gospodarowicz D: Purification of a fibroblast growth factor from bovine pituitary. J Biol Chem 250: 2515–2520, 1975
30. Conover A, Hintz RL, Rosenfield RG: Direct evidence that the insulin receptor mediates a mitogenic response in cultured human fibroblasts. Horm Metab Res 21: 59–3, 1989
31. Uneno S, Yamamoto I, Yamamuro T, Okumura H, Ohta S, Lee Y, Kasai R, Konish J: Transforming growth factor beta modulates proliferation of osteoblastic cells: Relation to its effect on receptor levels for epidermal growth factor. J Bone Miner Res 4: 165–171, 1989
32. Zhang ZW, Herrington AC, Carson RS, Findlay JK, Burger HG: Direct inhibition of rat granulosa cell inhibin production by epidermal growth factor. Mol Cell Endocrinol 54: 213–220, 1987
33. Tramontano D, Moses AC, Veneziani BM, Ingbar SH, Charles A: Adenosine 3',5'-monophosphate mediates both the mitogenic effect of thyrotropin and its ability to amplify the response to insulin-like growth factor 1 in FRTL5 cells. Endocrinology 122: 127–132, 1988
34. Gospodarowicz D, Chen I: Heparin protects acidic and basic FGF from inactivation. J Cell Physiol 128: 475–484, 1986
35. Saksela O, Moscatelli D, Sommer A, Rifkin D: Endothelial cell-

derived heparan sulfate binds basic fibroblast growth factor and protects it form proteolytic degradation. J Cell Biol 107: 743–751, 1988

36. Mansukhani A, Dell'Era P, Moscatelli D, Kornbluth S, Hanafus H, Basilico C: Characterisation of the murine BEK fibroblast growth factor (FGF) receptor: Activation by three members of the FGF family and requirement for heparin. Proc Natl Acad Sci (USA) 89: 3305–3309, 1992
37. Yayon A, Klagsbrun M, Esko JD, Leder P, Ornitz DM: Cell surface, heparin-like molecules are required for binding of basic fibroblast growth factor to its high affinity receptor. Cell 64: 841–848, 1991
38. Klagsbrun M: Mediators of angiogenesis: The biological significance of basic fibroblast growth factor (bFGF)-heparin and heparan sulfate interactions. Semin Cancer Biol 3: 81–87, 1992
39. Moscatelli D: High and low affinity binding sites for basic fibrobast growth factor on cultured cells: Absence of a role for low affinity binding in the stimulation of plasminogen activator production by bovine capillary endothelial cells. J Cell Physiol 131: 123–130, 1987