[19] Another receptor, Gua-Glu(OBnz)-Trp-Glu(OBnz)-NH_2, which is one of the weak binding receptors according to the on-bead assay ($K_{ass} = 90\,M^{-1}$), did not give any reasonable data. The binding constant was too low to be measured accurately in free solution by UV titration, which is again in good agreement with the rather poor binding affinity of this specific receptor observed in the on-bead assay.
[20] M. Conza, H. Wennmers, *Chem. Commun.* **2002**, 866 – 867.
[21] F. Mohamadi, N. G. J. Richards, W. C. Guida, R. Liskamp, M. Lipton, C. Caufield, G. Chang, T. Hendrickson, W. C. Still, *J. Comput. Chem.* **1990**, *11*, 440 – 467.
[22] Because of the limited solubilities both of the free peptide and of the receptors at concentrations $>10\,mM$ in aqueous solvents, the NMR studies had to be performed in DMSO. Although the relative importance of H-bonds/ion-pairing versus hydrophobic interactions should be shifted towards the hydrophobic interactions in water, the observation of the H-bonded pattern required for a β sheet in DMSO is also a necessary prerequisite for its occurrence in water.

Received: April 3, 2003 [Z613]

Synthetic Peptides Derived from the Angiostatin K4 Domain Inhibit Endothelial Cell Migration

Monica Dettin,*[a] Silvio Bicciato,[a] Claudia Scarinci,[a] Edith Cline,[b] Mark W. Lingen,[c] and Carlo Di Bello[a]

Angiogenesis, the process by which new capillaries are formed from preexisting blood vessels, represents a vital function for the growth of normal tissues during embryogenesis as well as for the pathological growth of tumors. Experimental evidence has shown that both primary and metastatic tumors need to recruit angiogenic vessels for their growth, and form their own circulatory system by up-regulating angiogenic stimulators and down-regulating angiogenesis inhibitors.[1] Blocking of positive regulators or utilization of negative regulators to suppress angiogenesis results in a delay or regression of induced tumors. In particular, negative regulators of angiogenesis, such as angiostatin, endostatin, and antagonists for integrin $\alpha v\beta 3$, displayed profound antitumor activities in vivo.[2]

Angiostatin is a 38 kDa internal fragment of plasminogen, produced by proteolytic cleavage, and consists of four triply-disulfide-bridged kringle structures. In mice, angiostatin inhibits primary tumor growth as well as angiogenesis-dependent growth of metastases. In vitro, angiostatin inhibits endothelial cell migration and reduces endothelial cell growth in proliferation assays. Furthermore, it has been shown that angiostatin induces endothelial cell-specific apoptosis in vitro.[3]

Despite intense research efforts, the exact mechanisms by which angiostatin down-regulates neovascularization remain to be determined. Little is also known about the nature of receptors mediating inhibitory effects on endothelial cells, although several receptors for angiostatin have been identified. ATP synthase has been described as a high-affinity angiostatin-binding protein, and antibodies to ATP synthase have been reported to inhibit the antiproliferative activity of angiostatin on endothelial cells. Plasminogen itself has been shown to bind to other cell-surface proteins such as annexin II and alpha enolase.[4] Recently, angiomotin has been identified in a yeast two-hybrid screen as a functional angiostatin-binding protein that mediates the inhibition of endothelial cell motility and tube formation in vitro.[5]

A fundamental step for deciphering the biology of angiostatin is to understand the role of the kringle functional domains in the structure/function of angiostatin. Each kringle is composed of approximately 80 amino acids in a double-loop conformation, held together by three disulfide bonds. Angiostatin was originally described as consisting of kringles 1 – 4, although the −COOH terminus was identified later on by Soff and co-workers, who described an angiostatin consisting of kringles 1 – 4, plus approximately 85 % of plasminogen kringle 5.[6] Early attempts to generate recombinant angiostatin indicated the importance of the disulfide-bonded kringle in the inhibition of angiogenesis. Several other findings suggested that the kringle structure itself confers a large part, if not all, of the antiangiogenic activity.[7] While sharing structural similarity and sequence homology, kringle domains appear to have distinct inhibitory profiles on angiogenesis-associated endothelial cell activities.[8] Specifically, the in vitro activity of angiostatin in endothelial-cell proliferation assays resides in kringles 1 – 3, with kringle 1 being the most potent inhibitor, while kringle 4 is relatively ineffective. In contrast, K4 is the smaller fragment with the most potent antimigratory activity. Finally, Cao and co-workers demonstrated that K5 of plasminogen presents the most potent activity against endothelial-cell proliferation.[9] The reasons why some of the kringle domains are significantly more potent than others and the mechanisms explaining the dissociation of antiproliferative and antimigratory activity attributed to K4, compared with other kringle domains, are still unknown. However, comparisons of different angiostatin-related proteins require careful scrutiny since differences in activity between different kringle domains, or different angiostatin isoforms with varying kringle compositions, may reflect artifacts of the recombinant technology, and not necessarily an inherent difference in the kringle composition.

To investigate the different contribution of K4 regions to the antimigratory activity, three linear peptides that mapped the entire K4 sequence were designed (Figure 1), synthesized, and tested for biological activity, the quantification of the biological activity being regarded as the first step in the process of identification of the minimum active sequence in the design of peptides of reduced length. All linear peptides present N- and C-terminal Cys residues protected by Acm or *t*Buthio groups. In

[a] *Dr. M. Dettin, Dr. S. Bicciato, Dr. C. Scarinci, Prof. C. Di Bello*
Department of Chemical Process Engineering
University of Padova, 35131 Padova (Italy)
Fax: (+39) 049-827-5555
E-mail: monica.dettin@unipd.it
[b] *Dr. E. Cline*
Department of Pathology, Cardinal Bernardin Cancer Center
Loyola University Medical Center, 60153 Maywood, IL (USA)
[c] *Prof. M. W. Lingen*
Department of Pathology, The University of Chicago
60637, Chicago, IL (USA)

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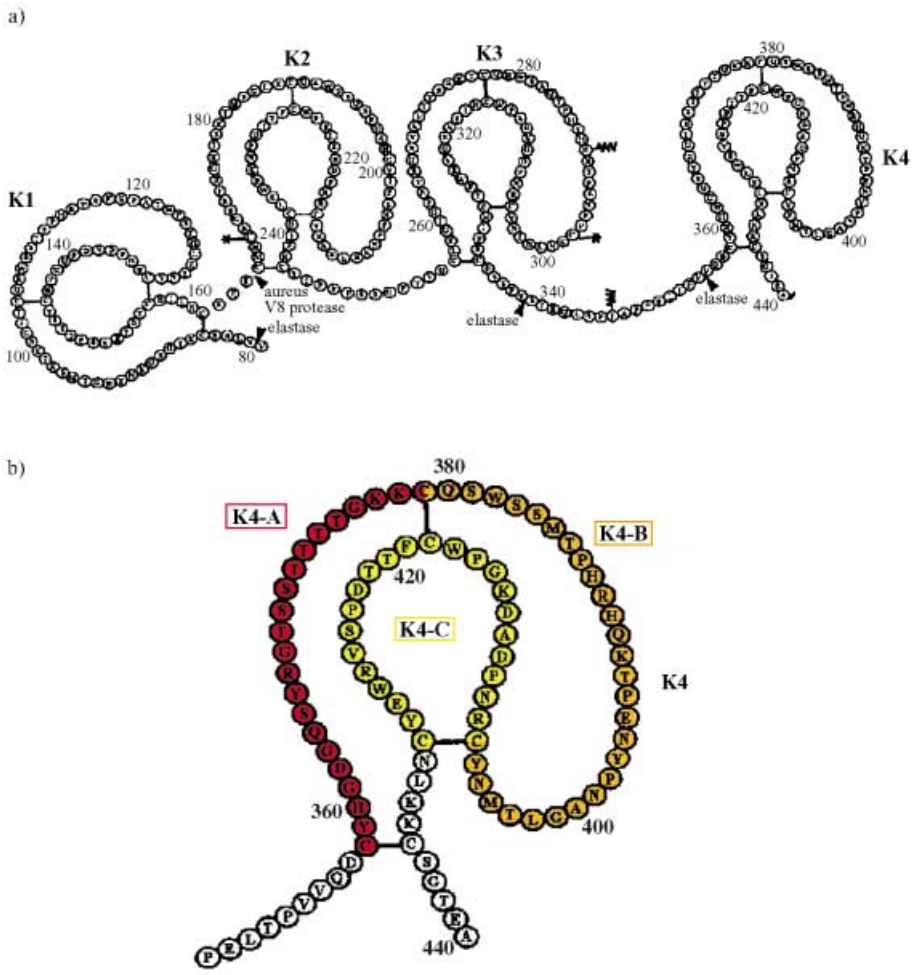

Figure 1. a) Structure of angiostatin as originally described by O'Reilly et al., consisting of the first four of the five kringle domains of plasminogen;[2] b) Structure of kringle 4 with the different synthesized sequences highlighted.

view of the double-loop structure of the native K4 domain, we also cyclized the three fragments using the disulfide bridge formation as conformational constraint. Indeed, the presence of disulfide bonds seems to play an important role in the inhibition of angiogenesis. Consequently, we synthesized cyclic K4-A, cyclic K4-B, and cyclic K4-C (Table 1), with only K4-C reproducing a natural K4 domain motif. The cyclic heterodimer cyclic K4-AB was synthesized to mimic the bigger external loop of the K4 domain (Figure 1). The synthetic molecule differs from the natural domain since: i) an unnatural disulfide bridge is present between Cys358 and Cys407, and ii) an additional Cys residue has been added in position 379 (Cys379 is present both as a C-terminal residue in K4-A and as a N-terminal residue in K4-B) to introduce a disulfide bridge between fragment K4-A and K4-B, replaced by a peptide bond in the K4 domain natural structure.

In the first synthetic protocol for intramolecular disulfide-bridge formation (syntheses of cyclic K4-A, K4-B, and K4-C), mercury(II) acetate was used to remove the Acm protecting group(s) from the Cys side chain(s) before potassium ferricyanide treatment. In each case, the cyclic analogue gave a complex with Hg^{2+}, as confirmed by mass spectroscopic data. The application of a different synthetic protocol, involving Ag^{I} removal of the Acm group and DMSO treatment, gave disulfide bridged cyclic peptides not conjugated with metallic ions.

In the synthesis of the cyclic peptide named K4-AB, a combination of *t*Buthio and Acm protection was used for the selective formation of two disulfide bonds. Closure of the disulfide bridge between the C-terminal Cys of K4-A and the N-terminal Cys of K4-B, after removal of the *t*Buthio groups, was carried out by thiolysis with di-*tert*-butyl azodicarboxylate, a reagent successfully applied in the synthesis of asymmetrical disulfides.[10] Subsequent Ag^{I} removal of the Acm groups from the N- and C-terminal cysteines and oxidation with aqueous HCl/DMSO in diluted solution resulted in the formation of the second disulfide bridge.

Table 1. *Sequences of peptides derived from the angiostatin K4 domain.*

Peptide		Form	Sequence
K4-A	Linear	K4(358 – 379)	[a]**C**YHGDGQSYRGTSSTTTTGKK**C**[b]
	Cyclic	K4(358 – 379)	**C**YHGDGQSYRGTSSTTTTGKK**C**
K4-B	Linear	K4 (379 – 407)	[b]**C**QSWSSMTPHRHQKTPENYPNAGLTMNY**C**[a]
	Cyclic	K4 (379 – 407)	**C**QSWSSMTPHRHQKTPENYPNAGLTMNY**C**
K4-C	Linear	K4 (407 – 430)	[a]**C**RNPDADKGPWCFTTDPSVRWEY**C**[a]
	Cyclic	K4 (407 – 430)	**C**RNPDADKGPWCFTTDPSVRWEY**C**
K4-AB	Cyclic	K4 (358 – 407)	**C**YHGDGQSYRGTSSTTTTGKKC CQSWSSMTPHRHQKTPENYPNAGLTMNY**C**

[a] Stands for Cys(Acm). [b] Stands for Cys(*t*Buthio).

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The antimigratory activities of synthetic peptides were examined on human microvascular endothelial cells in a Boyden chamber assay. Vascular Endothelial Growth Factor (VEGF), a positive regulator of angiogenesis, was used as chemoattractant to elicit cell migration. The assay results, reported in Table 2, can be summarized as follows:

i) Two peptides, linear K4-A and cyclic K4-C, exhibit an inhibitory activity on endothelial cell migration higher than that of the entire K4 domain ($IC_{50}^{K4} = 500$ nM).[9] Indeed, they show IC_{50} values of 386 and 441 nM, respectively.
ii) The 51 cyclic peptide K4-AB shows an IC_{50} of 600 nM.
iii) The cyclization of K4-C, unlike that of K4-A, gives an analogue more active than the linear peptide. It might be supposed that the cyclization of K4-C, by reproducing the natural constraint, improves the inhibitory effect whereas the introduction of a disulfide bridge in the K4-A sequence, not present in the K4 native structure, does not promote the biologically active conformation of the peptide.
iv) In all experiments, the cyclic peptides in complexation with Hg^{2+} show inhibitory activities lower ($IC_{50} > 1000$ nM) than those of cyclic analogues not complexed with Hg^{2+}. However, CD studies did not show any conformational variation between cyclic peptides with and without Hg^{2+} (data not shown). Most probably, the lower activity of cyclic peptides in complexation with Hg^{2+} may be ascribed to the presence of Hg^{2+} itself.
v) The experiments indicate that the inhibition of endothelial cell migration induced by the K4 domain is principally promoted by the (358–379) and (407–430) sequences.

Table 2. Inhibitory activity of synthetic peptides mapped from the K4 domain of angiostatin on human microvascular endothelial cell migration.

Peptide	Form	IC_{50} [nM]
K4-A	Linear	386
	Cyclic	852
K4-B	Linear	> 1000
	Cyclic	898
K4-C	Linear	> 1000
	Cyclic	441
K4-AB	Cyclic	600

Preliminary CD studies in water, 2,2,2-trifluoroethanol (TFE), and sodium dodecyl sulfate (SDS) show that the peptides are very flexible and that cyclization of K4-A, K4-B, and K4-C does not induce evident modifications in the population of conformers according to the large dimensions of the cycles (data not shown). Thus, it can be hypothesized that the inhibitory effect is mostly exerted through: i) the inhibition of VEGF action, and/or ii) the peptide mimicking K4 antimigratory activity.

In order to elucidate how the (358–379) and (407–430) regions of the angiostatin K4 domain can affect the cell migration mechanisms, experiments targeted towards the identification of the minimum sequence of bioactive peptides, the role of the single amino acids in eliciting the antimigratory activity, and the pharmaceutical potential of these synthetic molecules are underway in our laboratories.

Experimental Section

All peptides reported in Table 1 were synthesized by solid-phase methods by using a fully automated peptide synthesizer (Applied Biosystems Model 431 A) by Fmoc chemistry. Rink Amide MBHA resin (0.59 mmol g^{-1}) was used as solid support.

Synthesis of K4-A peptides: In the synthesis of the linear form of the K4-A sequence, side chains were protected by Acm and *t*Buthio (Cys, Table 1), Pmc (Arg), Trt (Gln and His), O*t*Bu (Asp), Boc (Lys), and *t*Bu (Thr, Ser, and Tyr). The cleavage and deprotection of 711 mg of peptide-resin was carried out with a reagent mixture composed of phenol (0.5 g), triethylsilane (0.2 mL), H_2O (0.5 mL), and trifluoroacetic acid (TFA, 8.8 mL) for 1 h. MALDI mass analysis confirmed the product identity (theoretical value: 2494 Da; experimentally determined value: 2501 Da).

The protocol for the synthesis of the cyclic form of K4-A started with *t*Buthio removal by reduction with thiols: K4-A (11.4 mg), ammonium bicarbonate (1.8 mL, 0.1 M), CH_3CN (0.4 mL), DMF (drops) at 25 °C under Ar for 5 min; then 1,4-dithiothreitol (DTT, 14.2 mg), ammonium bicarbonate (200 μL, 0.1 M) for 2 h under Ar, adjusted to pH 2 with AcOH.[11] The Acm removal was carried out first by Hg^{II} deprotection: *t*Buthio-deprotected K4-A (2.18 mg), AcOH/H_2O (225 μL, 30%), $(AcO)_2Hg$ (1.15 mg) at 25 °C for 1 h; 2-mercaptoethanol (2.54 μL) at 25 °C for 1 h, followed by ferricyanide oxidation *t*Buthio and Acm-deprotected K4-A (1.97 mg), H_2O (22 mL), pH 6.88, $K_3Fe(CN)_6/H_2O$ (≈1.8 mL, 100 mg:50 mL) at 25 °C for 30 min; addition of anionic resin (0.5 g), at 25 °C for 30 min.[12] This protocol produced the cyclic analogue in complexation with Hg^{2+}, as demonstrated by a mass spectrometric analysis (theoretical value: 2335+200 Da; experimentally measured value: 2538 Da).

Given this result, the cyclization was repeated after Acm deprotection with Ag^{I}: *t*Buthio-deprotected K4-A (9.27 mg), TFA (8.91 mL), anisole (90 μL), silver trifluoroacetate (85 mg) at 4 °C for 2 h; followed by DMSO oxidation of *t*Buthio- and Acm-deprotected K4-A (9 mg), CH_3COOH/H_2O 81 mL 5%), pH 6, DMSO (10 mL) at 25 °C for 2 h.[13] Mass spectroscopic data confirmed the product identity (theoretical mass: 2335 Da; experimentally measured mass: 2335 Da).

Synthesis of K4-B peptides: The following side chain protecting groups were used in the synthesis of the linear K4-B peptide: Acm or *t*Buthio (Cys, Table 1), Pmc (Arg), Trt (Asn, Gln, and His), O*t*Bu (Glu), Boc (Lys and Trp), *t*Bu (Thr, Ser, and Tyr). Cleavage and deprotection were carried out by use of the same trimethylsilane mixture as described for the K4-A synthesis. The identity of the synthetic peptide was checked by mass spectrometric analysis (theoretical value: 3539 Da; experimentally measured value: 3536 Da). The *t*Buthio removal was achieved by DTT treatment and Acm deprotection, and disulfide bond formation was carried out by the strategies described above. The protocol involving Hg^{II} treatment and ferricyanide oxidation produced a cyclic form of K4-B in complexation with Hg^{2+} (theoretical mass: 3378+200 Da; experimentally determined mass: 3583 Da), while the Ag^{I} treatment and DMSO oxidation resulted in the expected product (theoretical value: 3378 Da; experimentally determined value: 3378 Da).

Synthesis of K4-C peptides: In the synthesis of linear K4-C, amino acid side chains were protected by the following groups: Acm (Cys), Pmc (Arg), Trt (Asn), O*t*Bu (Asp and Glu), Boc (Lys and Trp), *t*Bu (Thr, Ser, and Tyr). Peptide cleavage and deprotection were carried out by use of a reagent mixture of phenol (1.5 g), ethane-1,2-dithiol (EDT, 0.5 mL), thioanisole (1 mL), H_2O (1 mL), and TFA (20 mL) over 1.5 h. The identity of the synthetic peptide was checked by mass spectrometry analysis (theoretical value: 2954 Da; experimentally measured value: 2956 Da).

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The cyclization of K4-C was achieved by disulfide bond formation after Acm removal. The Acm removal was first carried out by Hg^{II} deprotection K4-C (2.9 mg), AcOH/H_2O (0.5 mL 30%), $(AcO)_2Hg$ (1.25 mg) at 25 °C for 1 h; 2-mercaptoethanol (2.75 µL) at 25 °C for 1 h; followed by ferricyanide oxidation.[11] Again, the first protocol for Acm deprotection by Hg^{II} treatment and subsequent oxidation by ferricyanide resulted in a cyclic K4-C in complexation with Hg^{2+}, as demonstrated by a mass spectrometry analysis (theoretical value: 2810+200 (Hg^{2+}) Da; experimentally measured value: 3012 Da). The correct cyclic form of K4-C (theoretical mass value: 2810 Da; experimentally measured mass value: 2812 Da) was instead obtained by removal of the Acm protecting group with Ag^{I}, followed by DMSO oxidation.

Synthesis of K4-AB peptide: A combination of *t*Buthio and Acm protection for the selective formation of two disulfide bonds was used for the synthesis of the cyclic peptide spanning the (358–407) fragment of K4 (sequence K4-AB in Table 1). The first disulfide bond was formed after the selective removal of *t*Buthio protection by DTT from the C-terminal Cys of K4-A and N-terminal Cys of K4-B. The closure of the disulfide bridge was carried out by thiolysis with di-*tert*-butyl azodicarboxylate: a solution of Acm-deprotected K4-A (11 mg) in DMF (0.98 mL) was dropped into a solution of di-*tert*-butyl azodicarboxylate (3.15 mg) in DMF (51 µL) and left at 25 °C for 4 h under Ar.[10] Subsequent Ag^{I} removal of the Acm groups from the N- and C-terminal cysteines and treatment with aqueous HCl/DMSO in a very dilute solution (linear heterodimer (2.4 µmol), HCl (2.4 mL, 1 N), and DMSO (2.4 mL), 16 h) resulted in formation of the second disulfide bridge (theoretical mass: 5715 Da; experimentally measured mass: 5715 Da).

Crude peptides were purified by use of a semipreparative Delta Pak C_{18} column (15 µm, 100 Å, 7.8 × 300 mm, Waters). The identity and homogeneity of all synthetic products were demonstrated by analytical reversed-phase chromatography (Vydac C_{18}, 5 µm, 100 Å, 4.6 × 250 mm) and capillary electrophoresis (Applied Biosystems capillary electrophoresis system, Model 430 A). The formation of intramolecular disulfide bridges in the syntheses of cyclic analogues was confirmed by a negative response to the Elman test.[14]

Endothelial cell migration assay: The endothelial cell migration was performed as described in ref. [15]. Human dermal microvascular endothelial cells (Cell Systems, Kirkland, WA) were starved for 16 h in media containing 0.1% bovine serum albumin (BSA), harvested, resuspended into DME with 0.1% BSA, plated on the bottom side of a modified Boyden chamber (Nucleopore Corporation, MD), and allowed to attach in the inverted chamber for 2 h at 37 °C. The chamber was then reinverted, test substances were added to the wells of the upper chamber, and the cells were allowed to migrate for 4 h at 37 °C. Membranes were recovered, fixed, and stained, and the number of cells that had migrated to the upper chamber per 10 high power fields were counted. Background migration to DME + 0.1% BSA was subtracted, and the data were recorded as the number of cells migrated per 10 high power fields (400 ×). VEGF (R&D Systems, Minneapolis, MN) was used as a chemoattractant at a concentration of 100 pg mL^{-1} to elicit the migration of the endothelial cells. Each peptide in Table 1 was tested at varying concentrations of 50, 125, 250, 500, and 1000 nM. All experiments were repeated three times. The results of endothelial-cell-migration assays are reported in Figure 2. All synthetic peptides inhibited VEGF-induced cell migration in a dose-dependent manner, while only some of them presented IC_{50} values in the tested range of concentrations (Table 2).

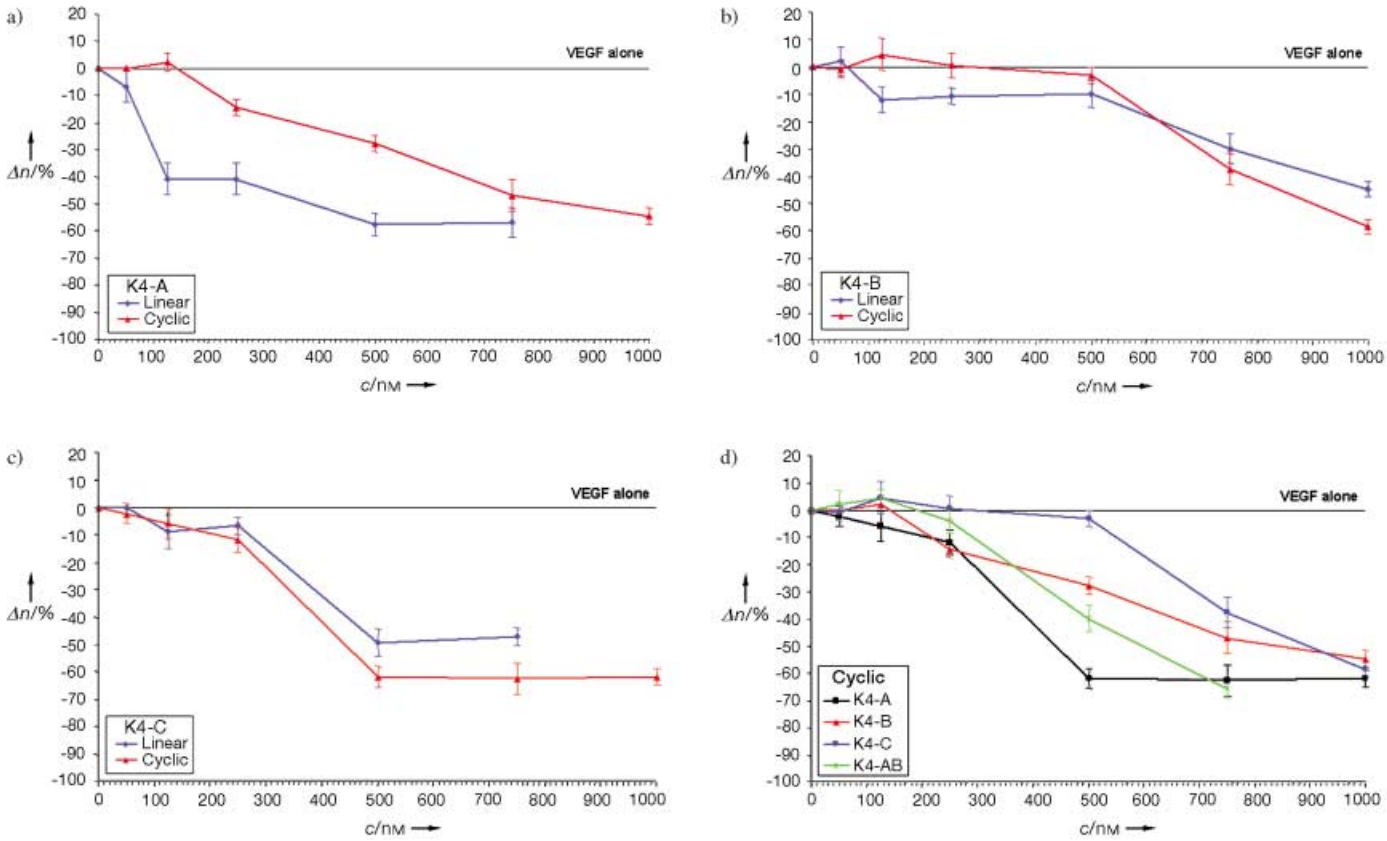

Figure 2. Comparison of inhibitory effects on endothelial cell migration by K4 fragments. Linear and cyclic peptides were assayed on human microvascular endothelial cells, stimulated with VEGF. The x axes represent peptide concentration (c), the y axes the changes in cell number (Δn).

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Acknowledgements

This work was supported in part by NIH grants DE12322 and DE00470 (M.W.L.), in part by the Italian MIUR grant PRIN2002034574 001 (M.D.), and in part by FIRB grant RBNE01TZZ8 (S.B.).

Keywords: angiogenesis · angiostatin · endothelial cell migration · kringle · peptides

[1] a) J. Folkman, *New Engl. J. Med.* **1971**, *285*, 1182 – 1186; b) J. Folkman, Y. Shing, *J. Biol. Chem.* **1992**, *267*, 10931 – 10934.

[2] a) M. S. O'Reilly, L. Holmgren, Y. Shing, C. Chen, R. A. Rosenthal, M. Moses, W. S. Lane, Y. Cao, E. H. Sage, J. Folkman, *Cell* **1994**, *79*, 315 – 328; b) M. S. O'Reilly, T. Boehm, Y. Shing, N. Fukai, G. Vasios, W. S. Lane, E. Flynn, J. R. Birkhead, B. R. Olsen, J. Folkman, *Cell* **1997**, *88*, 277 – 285; c) P. C. Brooks, S. Stromblad, R. Klemke, D. Visscher, F. H. Sarkar, D. A. Cheresh, *J. Clin. Investig.* **1995**, *96*, 1815 – 1822.

[3] a) G. Bergers, K. Javaherian, K. M. Lo, J. Folkman, D. Hanahan, *Science* **1999**, *284*, 808 – 812; b) L. Claesson-Welsh, M. Welsh, N. Ito, B. Anand-Apte, S. Soker, B. Zetter, M. O'Reilly, J. Folkman, *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 5579 – 5583.

[4] a) B. R. Zetter, *J. Cell. Biol.* **2001**, *152*, F35 – F36; b) T. L. Moser, M. S. Stack, I. Asplin, J. J. Enghild, P. Hojrup, L. Everitt, S. Hubchak, H. W. Schnaper, S. V. Pizzo, *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 2811 – 2816; c) L. A. Miles, C. M. Dahlberg, J. Plescia, J. Felez, K. Kato, E. F. Plow, *Biochemistry* **1991**, *30*, 1682 – 1691; d) K. A. Hajjar, A. T. Jacovina, J. Chacko, *J. Biol. Chem.* **1994**, *269*, 21191 – 21197.

[5] a) B. Troyanovsky, T. Levchenko, G. Mansson, O.Matvijenko, L. Holmgren, *J. Cell. Biol.* **2001**, *152*, 1247 – 1254; b) A. Bratt, W. J. Wilson, B. Troyanovsky, K. Aase, R. Kessler, E. G. Meir, L. Holmgren, *Gene* **2002**, *298*, 69 – 77.

[6] a) G. A. Soff, *Cancer Metastasis Rev.* **2000**, *19*, 97 – 107; b) G. A. Soff, J. Hong, D. Fishman, R. Schultz, D. Cundiff, S. Park, J. Enghild, M. S. Stack, S. Gately, *Proc. Am. Assoc. Cancer Res.* **1999**, *40*, 4088.

[7] a) Y. Cao, R. W. Ji, D. Davidson, J. Schaller, D. Marti, S. Sohndel, S. G. McCace, M. S. O'Reilly, M. Llinàs, J. Folkman, *J. Biol. Chem.* **1996**, *271*, 29461 – 29467; b) T. H. Lee, T. Rhim, S. S. Kim, *J. Biol. Chem.* **1998**, *273*, 805 – 812.

[8] a) S. Magnusson, L. Sittrup-Jensen, T. E. Petersen, G. Dudek-Wojciechowska, H. Claeys in *Proteolysis and Physiological Regulation* (Eds.: D. W. Ribbons, K. Brew), Academic Press, New York, **1976**, pp. 203 – 238; b) M. Llinàs, A. De Marco, S. M. Hochschwender, R. A. Laursen, *Eur. J. Biochem.* **1983**, *135*, 379 – 391.

[9] a) W. R. Ji, F. J. Castellino, Y. Chang, M. E. Deford, H. Gray, X. Villareal, M. E. Kondri, D. N. Marti, M. Llinas, J. Schaller, R. A. Kramer, P. A. Trail, *FASEB J.* **1998**, *12*, 1731 – 1738; b) Y. Cao, A. Chen, S. S. An, R. W. Ji, D. Davidson, M. Llinas, *J. Biol. Chem.* **1997**, *272*, 22924 – 22928.

[10] a) S. Romani, L. Moroder, R. Gohring, R. Schorf, E. Wunsch, Y. A. Barde, H. Thoenen, *Int. J. Pept. Protein Res.* **1987**, *29*, 107 – 117; b) E. Wunsch, L. Moroder, S. Gohring-Romani, H. J. Musiol, W. Gohring, G. Bovermann, *Int. J. Pept. Protein Res.* **1988**, *32*, 368 – 383.

[11] a) D. F. Veber, J. D. Milkowski, S. L. Varga, R. G. Denkewalter, R. J. Hirschmann, *J. Am. Chem. Soc.* **1972**, *94*, 5456 – 5461; b) S. N. McCurdy, *Pept. Res.* **1989**, *2*, 147 – 152.

[12] a) F. Albericio in *Fmoc Solid Phase Peptide Synthesis: A Practical Approach* (Eds.: W. C. Chang, P. D. White), Oxford University Press, **2000**, pp. 104; b) A. Otaka, T. Koide, A. Shide, N. Fujii, *Tetrahedron Lett.* **1991**, *32*, 1223 – 1226; c) J. P. Tam, C. R. Wu, W. Liu, J. W. Zhang, *J. Am. Chem. Soc.* **1991**, *113*, 6657 – 6662.

[13] M. Yoshida, K. Akaji, T. Tatsumi, S. Iinuma, Y. Fujiwara, T. Kimura, Y. Kiso, *Chem. Pharm. Bull.* **1990**, *38*, 273 – 275.

[14] G. L. Ellman, *Arch. Biochem. Biophys.* **1959**, *82*, 70 – 77.

[15] M. W. Lingen, P. J. Polverini, N. P. Bouck, *Lab. Invest.* **1996**, *74*, 476 – 483.

Received: July 9, 2003 [Z711]

Quantum Dots As A Visual Aid For Screening Bead-Bound Combinatorial Libraries

Hernando J. Olivos,[a] Kiran Bachhawat-Sikder,[a] and Thomas Kodadek*[a]

A commonly used method for identifying protein ligands is to screen combinatorial libraries or diverse compound collections. A particularly convenient way to do so is to create a library of compounds on beads by using the classical split-and-pool technique,[1] then incubate a labeled protein with the library. Ideally, beads that display ligands able to bind the target protein 'light up' above whatever background might be observed, which allows their identification.[2] This approach is particularly attractive if one wishes to employ the ligands thus identified as immobilized protein capture agents, which is the main focus of our research.[3] However, the technique places stringent demands on the resin to be utilized for both synthesis and screening, since it must exhibit good swellability in both aqueous and organic solvents, low nonspecific protein binding, and good mechanical stability. After considerable experimentation, we found that TentaGel (Rapp Polymere) resin, which consists of a cross-linked polystyrene core overlaid with long, amine-terminated polyethylene glycol chains, has by far the best combination of these attributes. Unfortunately, TentaGel, like many polystyrene-based resins, exhibits high-level, broad-wavelength autofluorescence (see Figure 1). This is unfortunate

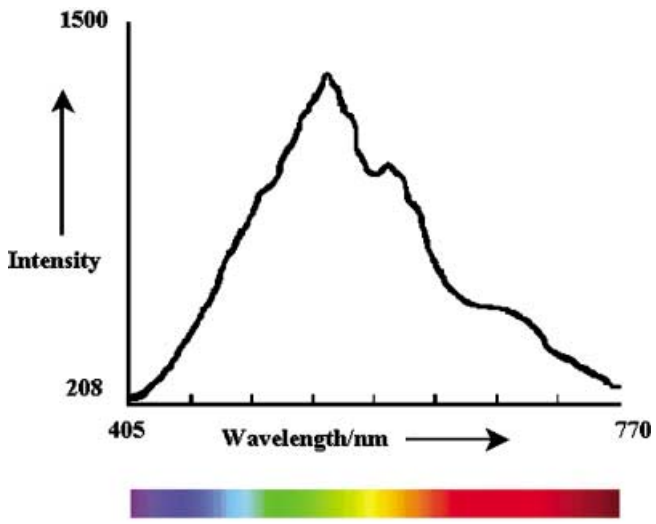

Figure 1. *Fluorescence emission spectrum of a TentaGel bead (excitation: 330 – 380 nm).*

[a] *Prof. T. Kodadek, Dr. H. J. Olivos, Dr. K. Bachhawat-Sikder*
Center for Biomedical Inventions
and the Departments of Internal Medicine and Molecular Biology
University of Texas Southwestern Medical Center
5323 Harry Hines Boulevard, Dallas, TX 75390 – 8573 (USA)
Fax: (+1) 214-6481450
E-mail: Thomas.kodadek@utsouthwestern.edu

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