Identification of a Heparin Binding Peptide on the Extracellular Domain of the KDR VEGF Receptor

A. MAUREEN DOUGHERTY, HEATHER WASSERSTROM, LAWRENCE TORLEY, LAITHA SHRIDARAN, PATRICK WESTDOCK, RONALD E. HILEMAN, JONATHAN R. FROMM, ROBERT ANDERBERG, STEWART LYMAN, ROBERT J. LINHARDT, JEFFREY KAPLAN, BRUCE I. TERNAN

*Wyeth-Ayerst Research, Pearl River, New York 10965; Division of Medicinal and Natural Products Chemistry, College of Pharmacy, University of Iowa, Iowa City, Iowa 52242-1112; Department of Molecular Genetics, Immunex Corporation, Seattle, Washington 98101-2936

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Vascular endothelial growth factor (VEGF), a potent and specific activator of endothelial cells, is expressed as multiple homodimeric forms resulting from alternative RNA splicing. VEGF121 does not bind heparin while the other three isoforms do, and it has been documented that the binding of VEGF165 to its receptor is dependent upon cell surface heparan sulfate proteoglycans. Little is known about the biochemical mechanism that allows for heparin regulation of growth factor binding. For example, it is not clear whether heparin interactions with growth factor or with cell surface receptors or both are essential for VEGF binding to its receptor. In this manuscript we provide results which are consistent with the hypothesis that an interaction between heparin and a site on the KDR receptor subtype is essential for VEGF binding. First, we demonstrate that expression of KDR into a CHO cell line deficient in heparan sulfate biosynthesis does not allow VEGF165 binding unless heparin is exogenously added during the binding assay. Secondly, we show that a ten amino acid synthetic peptide, corresponding to a sequence from the extracellular domain of the KDR, both inhibits VEGF165 binding to the receptor and also binds heparin with high avidity. Third, affinity purification of heparin molecules on a KDR-derived peptide affinity column, together with capillary electrophoresis and polyacrylamide electrophoresis analysis, was used to show that the KDR-derived peptide interacts with a specific subset of polysaccharide chains contained in the unfractionated heparin. Taken together, these results are consistent with the hypothesis that interactions between cell surface heparan sulfate proteoglycans and the VEGF receptor contribute to allowing maximal VEGF binding.

Keywords: VEGF, KDR Receptor Subtype

*Corresponding author: Dr. Bruce I. Terman, Wyeth-Ayerst Oncology Research, Pearl River, New York 10965. Tel: (914) 732-4589. Fax: (914) 732-2237.
INTRODUCTION

Vascular endothelial growth factor (VEGF), also known as vascular permeability factor, is a potent stimulator of angiogenesis in vitro (Senger et al., 1983; Gospodarowicz et al., 1989; Ferrara et al., 1992). Biological activities of VEGF on vascular endothelial cells include an increased proliferation rate of cells in culture and an increase in vascular permeability (Ferrara et al., 1992). High affinity binding sites for VEGF are ubiquitously distributed in vascular endothelial cells (Vaisman et al., 1990). The DNA encoding two VEGF receptors (FLK/TKDR and FLT1) have been cloned and expressed (Terman et al., 1989; Matthews et al., 1989; Shibuya et al., 1990; De Vries et al., 1992). The two receptor subtypes share many similarities, including tyrosine kinase activity, a kinase insert domain, and seven extracellular immunoglobulin domains.

There are four alternatively spliced forms of VEGF (Tischer et al., 1991). The 121-amino acid form (VEGF121) lacks a heparin binding domain, while VEGF165, VEGF189, and VEGF206 bind efficiently to heparin (Houck et al., 1991). All four forms induce permeabilization of blood vessels while only VEGF121 and VEGF165 stimulate endothelial cell mitogenic activity (Houck et al., 1991).

VEGF165 is the most prevalent protein form and its effects on angiogenesis in vivo as well as its binding characteristics to endothelial cells grown in culture have been well characterized. The binding of VEGF165 to its high affinity receptor is dependent upon cell surface heparan sulfate proteoglycans (HSPG), and this effect can be mimicked by exogenously added heparin (Gitay-Goren et al., 1992).

It is not clear whether the effect of heparin on VEGF165 binding to KDR is due to interactions of heparin with the growth factor or the receptor. Evidence supporting an essential interaction between heparin and growth factor have come from studies documenting the difference in binding between VEGF165 and VEGF121 (Cohen et al., 1995). An essential interaction between heparin and the receptor cannot be dismissed. In the accompanying manuscript we showed that a chimeric receptor consisting of the extracellular domain of KDR fused to the Fc portion of human IgG (KDR-Fc) binds VEGF165, even in the absence of heparin (Kaplan et al., 1997). Heparin augments binding to KDR-Fc 25-100%, and this increase in activity requires structural elements in the receptor IgG-like domains 4-7. The hypothesis that there are heparin binding domains on the VEGF receptor is strengthened by the finding that the binding of bFGF to its receptor requires heparin interactions with sites on both the growth factor and the receptor (Ornitz et al., 1992 and Kan et al., 1993).

An examination of the amino acid sequence of the extracellular domain of the KDR revealed R-K-T-K-R [amino acids 647-652 (Terman et al., 1992)], a highly basic sequence which fulfills some of the requirements expected of a heparin binding peptide (Margalit et al., 1993). This peptide is contained in a region of KDR between the sixth (from the amino end) and seventh immunoglobulin-like domains on the receptor. The goal of the current study was to test the hypothesis that the interaction of heparin with the region of KDR containing the peptide sequence plays a role in augmenting VEGF165 binding to the receptor.

EXPERIMENTAL PROCEDURES

Materials

VEGF165 was obtained from Peprotech (Rocky Hill, NJ). 125I-VEGF165 was obtained from Amersham Corp. (Arlington, Ill.). Heparin was purchased from Hepar Corp. (Franklin, OH). Human recombinant
Pgf was from R&D Systems (Minneapolis, MN). Synthetic peptides were synthesized as C-terminal acids and purified to greater than 90% homogeneity by Multiple Peptide Systems: San Diego, CA. The anti-KDR antibody was raised in rabbits (Berkeley Antibody Company, Richmond, CA) against a purified bacterially expressed protein fragment (amino acids 803-1356 (Terman et al., 1992)) of KDR. All other reagents were from standard sources.

Heparin lyase I (heparinase I, EC 4.2.2.7) was from Sigma Chemical Co. (St. Louis, MO). Heparin lyase II (no EC assigned) was a gift from IBEX technologies (Montreal, Canada). The disaccharide standard ΔUA2S(1-4)-D-GlcNpS6S was prepared from heparin and characterized (Linhardt et al., 1990; Merchant et al., 1985; Loganathan et al., 1990). Disaccharides ΔUA2S(1-4)-D-GlcNpS and ΔUA(1-4)-D-GlcNpAc6S were from Sigma Chemical Co. Disaccharides ΔUA(1-4)-D-GlcNpS, ΔUA2S(1-4)-D-GlcNpAc and ΔUA(1-4)-D-GlcNpAc were from Grampian Enzymes (Aberdeen, Scotland). Tetrascarahrade ΔUA2S(1-4)-D-GlcNpS6S(1-4)-L-IdoAp2S (1-4)-D-GlcNpS6S, hexaCascarhrade ΔUA2S(1-4)-D-GlcNpS6S(1-4)-L-IdoAp2S(1-4)-D-GlcNpS6S (1-4)-L-IdoAp2S(1-4)-D-GlcNpS6S and octaCascarhrade ΔUA2S(1-4)-D-GlcNpS6S(1-4)-L-IdoAp2S(1-4)-D-GlcNpS6S(1-4)-L-IdoAp2S(1-4)-D-GlcNpS6S were prepared and characterized as previously described (Pervin et al., 1995). Acrylamide (ultrapure), Tris (hydroxymethyl)aminomethane, Aldian Blue, Bromophenol Blue and ammonium persulfate were obtained from Boehringer Mannheim (Indianapolis, IN). EDTA, sucrose, N,N,N′-tetramethylthlenediamine, sodium phosphate and silver nitrate were from Fisher Chemicals Co. (Fairlawn, NJ).

Cloning and Expression of VEGF-C: A sequence (Z4272) that was related to the human VEGF sequence was identified in a collection of expressed sequence tags (EST) in the NCBI database (Bethesda, MD). Primers identical to the 5' end (5' CCGTCTACACATGGGGGGTT 3') and complementary to the 3' end (5' CAGGCTACTCTGCAGATGTGATT 3') were synthesized and used to PCR amplify the EST out of a human embryonic lung cell W126-VA4 lambda g10 cDNA library. The resulting 299 bp fragment was purified and cloned into the pCRII cloning vector using a TA cloning kit (Invitrogen, San Diego, CA). The resulting cloned fragment was excised using EcoRI and randomly primed using a Prime-it quick kit (Stratagene, La Jolla, CA). Approximately 200,000 plaque forming units from the W126 VA4 cDNA library were screened using the probe described above. 15 positive plaques were identified, and several of these were cloned into the EcoRI site of pBluescript SK(-) (Stratagene; La Jolla, CA).

The portion of the VEGF-C cDNA clone that contained the open reading frame was moved into a pDC302 expression vector. A FLAG sequence was added to the cDNA to facilitate purification of the expressed protein. This construct was transfected into CV-1/EBNA cells, and after three days the supernatants from these cells were collected and the VEGF-C protein was purified using anti-FLAG antibodies. Expression of the VEGF-C protein was confirmed by radioimaging a separate plate of cells with [35S]-Methionine/Cysteine at a concentration of 150 μCi/ml for 4 hours. Supernatants containing the radiolabeled proteins were run out on a 4-20% Tris/Glycine gel (NOVEX, San Diego, CA) under reducing conditions. Gels were fixed, dried, and autoradiographed.

Cell Lines

Bovine aortic endothelial (BAE) cells were grown in Dulbecco’s modified Eagle’s media (DMEM; Gibco BRL; Gaithersburg, MD) containing 10% calf serum, 1 ng/ml bFGF, 4.5 g/l glucose, penicillin G (100 U/ml) and streptomycin sulfate (1 mg/ml). For radioligand binding assay, the cells were plated and grown in wells containing this media minus bFGF. CHO745 cells (Esko et al., 1985) were a gift from Dr. J. Esko, University of California at San Diego. KDR-expressing cells were obtained by cotransfecting CHO745 cells with a eukaryotic expression vector (Terman et al., 1994) containing a 4.5 kb insert encompassing the full coding sequence of KDR with an expression vector containing DNA sequences for conferring G418 resistance. KDR-expressing cells
were selected by analyzing G418 resistance cells for expression of protein using a KDR-specific antibody together with their ability to bind $^{125}$I-VEGF-165. Cells were grown in Ham's F12 medium plus 7.5% FCS plus 150 μg/ml G418

**Western Blot Analysis**

Cells were grown to confluence on 24-well plates. The cells were washed three times with ice cold PBS and 200 μl of gel sample buffer (0.5M TRIS, pH 6.9, 2% SDS, 10% glycerol, 5% 2-mercaptoethanol, plus 0.02% bromophenol blue) were added. The samples were transferred to eppendorf tubes and boiled for 3 minutes. Samples, 40 μl, were loaded onto 7% SDS-PAGE gels. After electrophoresis, proteins were transferred to nitrocellulose filters. The filters were blocked for 2 hours using Tris-buffered saline plus 2% TWEEN (TBS-T) plus 5% dried milk. The primary antibody was a polyclonal antibody raised against the cytosolic domain of KDR expressed in bacteria. Detection of receptor was done using an ECL kit (Amersham; Chicago, Ill.).

**Radioligand Binding and Affinity Crosslinking**

Cells were plated in 24-well (6 x 10^5 cells/well) dishes. Twenty four hours later, the cells were washed twice with PBS, and 0.4 ml of DMEM containing 0.15% gelatin and 25 mM HEPES, pH 7.4, was added. $^{125}$I-VEGF and other additions were added to appropriate dishes adjusting the final volume to 0.5 ml. Cells were incubated with the radioligand for 60 minutes at room temperature, washed three times with ice cold PBS containing 0.1% BSA, and extracted from the dishes by incubating them for 30 minutes with 0.1% Triton X-100 in 10 mM sodium phosphate, pH 8.0. Radioactivity was determined in a gamma counter. Specific $^{125}$I-VEGF binding is defined as the difference seen in the presence and absence of 5 nM nonradioactive VEGF. Duplicate samples were analyzed. Each of the experiments utilizing radioligand binding is representative of at least three separate experiments.

For affinity crosslinking, radioligand binding was carried out as just described. At the end of the binding reaction, the wells were washed twice with PBS, and 0.5 mM disuccinimidyl suberate was added. After 15 minutes, the samples were washed twice with 300 mM sucrose plus 20 mM TRIS-HCL, pH 7.4. The sample was then subjected to SDS-PAGE autoradiography. Similar results were observed in each of the two separate experiments done.

**Purification of Active Heparin Moieties on a Peptide Affinity Column**

The peptide affinity column was prepared by coupling 4 mg of the QDRKTKKRHC peptide to 1.5 ml sulfolink gel (Pierce; Chicago, Ill.) as recommended by the manufacturer. The column was stored in 25 mM TRIS-HCl, pH 7.4, and 1 mM EDTA (wash buffer). Fractionation of heparin was done as follows. All steps were done at 4°C. A 0.5 ml solution containing 5 mg heparin (Hepar Corp.; Franklin, Ohio) was allowed to penetrate the resin and the column was left standing for one hour. The column was washed with 20 ml of wash buffer. Fractionation of bound heparin was done using a 0 to 2 M NaCl (in wash buffer) gradient. The gradient was run for 1 hour and 1.4 ml fractions were collected. The flow rate of liquid through the column was 0.7 ml per minute.

The quantity of heparin in each fraction was measured by a uronic acid carbazole reaction. Three ml of concentrated sulfuric acid was cooled to 4°C in an ice bath. Aliquots, 100 μl, of each fraction were carefully layered onto the acid. The samples were vortexed and heated to 100°C for 20 minutes. The samples were then cooled to 4°C. Carbazole in methanol, 100 μl of 0.125%, was added. The tubes were again heated in a boiling water bath for 10 min. The samples were cooled and UV absorption at 530 nm was measured.

The results shown (Fig. 4) are representative of six separate experiments using three independently constructed affinity columns.

**Structural Analysis of Heparin Fractions**

Enzymatic digestion of the KDR binding heparins: All digestions were done separately in 20 μl of
5 mM sodium phosphate, 100 mM NaCl, pH 7.4. Each sample (active, not active, and start) contained approximately 10 mg of heparin. Each of the three tubes received 1.3 mUnits heparin lyase II (IBEX) and 350 mUnits (Sigma Units) of heparin lyase I. The lyases were in gross excess. Digestions were carried out at 30°C for 27.5 h. Digestions were terminated by heating the digestion mixture for 5 min at 100°C.

Capillary electrophoresis: The experiments were performed on a Dionex capillary electrophoresis (CE) system (Sunnyvale, CA) equipped with a variable wavelength ultraviolet detector. System operation and data analysis were fully controlled using version 3.1 A1-450 chromatography software on a PC. The CE system was operated in the reverse polarity mode by applying the sample at the cathode and running using 20 mM phosphoric acid adjusted to pH 3.5 by a saturated solution of dibasic sodium phosphate as previously described (Pervin et al., 1994). Samples were applied to the capillary (75 µm i.d., 375 µm o.d., 68 cm long) using gravity injection (10s) by hydrostatic pressure (45 mm), resulting in a 9 nL sample volume. Each experiment was conducted at a constant 18,000 volts and data was collected at 232 nm. Peaks were identified by co-injection of standards.

Gradient PAGE analysis: Gradient polyacrylamide gel electrophoresis (PAGE) analysis was performed using a Protean II vertical slab gel unit, equipped with Model 1420B power source from BioRad (Richmond, CA). A polyacrylamide linear gradient gel (14 x 28 cm, 14.5 - 22% total acrylamide) was prepared and run as previously described (Pervin et al., 1995). The molecular sizes were determined by comparing oligosaccharide standards previously characterized (Edens et al., 1992). The oligosaccharides were visualized by Alcian blue staining followed by silver staining (Edens et al., 1992).

RESULTS

Expression of KDR in CHO745 Cells

The role of HSPG in binding VEGF_{165} to KDR was studied using a CHO cell line (CHO745 cells) which lacks the ability to synthesize heparan sulfates (Esako et al., 1985). These cells do not contain VEGF binding sites as determined by radioligand binding in the presence of heparin or by Western blot analysis using an anti-VEGF receptor antibody. CHO745 cells were transfected with KDR-containing expression vector and receptor expression was monitored using an anti-KDR antibody. Western blot analysis demonstrated expression of two molecular weight proteins (160 and 180 kDa) in KDR-transfected cells (Fig. 1A). The structural differences between the two molecular weight forms is not known, but may be due to altered glycosylation. Affinity cross-linking studies using DSS demonstrated that 125I-VEGF_{165} binds to both of these expressed proteins.

![Figure 1](image_url)  
**Figure 1:** Expression of KDR in CHO745 cells. Panel A: Western blot analysis of KDR expression. KDR-transfected CHO745 cells (lane 2), and cells transfected with vector alone (lane 1) were grown in 2 wells of a 24-well plate until confluence. The cells were washed twice with PBS and then solubilized in 200 µL of gel sample buffer. Western blot analysis was done using an anti-KDR antibody. Panel B: Affinity cross-linking 125I-VEGF to KDR-transfected CHO745 cells. Cells were grown in a 24-well plate until confluent. The cells were washed twice with PBS and then incubated with 125I-VEGF with or without heparin for 90 minutes at room temperature. The cells were washed twice with PBS and 0.5 mM DSS was added. After 15 minutes, the cells were washed with 300 mM sucrose plus 20 mM TRIS, pH 7.4. The cells were scraped from the wells and suspended in SDS-PAGE autorigidography. Lane 1 is the result of affinity cross-linking after carrying out the binding of 125I-VEGF in the absence of heparin. For Lanes 2 and 3, 1 µg/ml heparin (HEPAR Corporation) was added during the binding reaction. For Lane 3, the binding reaction included 5 mM VEGF. For Lane 4, 1 µg/ml purified heparin (see Figure 4) was included in the binding reaction.
in a heparin-dependent manner (Fig. 1B). Scatchard (Scatchard, 1949) analysis of ^125^I-VEGF^165^ binding to the KDR-transfected CHO745 cells indicated an affinity of 1000 PM and a receptor density of 8,000 per cell (Fig. 2A). The dose dependency of heparin in augmenting growth factor binding is shown in Figure 2B. VEGF is one member of a family of four (VEGF, PIGF, VEGF-B, and VEGF-C) related growth factors. In order to further demonstrate that KDR expressed in the CHO cell mutants are functionally identical to the endothelial cell receptor, we did a preliminary experiment to characterize the binding properties of the KDR-transfected CHO cells towards VEGF related proteins. It has been previously shown that VEGF-C but not PIGF binds to KDR (Park et al., 1994; Kendall et al., 1994; Joukov et al., 1996), and this was the case for the KDR-transfected CHO745 cells (Fig. 2C).

**Identification of a Heparin Binding Peptide in the Extracellular Domain of KDR**

We have previously shown that a chimeric protein consisting of the extracellular domain of KDR and the Fc portion of human IgG (KDR-Fc) efficiently binds VEGF^165^ in the absence of added heparin, and that heparin augments binding 25-100% (Kaplan
It was shown that the ability of heparin to augment binding was lost for a truncated receptor (consisting of receptor IgG-like domains 1–3) suggesting that IgG-like domains 4–7 contains structural features required for the heparin effect. An examination of the amino acid sequence of the receptor’s extracellular domain revealed the sequence QDRKTKKIHC between the 6th and 7th IgG-like domains. This sequence fulfills some of the properties expected of a heparin binding peptide (Margalit et al., 1993).

Figure 3A shows that a synthetic peptide corresponding to this region of the receptor inhibits $^{125}$I-VEGF$_{165}$ binding to BAE cells. Radioligand binding was done in the presence of 0, 25, and 100 µg/ml heparin. The ability of the peptide to inhibit radioligand binding to BAE cells, even in the absence of added heparin, is consistent with the peptide’s ability to bind endogenous HSPG, thereby blocking their ability to augment growth factor binding to the receptor. The finding that the potency of peptide inhibition diminished with increasing heparin concentrations is consistent with competition between the added heparin and cellular HSPG for the peptide. Results similar to these were also observed using KDR-transfected CHO745 cells (data not shown).

In the accompanying manuscript we showed that heparin, while not being required for $^{125}$I-VEGF$_{165}$ binding to the KDR-Fc, does enhance binding of...
25–100%. Figure 3B shows that the synthetic peptide had no effect on radioligand binding to the KDR-Fc in the absence of heparin, but did inhibit heparin's ability to stimulate binding. This result is consistent with the hypothesis that the peptide inhibits VEGF₁₆₅ binding to KDR by interacting with heparin and not with either the growth factor or the receptor.

Figure 3C shows that the synthetic peptide inhibited the proliferation of BAE cells. An effect by peptide was observed only when VEGF₁₆₅ was added to the cells.

To obtain more direct evidence that the KDR-derived peptide is heparin binding, we coupled the peptide to agarose and tested for heparin binding to the affinity resin. Heparin was mixed with the affinity column in low salt buffer, and then eluted with a NaCl gradient. Greater than 90% of the starting heparin bound to the column (less than 5% of labeled heparin binds to resin not coupled with the peptide), and the peak of heparin eluted at 0.8 M NaCl (Fig. 4). These results confirmed that the peptide does bind heparin.

We also tested an aliquot of each fraction from the affinity elution for its effect on the binding assay. Figure 4 shows that fractions 29–37 augmented radioligand binding; thus, it was possible to fractionate active heparin samples from the bulk of the heparin using the peptide affinity column. Figure 5 compares the dose dependence of the starting heparin with aliquots from fraction 32 in augmenting ¹²⁵I-VEGF₁₆₅ binding to BAE cells. The concentration of starting heparin giving a one-half maximal increase in binding was 0.5 μg/ml; for fraction 32 it was 0.05 μg/ml. A similar dose dependence is seen when using KDR-transfected CHO745 cells (data not shown).

CE analysis of the heparin lyase I and II depolymerized samples are shown in Figure 6. CE separates the seven standards corresponding to the disaccharides commonly found in heparin and heparan sulfate (Fig. 6a) in amounts given in Table I. Application of this sample to the peptide affinity column affords a low affinity fraction that shows these four peaks together with a number of additional minor peaks observed in the baseline (Fig. 6c). The high affinity fraction also shows the same four peaks together with a prominent, fast moving component migrating at 5 min (Fig. 6b). A migration time of

![Figure 4](image-url)  
**Figure 4.** Fractionation of heparin using a peptide affinity column. 5 mg of heparin was dissolved in 0.5 ml 20 mM Tris, pH 7.0 and loaded onto 2 ml of the affinity resin. The sample was allowed to sit in the resin for one hour, and the resin was then washed with 20 ml of wash buffer. Elution of the column with a NaCl gradient was done as described in the Experimental Procedures. 100 μl of each fraction was assayed for heparin mass using the uronic acid carbazole method (closed circles). 10 μl of each fraction was tested for its effect on ¹²⁵I-VEGF₁₆₅ binding to BAE cells (open circles). The data shown is the average of duplicate samples.

![Figure 5](image-url)  
**Figure 5.** Comparison of the crude vs purified heparin in stimulating ¹²⁵I-VEGF₁₆₅ binding to BAE cells. Samples of the heparin sample loaded onto the column (diamonds) and fraction 32 (squares) were tested for their effect on radioligand binding to BAE cells. 40 μM ¹²⁵I-VEGF₁₆₅ was used. The heparin mass in fraction 32 was determined using the uronic acid carbazole technique. The data shown is the average of duplicate samples.
5 min is consistent with a higher oligosaccharide, i.e., a tetrasaccharide or a hexasaccharide.

PAGE analysis of these samples was also performed (Fig. 7). Standard disaccharide 1, tetrasaccharide, hexasaccharide and octasaccharide is analyzed in lane a. While each standard is present in approximately equal amounts, the band corresponding to the disaccharide (labeled 2 in lane a) is faint. This is expected since the relative staining intensity is proportional to the net negative charge of the sample being visualized. The low affinity sample (Fig. 7, lane c) shows many intense bands and is nearly identical to the unfractionated sample (not shown). The high affinity sample shown in lane b is stained faintly because of the small quantity of this sample available for analysis. Bands corresponding to the disaccharide 1, hexasaccharide and octasaccharide standards can be seen, and are marked with small arrows (Fig. 7, lane b). Interestingly, the tetrasaccharide standard is not observed. Instead a larger oligosaccharide running just above the position for the tetrasaccharide standard, probably corresponding in size to an undersulfated hexasaccharide, is seen and is indicated with the large arrow.

These results suggest that KDR interacts with a specific subset of polysaccharide chains contained within unfractionated heparin. CE analysis of the disaccharide composition of this subset of high affinity chains shows that it contains a heparin lyase I and II resistant oligosaccharide and PAGE analysis...
which is consistent with the hypothesis that receptor IgG-like domains 4–7 contain a heparin binding amino acid sequence. Second, the peptide inhibited binding of [125I]-VEGF165 to KDR-expressing cells by competing with cell surface HSPG. Third, heparin samples active in [125I]-VEGF165 binding assays were fractionated using a peptide affinity column.

The concentration of peptide required to inhibit [125I]-VEGF165 binding is similar to that reported necessary for synthetic peptide inhibition of other heparin binding proteins. A FGF-derived peptide inhibits [125I]-FGF binding to its receptor at a dose slightly greater than that seen for the KDR-derived peptide (Baird et al., 1988). In addition, a FGF receptor-derived peptide inhibits radioligand binding at a dose very similar to that seen for the KDR-derived peptide (Kan et al., 1993).

The described results do not rule out the possibility that multiple interactions between heparin and either receptor and/or growth factor are required for [125I]-VEGF binding, and heparin binding to the 10 amino acid peptide is just one of those interactions. There are four alternatively spliced forms of VEGF (Houck et al., 1991; Tischer et al., 1991), and three of these (including the 165-amino acid form used in this study) are heparin-binding. The role of the heparin binding domains within VEGF for binding of growth factor to receptor is not known. It is of significance that the 121 isoform of VEGF, while not a heparin binding protein, requires cell-surface heparan sulfates for efficient binding to its receptor (Cohen et al., 1995). This finding is consistent with an essential interaction of heparan sulfate with VEGF receptors.

The biochemical mechanism by which heparin interactions with the receptor augments binding of growth factor is not clear; though several possible mechanisms can be speculated. It is possible that one molecule of heparin binds to two molecules of receptor, thus facilitating dimerization of the receptor. An alternative mechanism is that the highly basic peptide sequence prevents two receptor proteins from interacting through electrostatic repulsion, and binding of heparin to the receptor

suggested the presence of a hexasaccharide having a reduced level of sulfation. Further studies will be required to elucidate the precise structure(s) within the high affinity fraction responsible for its interaction with KDR.

**DISCUSSION**

The results presented are consistent with a model which states that the interaction of heparin with a 10 amino acid peptide on the extracellular domain of KDR is involved in allowing for VEGF165 binding to the receptor. Three experimental findings from this and the preceding manuscript support this hypothesis. First, heparin had no effect on VEGF165 binding to the three IgG-like domain form KDR-Fe.
neutralizes the positive charge. A third possible mechanism is that heparin causes a conformational change in the ligand binding site of the receptor allowing VEGF to bind with high affinity.

References


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