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Expression of a Functional Recombinant C-Type Lectin-Like Protein Lebecetin in the Human Embryonic Kidney Cells

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Lebecetin is an anticoagulant C-type lectin-like protein that was previously isolated from Macrovipera lebetina venom and described to consist of two subunits (alpha and beta). It was reported to potently prevent platelet aggregation by binding to glycoprotein Ib and to exhibit a broad spectrum of inhibitory activities on various integrin-mediated functions of tumor cells, including adhesion, proliferation, and cell migration. This study aimed to investigate the structure-function of lebecetin. Accordingly, the cDNA of each subunit was cloned and separately or jointly expressed in the human embryonic kidney cells using two vectors with different selectable tags. The immunofluorescence analysis of transfected cells revealed significant expression levels and co-localization of the two lebecetin subunits. The recombinant proteins were efficiently secreted and purified using metal-chelating affinity chromatography. We found that the Lebecetin alpha and beta subunits were produced as a mixture of homodimers and heterodimers and that the heterodimerization represents a prerequisite for functioning. © 2012 American Institute of Chemical Engineers *Biotechnol. Prog.,* 000: 000–000, 2012
Keywords: snake venom, lebecetin, C-type lectin, heterodimerization, integrin

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Introduction

Viperidae snake venoms are complex mixtures of biologically active proteins and peptides that interfere with blood clotting and platelet function. C-type lectin-like proteins (CLPs) constitute a particular group of nonenzymatic components of Viperidae snake venoms that are structurally related to Ca^{2+}-dependent animal lectins.[1] They are not considered as true lectins because they display no carbohydrate-binding activity.[2,3] Lebecetin is a 30 kDa heterodimeric CLP from *Macrovipera lebetina* venom consisting of two homologous subunits (named α and β chains) linked by a single disulfide bond.[4] It displays an inhibitory activity on various integrin-mediated functions in tumor cells, including adhesion, proliferation, and cell migration.[5]

The cDNAs encoding α and β subunits of lebecetin have been recently cloned from RT-PCR amplified fragments of *M. lebetina* venom gland RNA.[6] Typical snake venom CLPs are heterodimers with seven conserved disulfide bonds between the subunits,[7] which might explain the difficulties with which the expression and purification of large amounts of correctly folded subunits can be achieved for functional studies. In addition to animal cell systems,[8] *Pichia pastoris* yeast has been proved successful for the expression of heterologous proteins CLP.[9]

This study aimed to develop a human cell-based system for the expression and production of recombinant lebecetin as a new promising opportunity for pharmacological studies and possible therapeutic applications. Accordingly, the cDNAs of lebecetin subunits were cloned into pAMoA vector, allowing for their fusion to the $(His)_6$ tag and expressed in human embryonic kidney (*HEK*) cells. The findings revealed that this system helped retain about 60% of the native inhibitory activity on adhesion, proliferation, and cell migration. The results also demonstrate that only heterodimers are functional and that *HEK* cells are a promising system to produce biologically active snake CLPs in adequate amounts for structure-function studies.

Experimental Procedures

Materials and reagents

Venom was collected from *M. lebetina* snakes at the serpentarium of the Pasteur Institute, Tunis, Tunisia. Lebecetin was purified as previously described elsewhere.[5,4] Dulbecco's modified Eagle's medium (DMEM) was purchased from GIBCO (Cergy-Pontoise, France), rat type I collagen was from Upstate (Lake Placid, NY), and human fibronectin was from Chemicon (Temecula, CA).

Construction of recombinant plasmid

The peptide purification and the determination of the N-terminal sequence of lebecetin were previously described.[10] The cDNAs encoding the subunits of lebecetin were cloned from the RT-PCR fragments amplified from total RNA venom glands of *M. lebetina* according to procedures previously described.[10] Two of the amino acid sequences deduced for the cloned cDNAs were identified, exhibiting complete matches with the N-terminal sequences already determined for α and β chains.[4] The nucleotide sequences of the two clones, used in the present work, were deposited in GenBank under the following accession numbers: α chain (MVLa1:EU085446) and β chain (MVLb1: EU085462).[6]

For lebecetin expression and secretion, the pAMoA-GD3 expression vector[11] was selected thanks to its signal peptide sequence derived from the human granulocyte colony-stimulating factor (referred to as hG-CSF). The GD3 sequence was deleted from the vector together with the sequence coding for the IgG-binding domain, to avoid problems that could arise from immunoreactivity studies. For this purpose, the *HindIII* restriction site was swapped to an *EcoRV* by site-directed mutagenesis according to Ansaldi et al.[12] The resulting mutated plasmid was digested by *EcoRV* and *XhoI* restriction enzymes to remove the ProtA coding sequence together with the sequence encoding GD3 synthase. The same plasmid was used to subclone the PCR products of each lebecetin subunit between these sites. Accordingly, the cDNA encoding the alpha-subunit was prepared by PCR using the MVLa1 clone[9] with the following primer: upstream: 5′-cgtgat*ATCGAT*CAGGATTGTCTCCCT-3′ (italicized: *EcoRV* site; underlined: alpha sequence) and downsteam: 5′-cgtagt*CTCGAG***TTA**GATCTCACAGGTGAAACG-3′ (italicized: *XhoI* site; bold: the stop codon; underlined: the complementary alpha sequence). The putative lebectein "signal peptide" sequence (from the start codon to codon34) was omitted and the PCR product, encompassing 128 codons starting from codon 35 (with respect to the start codon) up to the stop codon. The upstream and downstream primers used for the beta-subunit were 5′-cgtctt*GATATC*GCTTT-GAATTGTGCCTCTGGT-3′ (italicized: *EcoRV* site; underlined: β sequence) and 5′-gttacg*CTCGAG***TTA*ATGGTGATG GTGATGGTG** TGCCGGGCTCTTGCAGAC-3′ (italicized: XhoI site; bold: stop codon; italicized bold: polyhistidine sequence; underlined: the beta complementary sequence), respectively. Again, the putative lebecetin signal peptide sequence (from the start codon to codon 34) was omitted and the PCR product encompassing 118 codons starting from codon 35 (with respect to the start codon). Following PCR, the amplified products were gel purified and digested with *EcoRV* and *XhoI* and cloned into pAMoA digested with the same restriction enzymes, yielding the pAMo-α and pAMo-β $(His)_6$ recombinant plasmids.

Expression of recombinant lebecetin α and β subunits

Chinese hamster ovary (CHO) cells were selected because they were relatively appropriate in terms of rapid growth, transient transfection and heterologous expression. After 48 h post-transfection, lebecetin expression was checked by fluorescence microscopy using antilebecetin polyclonal antibody (pAb). Briefly, cells were fixed in 4% paraformaldehyde in PBS for 15 min at room temperature and incubated with for 15 min in the permeabilization buffer: PBS containing 1% fetal calf serum (FCS) and 0.5% Triton (PST). Cells were then incubated for 30 min with anti-lebecetin pAb (1/000 dilution in PST), rinsed three times with PBS and incubated with Alexa 595-labeled secondary anti-rabbit antibody for 15 min at room temperature. Fluorescence was analyzed using an Olympus IMT-2 microscope (Olympus Optical, Tokyo, Japan).

Recombinant lebecetin was detected in the culture medium by dot-blotting aliquots (2 μL) of supernatants onto the nitrocellulose filter. After washing twice with 200 mL of phosphate-buffered NaCl (NaCl/P, pH 8.5) and saturation with 5% BSA in NaCl/P containing 0.2% Tween-20, the nitrocellulose filter was incubated with the anti-lebecetin pAb at 1/25,000 in NaCl/P, containing 0.5% BSA in NaCl/P

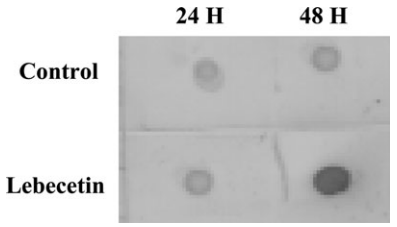

Figure 1. Secretion of recombinant lebecetin in culture media.

20 μL of supernatant from CHO cells transfected with pAMo without insert (control) or with pAMO-α/pAMo-β $(His)_6$ (lebecetin) were aspirated through a nitrocellulose filter in a dot-blot apparatus. Recombinant lebecetin was detected using rabbit polyclonal antibodies directed against purified natural lebecetin.

0.2% Tween-20). The detection system consisted of alkaline phosphatase-conjugated goat anti-rabbit immunoglobulins.

For the production of recombinant lebecetin, monolayers of HEK cells were grown in 10 cm Petri dishes until 70% confluency and transfected with 10 μg DNA of α and β subunits (separately or combined) using Lipofectamine 2000, according to the manufacturer instructions. Cell plates were then incubated at 37°C in a humidified incubator with 5% CO_2.

Two days after transfection, the cell monolayers were washed with Opti-MEM I and incubated with 4 mL serum-free DMEM. After 24 h of incubation at 37°C, the conditioned media were collected and centrifuged to remove any cells and debris.

Purification of the recombinant protein

Media containing the recombinant lebecetin subunits were dialyzed against Ni-NTA column binding buffer (20 mM sodium phosphate, 300 mM NaCl, and pH 7.2) and loaded onto Ni^{2+} Chelating gel (Invitrogen). The unbound materials were washed away with binding buffer containing 10 mM imidazole. Recombinant proteins were eluted with 100 mM imidazole in binding buffer. The appropriate fractions were pooled, buffer exchanged to TBS (20 mM Tris-HCl, pH 7.5, 150 mM NaCl) to remove imidazole using Amicon centrifugal devices from Millipore, and concentrated by ultrafiltration through 3 kDa cut-off membranes (Amicon, Millipore). The proteins were analyzed by electrophoresis in 12% polyacrylamide gels under reducing and nonreducing conditions. After blotting on a nitrocellulose membrane, recombinant lebecetin was detected with the polyclonal rabbit antibody, as indicated above. Protein contents were quantified by Bradford colorimetric protein assay.[13]

Cell culture and adhesion assay

The human cell lines derived from melanoma (IGR39) were routinely cultured in DMEM containing 10% FCS. Adhesion assays were performed as previously described elsewhere.[14,15] Briefly, cells in single cell suspension were added to wells coated with purified extra cellular matrix (ECM) proteins and allowed to adhere to the substrata for 1 h at 37°C. After washing, attached cells were fixed, stained by 0.1% crystal violet, and lysed with 1% SDS. Absorbance was then measured at 600 nm.

Cell migration assay

In vitro cell migration assays were performed using modified Boyden chambers (NeuroProbe, Bethesda, MD) as previously described,[14,15] except that the incubation medium was DMEM/10% FCS and that the cells were stained with 0.1% crystal violet. Cell migration was quantified by the measurement of absorbance at 600 nm.

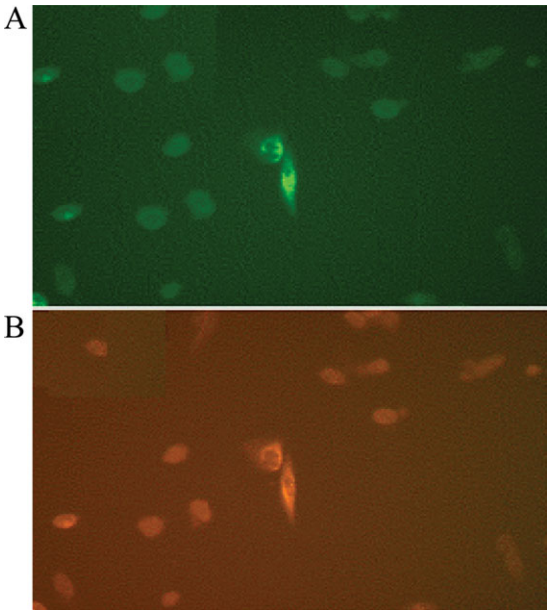

Figure 2. Expression of recombinant lebecetin visualized by fluorescence microscopy.

(A) Cells were incubated with the anti-his, followed by a FITC-conjugated anti-mouse antibody (green); (B) Cells were incubated with the antibody anti-lebecetin, followed by a RITC-conjugated anti-rabbit antibody (red). The staining was observed by fluorescence microscopy (Olympus Optical, Tokyo, Japan).

Cell proliferation assay

IGR39 cells were seeded at 5000 cells/cm^2 in the presence or absence of 25 μg/mL lebecetin and cultured in standard conditions. Cell proliferation was quantified on a daily basis through crystal violet staining as performed for the adhesion assays.

Results

Construction of lebecetins α and β recombinant plasmids

The inserts encoding the two chains of lebecetin were amplified from the cDNA clones[6] and were inserted in the mutated plasmid pAMo used as an expression vector (see Experimental Procedures). Each subunit was fused in-frame with the signal peptide sequence of the hG-CSF. It is worth noting that α subunit was expressed as a nontagged protein whereas the β subunit was fused to the (His) 6-tag at its C-terminus.

Expression of recombinant lebecetin

The transient expression of lebecetin was tested in CHO cells, which were transfected with the expression vectors encoding the α chain (pAMo-α), the β chain (pAMo-β $(His)_6$) or both of them concurrently. Two days after transfection, the expressed proteins were visualized either by dot-blot analysis using a pAb (Figure 1) or by fluorescence microscopy using an anti-lebecetin antibody which showed a

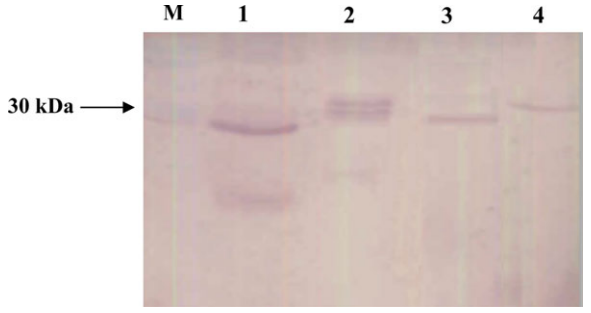

Figure 3. Western blot analysis of lebecetin and subunits secreted by HEK cells.

After electrophoresis in 12% polyacrylamide gels under nonreducing conditions, lebecetin and homodimers subunit α/α, β/β as detected using alkaline phosphatase conjugate of goat anti-rabbit IgG. M: marker; 1, purified native lebecetin; 2, cotransfection pAMO-α chain, pAMo-β(His)6; 3, pAMo-α chain; 4, pAMo-β(His)6.

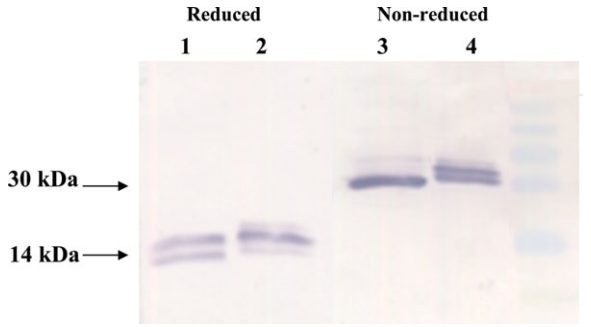

Figure 4. Western blot analysis of proteins secreted in culture media.

After electrophoresis in 12% polyacrylamide gels under nonreducing or reducing conditions using β-mercaptoethanol. Recombinant lebecetin was detected using alkaline phosphatase conjugate of goat anti-rabbit IgG. 1, 3: purified native lebecetin; 2, 4: recombinant lebecetin.

marked positive internal labeling of cell membranes (Figure 2). The expression of recombinant proteins was also confirmed after electrophoresis in 12% polyacrylamide gels under nonreducing conditions followed by western blot. Figure 3 shows heterodimer of α and β subunits (Figure 3, lane 2) and homodimers of each subunit α or β were detected (Figure 3, lanes 3 and 4). Indeed, the culture media from the cells separately transfected with vectors pAMo-β (his6) and pAMo-α contained immunoreactive material that was recognized by the anti-lebecetin Ig. Indeed, as shown in Figure 3, a 30 kDa protein was detected in the supernatant of the pAMo-β (His)6-transfected cells (Figure 3, lane 4) under nonreducing conditions. Similarly, the supernatant of vector pAMo-α transfected cells (Figure 3, lane 3) contained an immuno-reactive protein of 29 kDa only under nonreducing conditions. According to the predicted MW of α and β chains, these results suggested that the secreted β-$(His)_6$ and α subunit would be folded into disulfide-linked homodimers (30 and 29 kDa, respectively). The β-chain homodimers were further confirmed by western-blotting using anti-(His)6 tag antibodies under reducing conditions (data not shown). These findings are in agreement with previously reported results observed for CLPs proteins expressed in other expression systems.[8,9]

When co-transfected by pAMo-β(his_6) and pAMo-α constructs, *HEK* cells secreted proteins that were purified by

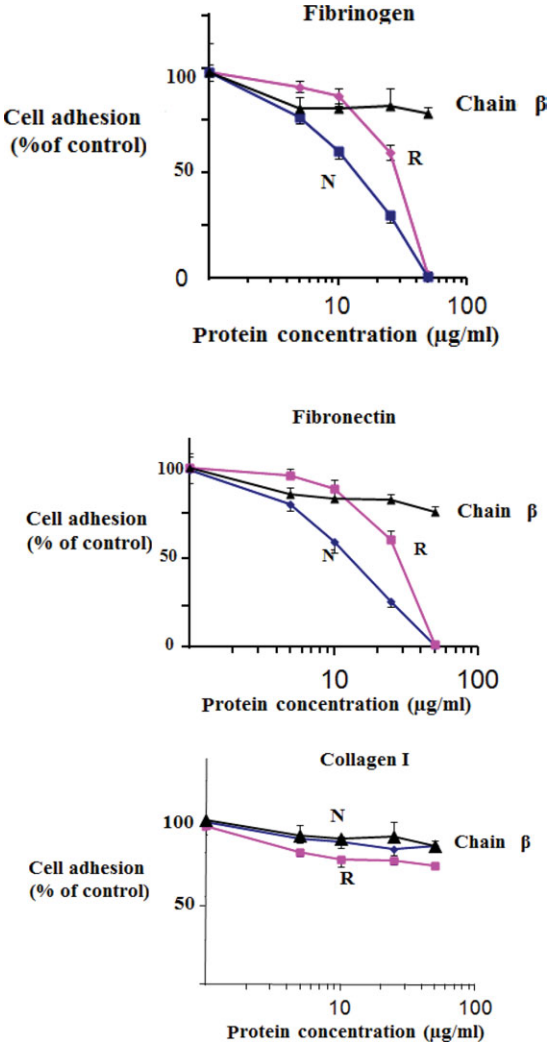

Figure 5. Lebecetin inhibits integrin-mediated functions in tumor cells.

Melanoma IGR39 cells were preincubated with the indicated concentration of natural lebecetin (N), recombinant lebecetin (R) or homodimer β/β for 30 min at room temperature. Cells were then added to 96-well microtiter plates, previously coated with 10 µg/mL fibrinogen, fibronectin or type I collagen and allowed to adhere for 1 h at 37°C. After washing, adherent cells were stained with crystal violet, solubilized by SDS and absorbance was measured at 600 nm. Data shown are means (±SD) from two to four experiments performed in triplicate. They are expressed as a percentage of adhesion in the absence of peptide.

Ni^{2+} chelating affinity chromatography. The purified proteins migrated as a couple of 29 and 31 kDa proteins under nonreducing conditions (Figure 4, lane 4) and a couple of 17 and 15 kDa under reducing conditions (Figure 4, lane 2). This suggested that the secreted homodimers β (His6) subunit (17 kDa) folded into disulfide-linked homodimers (31 kDa) that were co-purified on the Ni column with heterodimers encoded from pAMo-α and pAMo-β (his_6) constructs. It should be noted here that α subunit, expressed as nontagged protein, can bind on Ni^{2+} column only through its β subunit partner which carries the His-tag. The yield of purified heterodimers was approximately 3 mg/L.

Activity of recombinant Lebecetin

In a previous work, we reported that CLPs isolated from *M. lebetina* venom have a potent effect on adhesion and

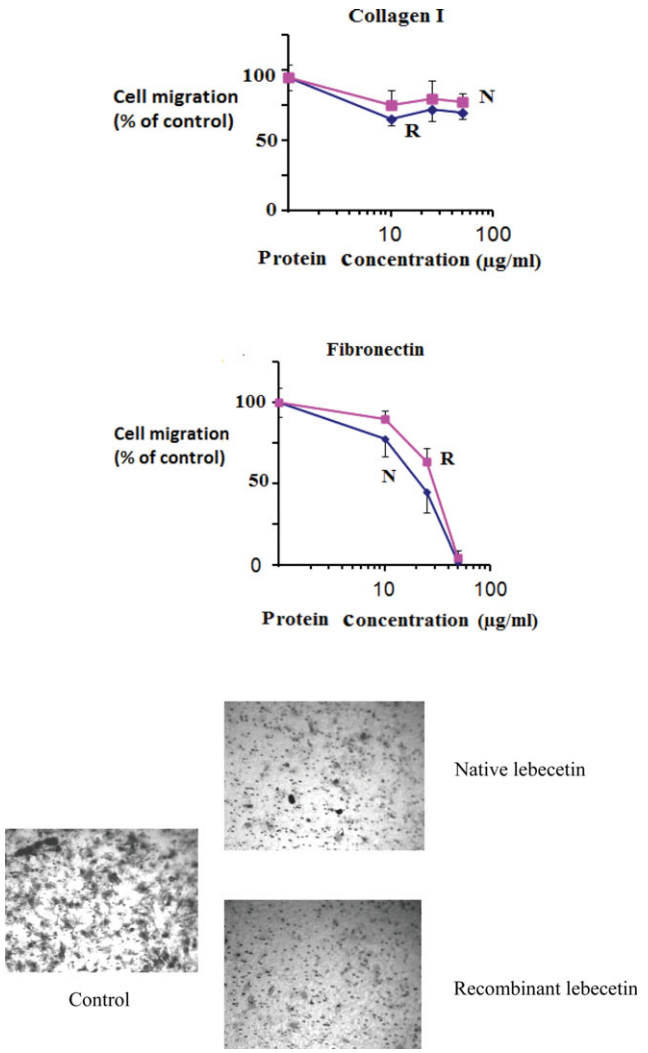

Figure 6. Recombinant lebecetin inhibited cell migration to fibronectin.

IGR39 cells were pre-treated with natural (N) or recombinant (R) lebecetin for 30 min at room temperature. Cells were then seeded into the upper reservoir of Boyden chambers and allowed to migrate through the filter toward the lower reservoir for 5 h at 37°C. Cells that migrated to the underside of the filter were stained with 0.1% crystal violet. Data shown (±SD), from one experiment representative of three performed in triplicate, are expressed as a percentage of migration in the absence of peptide.

migration of IGR39 melanoma cells by inhibiting $\alpha 5\beta 1$ and αv integrins.[16] The anti-integrin activity of lebecetin was, therefore, first checked by performing adhesion assays on a panel of purified ECM proteins.[16] Therefore, the effects of both the recombinant lebecetin [heterodimers α and β (his6)] and β homodimers were tested on the behavior of melanoma cells (IGR39) and compared to that of the native lebecetin. As illustrated in Figure 5, while having no observable effect on type I collagen, heterodimers α and β (his6) readily impaired the attachment of IGR39 melanoma cells to fibrinogen and to fibronectin in a dose-dependent way with an IC50 of about 39 μg/mL. Interestingly, while, under the same conditions, the same inhibition pattern was observed with native lebecetin in a dose-dependent way with an IC50 of about 25 μg/mL, no inhibition was observed with the homodimers β(his6). These results indicate that the association between α and β (his6) subunits is a prerequisite for the acquisition of biological functions. The functional recombinant lebecetin was also able to impair cell migration to fibronectin in haptotaxis assays, without affecting motility to type I collagen, this effect was concentration-dependent with an IC50 of about 38 μg/mL. The same inhibition pattern was observed with native lebecetin in a dose-dependent way with an IC50 of about 25 μg/mL (Figure 6).

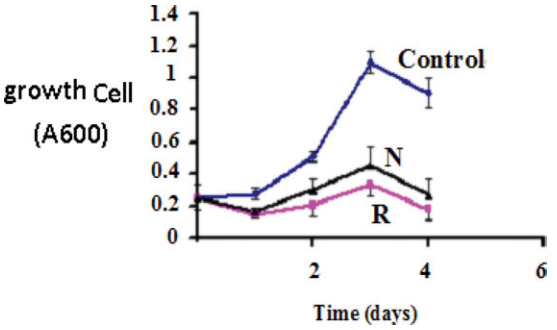

Figure 7. Lebecetin inhibited tumor cells proliferation.

IGR39 cells were cultured for the indicated periods of time in the absence (control) or in the presence of 25 μg/mL recombinant (R) or natural (N) lebecetin. IGR39 cells were quantified by staining with 0.1% crystal violet, solubilization with 1% SDS and measure of absorbance at 600 nm.

Integrins are primarily responsible of cell adhesion to ECM and are, therefore, involved in anchorage-dependent cell proliferation.[17] Accordingly, it is not unexpected that the recombinant lebecetin, which inhibited cell adhesion, also interfered with cell growth (Figure 7). Similarly to native lebecetin, recombinant lebecetin inhibited the proliferation of melanoma IG39 cells.[17]

Discussion

Lebecetin, a CLP from the viper *M. Lebetina* venom, is a potent inhibitor of adhesion, migration, and invasion of tumor cells.[5] Lebecetin is composed of two disulfide-linked α and β chains that are structurally similar to other snake venom CLP. We previous reported the cloning of the cDNAs encoding both chains from *M. lebetina* venom glands and the deduction of their complete peptide sequences.[6] This study aimed to express a functional recombinant lebecetin by co-transfecting *HEK* cells with α and β chain constructs. Such recombinant protein appeared to possess functional characteristics comparable to those of native lebecetin. In addition to the heterodimers, the study managed to produce α/α and β/β homodimers by the intracellular expression of each subunit alone. Although β chains were able to form homodimers, they were not able to compete for adhesion with purified α/β heterodimers. Moreover, the culture media from cells transfected with α chain vector alone (data not shown) or β chain did not inhibit adhesion, because the α and β chain homodimers were inactive. In agreement with these results, we previously showed that the reduced forms of α or β subunits from native lebecetin were unable to bind the glycoprotein Ib of the platelet membrane.[4] However, the secretion of dimers was detected in the culture media owing to the presence of a signal peptide upstream of the recombinant subunit. The β/β dimers were shown to be expressed simultaneously with α/β dimers in transfected cells and to be recognized by antibodies. The results presented in the current work show, however, that in contrast to the α/β heterodimers, these β/β homodimers failed to inhibit adhesion.

Recombinant lebecetin (α/β dimers) exhibited functional characteristics comparable to those of the native lebecetin since those recombinant heterodimers were noted to retain approximately 60% of inhibition of cell adhesion, migration, and invasion of tumor cells. For example, both native and recombinant lebecetin inhibit adhesion cells in a concentration dependent manner; the concentration for half maximal inhibition was 25 and 39 μg/mL for native and recombinant lebecetin, respectively. These results would become even more interesting when comparisons are drawn with similar recombinant lectins previously reported in the literature. For instance, the recombinant ACFI, an anticoagulant CLP isolated from *Agkistrodon acutus* venom and expressed in *P. pastoris* yeast, was previously reported to retain approximately 30% of the native binding activity to factor X.[9] Likewise, the recombinant CLP bothrojaracin from *Bothrops jararaca* snake venom, expressed in COS cells, was reported to exhibit about 20% of the activities of native protein for thrombin.[8]

Taken together, the results of the present work and those previously reported for recombinant CLP in various systems, either in animal cells or in *P. pastoris* yeast[8,9] show that neither of the recombinant homodimers (α and β) or used separately were active, thus firmly suggesting that the heterodimerization of the two subunits is required for their function. In addition, this work shows that the two-chain snake venom C-type lectin can be expressed in an active form in animal cells. It particularly showed that the association of both lebecetin chains and their correct folding are required for the attainment of inhibitory activity.

Acknowledgments

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Literature Cited

1. Markland FS. Snake venoms and the hemostatic system. *Toxicon*. 1998;36:1749–1800.
2. Drickamer K. Evolution of Ca^{2+}dependent animal lectins. *Prog Nucl Acid Res Mol Biol*. 1993;45:207–232.
3. Drickamer K. C-type lectin-like domains. *Curr Opin Struct Biol*. 1999;9:585–590.
4. Sarray S, Srairi N, Hatmi M, Luis J, Louzir H, Regaya I, Slema H, Marvaldi J, El Ayeb M, Marrakchi N. Lebecetin, a potent antiplatelet C-type lectin from *Macrovipera lebetina* venom. *Biochim Biophys Acta*. 2003;1651:30–40.
5. Sarray S, Berthet V, Calvete JJ, Secchi J, Marvaldi J, El-Ayeb M, Marrakchi N, Luis J. Lebectin, a novel C-type lectin from *Macrovipera lebetina* venom, inhibits integrin-mediated adhesion, migration and invasion of human tumour cells. *Lab Invest*. 2004;84:573–581.
6. Jebali J, Bazaa A, Sarray S, Benhaj K, Karboul A, El Ayeb M, Marrakchi N, Gargouri A. C-type lectin protein isoforms of *Macrovipera lebetina*: cDNA cloning and genetic diversity. *Toxicon*. 2009;53:228–237.
7. Clemetson KJ, Lu Q, Clemetson JM. Snake C-type lectin-like proteins and platelet receptors. *Pathophysiol Haemost Thromb*. 2005;34:150–155.
8. Arocas V, Castro HC, Zingali RB, Guillin MC, Jandrot-Perrus M, Bon C, Wisner A. Molecular cloning and expression of bothrojaracin, a potent thrombin inhibitor from snake venom. *Eur J Biochem*. 1997;248:550–557.
9. Hu S, Li W, Chen L, Liu J. Expression of a recombinant anticoagulant C-type lectin-like protein ACFI in *Pichia pastoris*: heterodimerization of two subunits is required for its function. *Toxicon*. 2005;46:716–724.
10. Sarray S, Srairi N, Luis J, Marvaldi J, El Ayeb M, Marrakchi N. Lebecetin, a C-lectin protein from the venom of *Macrovipera lebetina* that inhibits platelet aggregation and adhesion of cancerous cells. *Haemostasis*. 2001;31:173–176.
11. Sasaki K, Kurata K, Kojima N, Kurosawa N, Ohta S, Hanai N, Tsuji S, Nishi T. Expression cloning of a GM3-specific alpha-2,8-sialyltransferase (GD3 synthase). *J Biol Chem*. 1994;269:15950–15956.
12. Ansaldi M, Lepelletier M, Méjean V. Site-specific mutagenesis by using an accurate recombinant polymerase chain reaction method. *Anal Biochem*. 1996;234:110–111.
13. Bradford M. Description originale de la méthode de Bradford. *Anal Biochem*. 1976;72:248–254.
14. Kadi A, Pichard V, Lehmann M, Briand C, Braguer D, Marvaldi J, Rognoni JB, Luis J. Effect of microtubule disruption on cell adhesion and spreading. *Biochem Biophys Res Commun*. 1998;246:690–695.
15. Rigot V, Lehmann M, André F, Daemi N, Marvaldi J, Luis J. Integrin ligation and PKC activation are required for migration of colon carcinoma cells. *J Cell Sci*. 1998;111:3119–3127.
16. Sarray S, Siret C, Lehmann M, Marrakchi N, Luis J, El Ayeb M, André F. Lebectin increases N-cadherin-mediated adhesion through PI3K/AKT pathway. *Cancer Lett*. 2009;285:174–181.
17. Cruet-Hennequart S, Maubant S, Luis J, Gauduchon P, Staedel C, Dedhar S. alpha(v) integrins regulate cell proliferation through integrin-linked kinase (ILK) in ovarian cancer cells. *Oncogene*. 2003;22:1688–1702.
18. Bharati K, Hasson SS, Oliver J, Laing GD, Theakston RD, Harrison RA. Molecular cloning of phospholipases A(2) from venom glands of *Echis* carpet vipers. *Toxicon*. 2003;41:941–947.

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