Proc. Natl. Acad. Sci. USA
Vol. 88, pp. 2227–2231, March 1991
Biochemistry

An angiogenin-binding protein from endothelial cells

(angiogenesis/receptors/heparan sulfate)

GUO-FU HU, SOO-IK CHANG, JAMES F. RIORDAN, AND BERT L. VALLEE*

Center for Biochemical and Biophysical Sciences and Medicine, Harvard Medical School, 250 Longwood Avenue, Boston, MA 02115

Contributed by Bert L. Vallee, December 26, 1990

ABSTRACT **A 42-kDa bovine protein that binds bovine angiogenin [angiogenin binding protein (AngBP)] has been identified as a dissociable cell-surface component of calf pulmonary artery endothelial cells and a transformed bovine endothelial cell line, GM7373. Binding of ^{125}I-labeled bovine angiogenin (^{125}I-Ang) to AngBP occurs with an apparent $K_d \approx 5 \times 10^{-10}$ M and is specific, saturable, and inhibited by excess unlabeled angiogenin. ^{125}I-Ang can be crosslinked efficiently to AngBP by a water-soluble carbodiimide, 1-ethyl-3-(3-dimethylaminopropyl)carbo-diimide. Bovine ribonuclease A competes with the binding of ^{125}I-Ang to AngBP, but lysozyme does not. Direct binding to AngBP of ^{125}I-labeled bovine ribonuclease A is, however, much weaker than that of ^{125}I-Ang. Two enzymatically active derivatives of angiogenin cleaved at residues 60–61 and 67–68, respectively, fail to induce angiogenesis and also bind to AngBP only weakly. AngBP has been isolated by treatment of cells with heparan sulfate, affinity chromatography on angiogenin-Sepharose of the material dissociated from the cell surface, and gel filtration HPLC. The results suggest that AngBP has the characteristics of a receptor that may likely function in angiogenesis.**

Angiogenin, a 14-kDa protein first isolated from HT-29 human colon adenocarcinoma cells (1), is a potent blood vessel inducer present in mammalian plasma and milk (2–4). It has a unique ribonucleolytic activity (5, 6) that is essential for neovascularization. It also stimulates endothelial cells to form diacylglycerol (7) and to secrete prostacyclin (8) by activating phospholipase C and phospholipase A_2, respectively. It binds to calf pulmonary artery endothelial (CPAE) cells (9) and has been found to modulate a mitogenic effect in certain other cells (10). When angiogenin is modified by mutagenesis (11) or by limited proteolysis (12) in the region of residues 61–67, it loses angiogenin activity and will not compete with the native protein in the chicken embryo chorioallantoic membrane (CAM) assay. In contrast, angiogenin modified at either of the active-site histidine residues involved in ribonucleolytic activity, although inactive itself on the CAM, will compete with the native protein in this angiogenesis assay (13). These second-messenger binding and modulatory effects have led to the postulation of a dual-site model for the organogenic activity of angiogenin (12), which assumes that angiogenin interacts directly with cells, presumably through a membrane receptor, to exert this biological function.

We report here the identification, isolation, and initial characterization of a bovine endothelial cell angiogenin-binding protein (AngBP) whose properties are consistent with its being a component of a cellular receptor of angiogenin.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

MATERIALS AND METHODS

Materials. Bovine angiogenin, isolated from bovine milk as described (4), migrated as a single band in SDS/PAGE, induced vascularization in the chicken embryo CAM assay, and stimulated a second-messenger response in endothelial cells. Single-site mutants H13A and H114A in which the ribonucleolytic active-site histidine residues are changed to alanine,† the regional mutant ARH-I,‡ native human angiogenin, placental ribonuclease inhibitor (PRI), and bovine angiogenin were provided by R. Shapiro. The two proteolytic derivatives of human angiogenin, angiogenin K (cleaved at residues 60–61) and angiogenin E (cleaved at residues 67–68) were from T. Hallahan (12). ^{125}I-Ang, prepared with Enzymobeads (Bio-Rad; immobilized glucose oxidase–lactoperoxidase system), had a specific activity of 1.08 μCi (40 kBq)/μg. Heparan sulfate was purchased from Sigma; RNase A, from Boehringer Mannheim; Na^{125}I, from Du Pont/New England Nuclear; and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC), disuccinimidyl suberate, and disuccinimidyl tartrate, from Pierce.

Cell Culture. GM7373 cells, obtained from the National Institute of General Medical Sciences, Human Genetic Mutant Cell Repository (Institute for Medical Research, Camden, NJ), were derived from fetal bovine aortic endothelial cells by transformation with benzo(*a*)pyrene (14). The cells were propagated at 37°C in humidified 5% CO_2/95% air as monolayer cultures in Dulbecco's modified Eagle's medium supplemented with 10% (vol/vol) heat-inactivated fetal bovine serum, 2 mM glutamine, 1 μg of Fungizone per ml, and 5 μg of gentamicin sulfate per ml. CPAE and COS-7 (African green monkey kidney) cells from American Type Culture Collection were cultured in the same way, except that the medium for CPAE cells contained 20% (vol/vol) fetal calf serum. Subconfluent cells were kept in serum-free Dulbecco's modified Eagle's medium for 24 hr before experimentation. Human lymphocyte (HL-60) cells, from American Type Culture Collection, were cultured in RPMI 1640 medium supplemented with fetal calf serum, L-glutamine, Fungizone, and gentamicin sulfate as above. Subconfluent cells were kept in serum-free RPMI 1640 medium for 24 hr before experimentation.

Solubilization of Cells. Cell monolayers previously exposed to ^{125}I-Ang and treated with EDC were solubilized with radioimmunoprecipitation assay buffer (RIPA buffer; 150 mM NaCl/1% Nonidet P-40/0.5% desoxycholate/0.1%

Abbreviations: AngBP, angiogenin-binding protein; CPAE, calf pulmonary artery endothelial; CAM, chorioallantoic membrane; EDC, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; PRI, human placental ribonuclease inhibitor.

*To whom reprint requests should be addressed.

†Mutant proteins are designated by the single-letter code for the original amino acid followed by its position in the sequence and the single-letter code for the new amino acid.

‡ARH-I is the extension derivative Met-angiogenin (methionyl residue at position −1) in which residues 58–70 have been replaced by residues 59–73 of RNase A.

SDS/10 mM Tris, pH 7.5). When cell monolayers were solubilized prior to addition of ^{125}I-Ang and carbodiimide crosslinking, Tris in the RIPA buffer was replaced with 10 mM phosphate (pH 7.5).

Release of Cell-Surface Proteins by Heparan Sulfate. Subconfluent starved-cell monolayers in 10-cm^2 dishes (Falcon) were washed three times with phosphate-buffered saline (PBS), and 0.4 ml of PBS containing 1 mg of heparan sulfate per ml was added. The cells were incubated at room temperature for 30 min with occasional shaking, and the released material was removed and clarified by centrifugation at 1500 rpm (700 × *g*) for 5 min.

Purification of AngBP. Saturated ammonium sulfate, adjusted to pH 7.4 with NaOH, was added at 0°C to crude AngBP from supernatant solutions of heparan-treated subconfluent starved and washed GM7373 cells to give 50% saturation. The precipitate was dissolved in PBS, dialyzed against PBS overnight at 4°C, and applied to an angiogenin-Sepharose affinity column. The column was washed sequentially with 5–10 column volumes each of PBS, 3 M NaCl, and H_2O. AngBP was eluted with 3% SDS that was 0.5% in 2-mercaptoethanol. Fractions were pooled, and protein was precipitated with equal volumes of 30% trichloroacetic acid. SDS was removed by washing the precipitate with 80% ice-cold acetone three times. The precipitate was dissolved in 0.5 M NH_4OH, diluted 1:1 with solvent A, and applied to a protein PAK-125 (Waters) HPLC column. Elution was achieved with 50:50 (vol/vol) solvent A/solvent B; solvent A was 0.1% trifluoroacetic acid, and solvent B was 3:2:2 (vol/vol) isopropanol/acetonitrile/water containing trifluoroacetic acid at a final concentration of 0.08%.

Densitometry. Autoradiogram intensities were recorded with a densitometer (E-C Apparatus), and areas were measured by weighing the excised peaks. The densitometric results were uncorrected for nonlinear response.

RESULTS

Identification of AngBP. The water-soluble carbodiimide EDC crosslinked ^{125}I-Ang to an AngBP present on the surface of CPAE or GM7373 cells. Crosslinking of either monolayers of CPAE cells (Fig. 1, lane A) or a crude cell lysate (Fig. 1, lane B) generated a single major product of 58 kDa detected by SDS/PAGE. A similar result was obtained with monolayers of GM7373 cells, a transformed endothelial cell line (Fig. 1, lane D), and its corresponding cell lysate (Fig. 1, lane E). The latter cell line is better suited to isolation of cell-surface proteins in quantity by virtue of its stability and rapid growth characteristics; it was therefore used for further experiments.

The intensity of the band for cell monolayer-derived AngBP (Fig. 1, lanes A and D) was much weaker than that for cell lysate-derived AngBP, and this suggested that some of the AngBP or its complex with angiogenin was lost, perhaps during one of the washing steps. Therefore, the dissociability of AngBP from GM7373 cells was examined by treating the cells with heparan sulfate, a procedure sometimes used for release of cell-surface proteins (15). AngBP was indeed present in the heparan sulfate-released material (Fig. 1, lane F) and was crosslinked to ^{125}I-Ang by EDC with the formation of a single, characteristic 58-kDa band. The same result was obtained with heparan sulfate-released material from CPAE cells (Fig. 1, lane C). Neither disuccinimidyl tartrate (Fig. 1, lane F) nor its longer-chain homolog, disuccinimidyl suberate (Fig. 1, lane G), was able to crosslink ^{125}I-Ang with AngBP as efficiently as EDC under conditions typically used with di(*N*-hydroxy)succinimidyl ester reagents. AngBP preparations obtained from GM7373 cells by this heparan sulfate method were used for subsequent experiments.

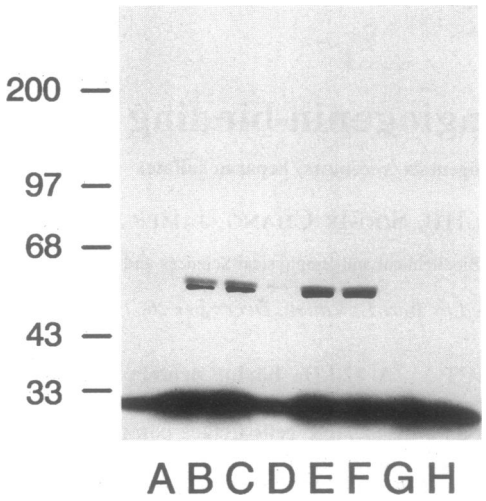

FIG. 1. SDS/PAGE of products of crosslinking ^{125}I-Ang to AngBP. Initial amounts of starved subconfluent CPAE (lanes A–C) or GM7373 (lanes D–H) cells from 10-cm^2 dishes and of ^{125}I-Ang are identical for all lanes. EDC crosslinking reactions (lanes A–G) were quenched with Tris at a final concentration of 100 mM (pH 8.0). For lane A, ^{125}I-Ang was crosslinked to AngBP in CPAE cell monolayers (passage 17). After a 30-min exposure of monolayers to 40 ng of ^{125}I-Ang, unbound ^{125}I-Ang was removed by washing three times with PBS. Bound ^{125}I-Ang was fixed with 10 mM EDC at room temperature for 30 min. After the reaction was quenched, the cells were washed three times with PBS and solublized with 0.4 ml of RIPA buffer. For lane B, ^{125}I-Ang was crosslinked to AngBP in a crude CPAE cell lysate. Cell monolayers were solubilized with the phosphate modification of RIPA buffer, and a 50-μl sample of the released material was incubated with 5 ng of ^{125}I-Ang for 30 min at room temperature. EDC was added to a final concentration of 10 mM, and the mixture was incubated at room temperature for 30 min and then quenched. For lane C, ^{125}I-Ang was crosslinked to AngBP in heparan sulfate-released material from CPAE cells as for lane B. Other lanes: D–F, as in lanes A–C except with GM7373 cells; G, as in lane F except the crosslinking agent was 0.2 mM disuccinimidyl suberate; H, as in lane G, except the crosslinking agent was 0.2 mM disuccinimidyl tartrate.

Specificity of Angiogenin Binding to AngBP. An excess (2 μg) of either unlabeled bovine or human angiogenin competed with ^{125}I-Ang for binding to AngBP under the conditions given for Fig. 1, and the intensities of the 58-kDa bands were reduced by factors of 50 and 7, respectively, as determined by densitometric analysis. RNase A, a close natural homolog of angiogenin with 35% sequence identity, competed with ^{125}I-Ang in a binding and crosslinking experiment with AngBP, though not as effectively as angiogenin. In the presence of 2 μg of RNase A the intensity of the ^{125}I-Ang–AngBP band was reduced by a factor of 3.4. However, only a very faint band was seen after attempting to bind and crosslink ^{125}I-labeled RNase A to AngBP (data not shown). Under the same conditions, lysozyme, a strongly basic protein, did not compete at all. No AngBP was detected in heparan sulfate-released preparations from COS-7 and HL-60 cells under the same conditions as were used with CPAE and GM7373 cells.

PRI Prevents the Binding of ^{125}I-Ang to AngBP. Preincubation of ^{125}I-Ang with PRI before addition to the heparan sulfate-released material prevented subsequent formation of a cross-linkable complex with AngBP (data not shown). Angiogenin binds with extraordinary avidity to PRI (16). The K_d for this interaction, 7×10^{-16} M, is 60-fold stronger than that for the interaction of PRI with RNase A. Remarkably, when an excess of AngBP and PRI in equal proportions is added to ^{125}I-Ang, only the ^{125}I-Ang–AngBP product is observed. This suggests that under these conditions angiogenin

binds to AngBP preferentially, although the stability of PRI in the released material has not been established.

Saturability of Binding of ^{125}I-Ang to AngBP. Formation of the ^{125}I-Ang–AngBP complex depends on the concentration of added angiogenin (Fig. 2). A plot of bound vs. free ^{125}I-Ang clearly shows that binding to AngBP is saturable. A Scatchard plot (not shown) indicates an apparent K_d of 5×10^{-10} M, computed on the assumptions that the yield of crosslinking of bound ^{125}I-Ang to AngBP is constant and occurs much faster than dissociation of ^{125}I-Ang–AngBP and that disappearance of free ^{125}I-Ang and free AngBP also occurs much faster than dissociation of ^{125}I-Ang–AngBP.

Specificity of Binding of Mutant and Clipped Angiogenins. Two mutant forms of angiogenin, H13A and H114A, in which active-site histidine residues are replaced by alanine with resultant loss of ribonucleolytic and angiogenic activity, also competed for ^{125}I-Ang binding to AngBP: in the presence of 2 μg of either H13A or H114A, a reduction in the 58-kDa band intensity by a factor of 3 or 5, respectively, was observed. These mutants also competed efficiently with angiogenin both in the chicken embryo CAM assay and in a second-messenger response assay, indicating that they both retain an intact receptor binding site. Two proteolytically clipped forms of angiogenin, on the other hand, angiogenins K and E, which do not compete with the native protein in the chicken embryo CAM assay when at 20-fold excess (12), competed only weakly (17–33% reduction in band intensity) in ^{125}I-Ang binding to AngBP. These two derivatives have single peptide bond cleavages at residues 60–61 and 67–68, respectively, which are thought to lie in the receptor binding region of angiogenin (12). Mutagenic and chemical changes in this region are accompanied by loss of angiogenic activity but retention of ribonucleolytic activity (12).

Purification of AngBP. Many proteins were released from bovine endothelial cells by treatment with heparan sulfate (Fig. 3, lane A). AngBP was purified from this mixture by starting with ammonium sulfate fractionation, in which all of the ^{125}I-Ang binding activity was recovered in the 50% saturation precipitate. This was taken up in PBS and applied to an angiogenin-Sepharose column to which AngBP bound tightly. Most impurities were removed by washing with PBS, and no ^{125}I-Ang binding was found in subsequent 3 M NaCl and H_2O washes. AngBP was eluted by washing with 3% SDS/0.5% 2-mercaptoethanol and was freed from SDS by precipitation with trichloroacetic acid. Subsequent washing with aqueous acetone afforded material that migrated as a single major 42-kDa band in SDS/PAGE (Fig. 3, lane B).

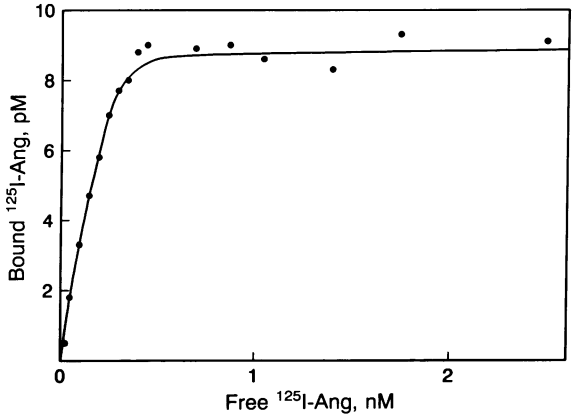

FIG. 2. Saturability of binding of ^{125}I-Ang to AngBP. ^{125}I-Ang samples at various concentrations were incubated with crude native AngBP and crosslinked with EDC as in Fig. 1. The gel slices containing the ^{125}I-Ang–AngBP product were located by autoradiography, cut, and assayed for radioactivity. The resulting cpm were defined as bound ^{125}I-Ang.

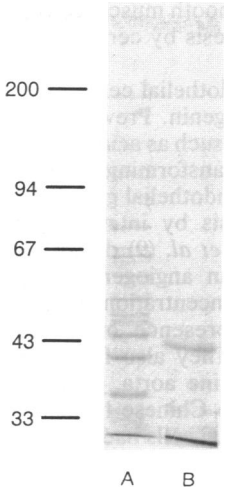

FIG. 3. SDS/PAGE analysis of AngBP samples. Lane A shows cell-surface species released by heparan sulfate treatment. The cell-free supernatant solution, which contained crude AngBP, was analyzed with SDS/7% PAGE and Coomassie brilliant blue stain. Lane B shows purified AngBP prepared as described in *Materials and Methods*. A sample taken after the acetone wash was dissolved in 0.5 M NH_4OH and analyzed by SDS/PAGE as in Fig. 1.

AngBP was isolated from a gel slice containing this band by electroelution in 0.1 M NH_4HCO_3 buffer. After lyophilization, AngBP was renatured by incubation in PBS at 4°C overnight. This highly purified product binds ^{125}I-Ang as measured by crosslinking with EDC and autoradiography (see Fig. 1).

An aliquot of the trichloroacetic acid precipitate dissolved in 0.5 M NH_4OH and applied to a HPLC gel filtration column, PAK-125, was eluted as a single peak of AngBP (Fig. 4). An amino acid analysis of the pooled fractions from this single peak showed that AngBP differs markedly from PRI (data not shown). The latter is characterized by seven leucine-rich repeats and a cysteine content much higher than observed for AngBP. Anti-PRI polyclonal antibodies did not recognize AngBP (data not shown).

DISCUSSION

Characterization of an angiogenin receptor is essential to an understanding of not only the mechanism of action of angiogenin and its signal-transduction pathway but also the control of angiogenesis. The preceding paper in this issue (12) showed that two distinct regions of angiogenin are crucial for the expression of its biological activity. One region encompasses the residues involved in ribonucleolytic activity, and the other is a cellular recognition site that spans residues 60–68. A functional catalytic site and an intact cell-binding site are both required for angiogenin's organogenic activity. This dual-site mechanism also implicates a complementary recognition site on the outer membrane of the target cell. Evidence that the angiogenic process is receptor-mediated includes the activation by angiogenin of phospholipases C

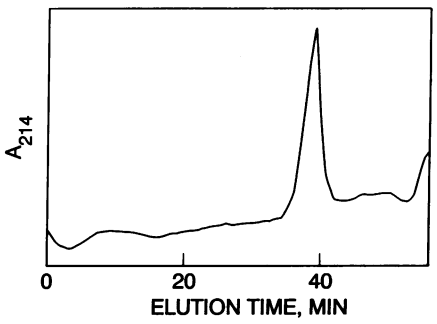

FIG. 4. Gel filtration HPLC of purified AngBP. A sample from the active fraction from affinity chromatography, prepared as in Fig. 1, lane B, was subjected to HPLC gel filtration on a PAK-125 column. The resultant single peak at 39 min was subjected to amino acid analysis.

and A_2 in vascular endothelial and smooth muscle cells (7, 8) as well as the inhibition of angiogenesis by certain inactive angiogenin mutants (13).

This study identifies a 42-kDa endothelial cell membrane protein that specifically binds angiogenin. Previous studies had indicated that angiogenic factors such as acidic and basic fibroblast growth factors (17, 18), transforming growth factors α and β (17, 18), and vascular endothelial growth factor (19) most likely induce angiogenesis by interacting with endothelial cells. In addition Badet *et al.* (9) demonstrated that ^{125}I-labeled recombinant human angiogenin binds to CPAE cells in a manner that is concentration dependent, reversible, and saturable in the presence of increasing amounts of unlabeled angiogenin. They also found that it binds to endothelial cells from bovine aorta, cornea, and adrenal cortex capillaries but not to Chinese hamster lung fibroblasts. The binding sites on CPAE cells had an apparent K_d of 5×10^{-9} M and were considered to satisfy the criteria for an angiogenin receptor.

Preliminary investigations in this laboratory (S.-I.C., unpublished observations) revealed a marked degree of species dependency for angiogenin binding to endothelial cells. Consequently, subsequent work was carried out with bovine angiogenin isolated from bovine milk and bovine endothelial cells, primarily GM7373 cells. Initial efforts to identify an angiogenin receptor used conventional crosslinking techniques with both cell monolayers and membrane preparations. Although a wide variety of conditions and reagents were tested, the results were largely inconclusive. Both high (>200,000) and low (≈60,000) molecular weight bands could be detected by autoradiography after crosslinking with labeled angiogenin but not consistently nor of appreciable intensity. It is now apparent that at least two technical reasons contributed to this problem. For one, the di(*N*-hydroxy)succinimidyl esters that were used are not very effective in cross linking ^{125}I-Ang and AngBP. More importantly, perhaps, is the tendency of AngBP to dissociate from the endothelial cell surface. Although the circumstances under which this occurs have not been investigated completely as yet, dissociation is clearly enhanced by heparan sulfate (Fig. 1). It is therefore possible that loss of AngBP or ^{125}I-Ang–AngBP from the cell surface during washing steps could have contributed to the low yields of product seen in these early experiments.

Materials released from cell surfaces with heparan sulfate (15) are largely proteoglycans, which are known to participate in cell–cell adhesion, intercellular communication, and ligand–receptor binding. Among the last are the receptors for transferrin (20), lymphocyte homing (21), lipoprotein lipase (22), and transforming growth factor β [TGF-β (type III)] (23). These peripheral membrane proteoglycans are thought to be an integral membrane receptor component noncovalently linked either through a glycosaminoglycan side chain or the core protein. Non-proteoglycan cell-surface proteins may also be released (24–28) [e.g., by proteolysis (26)]. Secreted forms of such proteins may arise as a result of alternative splicing of mRNA (27, 28) or from multiple genes (25).

Whatever the nature of AngBP, its release from the cell surface by heparan sulfate and crosslinking to ^{125}I-Ang with EDC provides a sensitive and specific assay for its purification. AngBP is the single angiogenin-specific binding species detected by this means. It is readily purified from the material dissociated from the cell surface to yield a single band in SDS/PAGE analysis. Whether AngBP is the entire receptor, the extracellular component, or a fragment will likely be apparent after sequence analysis and correlation with biological function.

The evidence obtained thus far indicates that the binding of angiogenin to AngBP is specific, saturable, and inhibited by PRI. These studies have been largely carried out with crude preparations of AngBP (heparan sulfate releasates) and obviously must be repeated with pure material before explicit interpretations are possible. The competition for binding by RNase A, for example, is not entirely consistent with the results of direct binding of ^{125}I-labeled RNase A to AngBP. The failure of PRI to compete with AngBP is unexpected since the K_i for PRI inhibition, 7×10^{-16} M, is vastly different from the apparent K_d for AngBP binding, 5×10^{-10} M. It may well be that this reflects an instability of PRI in the crude AngBP mixture. It should be noted, incidentally, that these results suggest that AngBP is probably not PRI or some modified form of PRI. The amino acid composition of AngBP is also consistent with this view.

Unlabeled bovine angiogenin competes with ^{125}I-Ang for binding to AngBP, but lysozyme, which is a similarly basic protein, does not. Thus, the binding is not due solely to a charge interaction. Competition is also seen with two catalytic-active-site mutants of angiogenin, H13A and H114A. These proteins are known to compete with angiogenin in the chicken embryo CAM assay and hence must have an intact receptor binding site (12). However, they lack ribonucleolytic activity and, therefore, are unable to induce blood vessel formation. Conversely, the two proteolytically cleaved derivatives of angiogenin examined (angiogenins K and E) do not compete with the native protein in the chicken embryo CAM assay presumably because of disruption of their receptor binding site (12). That they also fail to compete effectively in the AngBP binding assay is consistent with this view. Indeed, the results overall are further confirmation of the dual-site model for the mechanism of action of angiogenin.

The availability of purified AngBP will allow a more detailed investigation of its interaction with angiogenin as well as complete characterization of its structure. The latter, in conjunction with cDNA sequence information, should provide greater insight to its mode of association with the endothelial cell membrane and to its function as an angiogenin receptor. It is premature to define the specific biological role of AngBP. It could participate directly in transmembrane signalling. Alternatively, it could be involved in receptor presentation or delivery, potentiate angiogenin-RNA binding, or serve as an indirect mediator of angiogenin action. Further studies will clarify its function and perhaps provide a basis for the design of agents to modulate receptor binding and to control angiogenin-induced angiogenesis.

We thank Drs. Sigitas Verselis and Anatole Klyosov for early investigations on angiogenin receptors, Daniel J. Strydom for amino acid analysis, and Robert Shapiro and Thayer C. French for valuable discussions and help in preparation of the manuscript. This work was supported by funds from Hoechst, A. G. under agreements with Harvard University.

1. Fett, J. W., Strydom, D. J., Lobb, R. R., Alderman, E. M., Bethune, J. L., Riordan, J. F. & Vallee, B. L. (1985) *Biochemistry* **24**, 5480–5486.
2. Shapiro, R., Strydom, D. J., Olson, K. A. & Vallee, B. L. (1987) *Biochemistry* **26**, 5141–5146.
3. Bond, M. D. & Vallee, B. L. (1988) *Biochemistry* **27**, 6282–6287.
4. Maes, P., Damart, D., Rommeus, C., Montreuil, J., Spik, G. & Tostar, A (1988) *FEBS Lett.* **241**, 41–45.
5. Shapiro, R., Riordan, J. F. & Vallee, B. L. (1986) *Biochemistry* **25**, 3527–3532.
6. St. Clair, D. K., Rybak, S. M., Riordan, J. F. & Vallee, B. L. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 8330–8334.
7. Bicknell, R. & Vallee, B. L. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 5961–5965.
8. Bicknell, R. & Vallee, B. L. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 1573–1577.
9. Badet, J., Soncin, F., Guitton, J.-D., Lamare, O., Cartwright,

T. & Barritault, D. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 8427–8431.
10. Heath, W. F., Moore, F., Bicknell, R. & Vallee, B. L. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 2718–2722.
11. Harper, J. W. & Vallee, B. L. (1989) *Biochemistry* **28,** 1875–1884.
12. Hallahan, T. W., Shapiro, R. & Vallee, B. L. (1991) *Proc. Nat. Acad. Sci. USA* **88,** 2222–2226.
13. Shapiro, R. & Vallee, B. L. (1989) *Biochemistry* **28,** 7401–7408.
14. Grinspan, J., Mueller, S. N. & Levine, E. M. (1983) *J. Cell. Physiol.* **114,** 328–338.
15. Höök, M., Kjellén, L., Johansson, S. & Robinson, J. (1984) *Annu. Rev. Biochem.* **53,** 847–869.
16. Lee, F. S., Shapiro, R. & Vallee, B. L. (1989) *Biochemistry* **28,** 225–230.
17. Blood, C. H. & Zetter, B. P. (1990) *Biochim. Biophys. Acta* **1032,** 89–118.
18. Folkman, J. & Klagsbrun, M. (1987) *Science* **235,** 442–447.
19. Vaisman, N., Gospodarowicz, D. & Neufeld, G. (1990) *J. Biol. Chem.* **265,** 19461–19466.
20. Fransson, L.-A. (1987) *Trends Biochem. Sci.* **12,** 406–411.
21. Jalkanen, S., Jalkanen, M., Bargatze, R., Tammi, M. & Butcher, E. C. (1988) *J. Immunol.* **141,** 1615–1623.
22. Saxena, U., Klein, M. G. & Goldberg, I. J. (1990) *J. Biol. Chem.* **265,** 12880–12886.
23. Andres, J. L., Stanley, K., Cheifetz, S. & Massagué, J. (1989) *J. Cell Biol.* **109,** 3137–3145.
24. Gavin, J. R., Buell, D. N. & Roth, J. (1972) *Science* **178,** 168–169.
25. Kress, M., Cosman, D., Khoury, G. & Jay, G. (1983) *Cell* **34,** 189–196.
26. Fujimoto, J., Stewart, S. J. & Levy, R. (1984) *J. Exp. Med.* **160,** 116–124.
27. Weber, W., Grill, G. N. & Spiess, J. (1984) *Science* **224,** 294–297.
28. Krangel, M. S. (1986) *J. Exp. Med.* **163,** 1174–1190.