A laminin-antagonist peptide, comprising amino acids 33–42 of murine epidermal growth factor (mEGF-(33–42)), interacts with a breast cancer- and endothelial cell-associated receptor, which is specific for the laminin B1 chain sequence, CDPGYIGSR-NH₂ (Lam.B1-(925–933)), and is immunologically similar to a previously described 67-kDa laminin receptor. In whole cell receptor assays, mEGF-(33–42), Lam.B1-(925–933), and laminin all have IC₅₀ values for displacement of 1²⁵I-laminin in the range 1–5 nM. Cell attachment to solid-phase laminin is also blocked by all three ligands, but in contrast to the receptor assays, mEGF-(33–42) or Lam.B1-(925–933), while equipotent with each other, were less effective than laminin. The concentrations of the peptides required to produce half-maximal inhibition of attachment were in the range 230–390 nM, but those for laminin were 1000-fold lower, in the range 0.2–0.3 nM. Like laminin, solid-phase mEGF-(33–42) supports cell attachment, and this ability is blocked by anti-67-kDa receptor antibodies. Modeling studies suggest that both peptides present a tyrosyl and an arginyl residue on the same face of a right-handed helical fold with elliptical cross-section.

Laminin, a basement membrane glycoprotein of approximately 900 kDa, has multiple tissue domains that bind to several integrin and nonintegrin receptors (1). Alterations in cellular interactions with laminin are implicated in the progression of several angiogenic diseases, notably diabetic retinopathy (2) and metastatic cancers (3).

Adhesion, motility, and differentiation of endothelial cells are stimulated by laminin in vitro (4) and, in vivo, laminin promotes angiogenesis in chick embryos (5). Laminin stimulates tumor cell chemotaxis (6) and increases experimental metastatic potential (7).

Peptides derived from amino acid sequence 925–933 of the murine laminin B1 chain (Lam.B1-(925–933)) are bioactive; both Lam.B1-(925–933) (CDPGYIGSR-amide) and YIGSR-amide inhibit cell attachment to laminin, thus accounting for their antimetastatic effects in vivo (8, 9). However, Lam.B1-(925–933) acts as an agonist in cell motility assays (5).

Lam.B1-(925–933) is a ligand domain for a high affinity 67-kDa laminin receptor (67-LR) found on both tumor (3, 10) and endothelial cells (11). CDNA clones of this receptor encode a 32-kDa precursor (12, 13), which contains a YIGSR-binding domain (14). Antibodies raised to residues 263–282 of the gene product (peptide Pro-20-Ala) block both cell attachment and cell haptotaxis toward laminin (15). 67-LR immunoreactivity has a surface location on whole cells (16), but a large pool of the precursor is also found in the cytoplasm (17).

We found that a linear peptide encompassing residues 33–42 of murine EGF C-loop (mEGF-(33–42); CVIGYSGRDC) blocks the stimulatory effects of laminin in cell motility and chick angiogenesis assays (5). Although derived from mEGF, mEGF-(33–42) has no demonstrable EGF receptor binding activity (18). The 67-LR agonist, Lam.B1-(925–933), is partially homologous with the decapetide C-loop sequence of all transforming growth factors α and EGFs (19, 20). Lam.B1-(925–934) represents the “C-loop” of one of eight EGF-like repeats found in domain III of both murine and human forms of the laminin B1 chain (21, 22) and contains the invariant EGF residues, CXGYYGXRRC.

Consequently, we have examined the ability of mEGF-(33–42) to interact with 67-LR in both radioreceptor and attachment assays and attempt to explain, in terms of a common predicted structure, the peptides’ shared abilities to block both specific laminin binding and laminin-dependent cell attachment.

MATERIALS AND METHODS

Polypeptides

Murine epidermal growth factor (receptor grade) was obtained from Biogenesis (Bournemouth, UK); murine laminin (affinity-purified) was from Sigma (Poole, UK); CDPGYIGSR-amide (Lam.B1-(925–933)) was from Novabiochem (Nottingham, UK), and acetyl-C-(S-Acm)-YIGYS-GDR-C-(S-Acm)-amide (mEGF-(33–42)) was synthesized as described previously (18). Control peptides were as follows: neureomedin B (obtained from Novabiochem) and a decapetide containing all residues from mEGF-(33–42) in a random sequence, which was synthesized on an Applied Biosystems automated peptide synthesizer (model 431A) using standard solid-phase Fmoc procedures. Peptides were purified after synthesis using reverse phase high performance liquid chroma-
tography, and purity was confirmed by automated amino acid analysis and electropray mass spectrometry.

A biotinylated analog of mEGF-(33–42) was synthesized as above, in which an N'-biotinylated lysine residue was incorporated as its Nα-Fmoc derivative to give acetyl-C(S-Acm)-VIGYSGDRC(S-Acm)-K(N-biotin)-amide.

In calculations of molarity, the following values for molecular mass (m) were used: laminin, m = 900,000 Da; mEGF-(33–42), m = 1,255 Da; Lamb, B (925–933), m = 968 Da; “random”, m = 1,255 Da; neuromedin B, m = 1,133 Da.

Antibodies

The peptide (PTEDWSAPQETDSWAAPA, peptide Pro-20-Ala), corresponding to a linear sequence from the C-terminal end of the human laminin receptor (15), was used as the antigen template. The peptide was synthesized as a multiple antigen presentation derivative (22), using standard Fmoc protocols.

New Zealand White rabbits were immunized subcutaneously with 500 µg of antigen in adjuvant (Alum Injekt, Pierce, Chester, UK), with boosts of 800 µg (at 21-day intervals). Test bleeds were taken 2 days after each boost, and serum was prepared.

The IgG fraction of antisera was purified using immobilized protein G-Sepharose (Pharmacia, Uppsala, Sweden). The titer and specificity of antisera as well as IgG fractions were confirmed using an enzyme-linked immunosorbent assay, as described (15) except that the multiple antigen presentation antigen was dried onto plates.

The R1 monoclonal antibody against the human EGF receptor was kindly provided by Dr. I. William (Hydromed Development Unit of the Imperial Cancer Research Fund (London, UK). This antibody has previously been shown to specifically block the biological effects of the EGF receptor (24).

Cell Culture

T-47D human breast cancer cells and SK HEP-1 human endothelial cells (25) were obtained from the European Animal Cell Culture Collection (Porton Down, UK), media, and fetal calf serum were from ICN Biomedicals (High Wycombe, UK). T-47D and SK HEP-1 cells were routinely passaged in Dulbecco’s modified Eagle’s medium (DMEM), containing 10% fetal calf serum.

Embryonic chick endothelial cells were isolated from the area vasculosa of the chick embryo, as described earlier (26), and cells were maintained in DMEM containing 10% fetal calf serum, penicillin (200 unit/ml), streptomycin (200 µg/ml), and fungizone (2.5 µg/ml).

Bovine retinal capillary endothelial cells (BRCE) were kindly provided by Dr. Usha Chakravarty (Department of Ophthalmology, Royal Victoria Hospital, Belfast, UK) and were cultured in DMEM containing 10% fetal calf serum.

Radioiodinated Laminin Receptor Assays

Affinity-purified murine laminin (Sigma) was radiiodinated using 125I-labeled sodium iodoide (Amersham, Buckinghamshire, UK) and immobilized chloramine-T (Iodobeads, Pierce), according to the manufacturer’s instructions. The reaction, excess Na125I and unincorporated 125I were separated from the iodinated protein by gel filtration on a GF-5 exclusion column (Pierce). Iodinated laminin fractions were recovered at a specific activity of approximately 1.2 µCi/µg protein (864 Ci/mmol).

Near confluent cultures of T-47D or SK HEP-1 cells were removed from flasks using 0.02% EDTA in calcium-free phosphate-buffered saline (CFS) and passed through a G-25 syringe needle to produce single-cell suspensions. Aliquots of each cell type (106 cells/ml) were dispensed into separate Eppendorf tubes (1 ml each) and pelleted. The cells were then resuspended in 1 ml of ice-cold serum-free DMEM containing 0.1% bovine serum albumin, either laminin or synthetic peptide (at the concentrations indicated), and iodinated laminin at a final 125I-laminin concentration of 0.1 nM (approximately 50,000 cpm). These mixtures were incubated overnight at 4°C.

The tubes were then microcentrifuged at high speed, and the supernatant was removed. After washing the pellet with 500 µl of CFS, the remaining radioactivity was determined using a γ radiation counter.

Non-specific binding was determined by incubating cells with a 1000-fold molar excess of unlabeled laminin. All estimations were carried out in triplicate.

IC50 (concentration of peptide required for 50% inhibition of radioligand binding), EC50 (effective concentration for 50% inhibition of cell attachment), and their S.D. values were calculated using computerized curve fitting (27).

Laminin Attachment Assay

Non-tissue culture grade 96-well plates (Sterilin Ltd., Middlesex, UK), coated with 2.5 µg of murine laminin (Sigma) in 50 µl of CFS/well, were air-dried overnight at room temperature. Preliminary experiments indicated that cell attachment was concentration-dependent, maximal binding occurred at a laminin coating of 2.5 µg/well (data not shown). After rinsing with CFS (100 µl), the plastic was saturated with casein (0.2% in CFS). Plates were incubated at room temperature for 45 min and then washed extensively with CFS (3 x 100 µl).

After removal of culture media, cells were detached from monolayers with 0.02% (0.02% in CFS) at 37°C. The tubes were then centrifuged at low speed, and the pellet was resuspended in serum-free DMEM. Peptides and soluble laminin (at the concentrations indicated) were incubated with aliquots of 105 cells (37°C for 1 h) in a volume of 1 ml. 100-µl samples of each cell suspension were then added to the precoated multwell plates and incubated for a further 60 min. Incubation media were then removed, and the wells washed with CFS (3 x 100 µl) to remove nonadherent cells.

Attached cell numbers were evaluated spectrophotometrically at 620 nm after fixing with 10% formaldehyde and staining with crystal violet (28). All incubations were carried out at least in triplicate.

mEGF-(33–42)-coated Plate Attachment Assay

In order to demonstrate cell adhesion to immobilized mEGF-(33–42), a modification of the above laminin attachment assay was used. Solid-phase streptavidin (coated onto 96-well plates) was used to capture the biotinylated derivative of mEGF-(33–42), since it had previously determined that undervatized mEGF-(33–42) bound to the plastic with low affinity (data not shown).

Briefly, non-tissue culture grade 96-well plates were coated with 100 µg/well streptavidin (Sigma) in 0.2 % carbonate buffer (pH 9.8). Following an overnight incubation at 37°C, the wells were washed with CFS (3 x 100 µl), and the plastic was blocked with casein (0.2% in CFS). The plates were then incubated at room temperature for 45 min and washed with CFS as previously detailed.

Biotinylated mEGF-(33–42) in CFS was then aliquoted into the wells (1.6 µg/well), and the plates were incubated for 3 h at 37°C. After a further block with 0.2% casein, the wells were washed with CFS (3 x 100-µl aliquots).

Control cells were prepared as above and preincubated for 1 h at 37°C in peptide-free medium, test cells were preincubated with serial dilutions of either the anti-laminin receptor polyclonal or anti-EGF (R1) receptor monoclonal antibodies, prior to addition to coated wells. Subsequent procedures were as detailed for the laminin attachment assay.

Molecular Modeling

Amino acid residues in the synthetic peptides are numbered by their position (P1–P10) when aligned with mEGF-(33–42).2

Modeling of mEGF-(33–42) Using Molecular Dynamics at 350 K

The Sybyl suite of programs (version 6.0, Tripos Associates Inc., St Louis, MO), running on an ESVS3+ workstation (Evans and Sutherland Inc., Salt Lake City, UT) was used throughout the modeling. The starting structure was obtained from the Brookhaven Protein Data Bank (29), (entry 1EPI, mEGF (30)). Residues 1–32 and 43–53 of mEGF were deleted, and the N and C termini of the remaining decapeptide structure (CVIGYSGDRC) were capped with acetyl and amide groups, respectively. Acm groups were built on the exposed sulfur atoms to give mEGF-(33–42) in the initial, native conformation S0, a starting point for all the dynamics simulations.

This structure was minimized to generate an initial structure for the dynamics run. Partial atomic charges were calculated using the Pullman method (31), and a distance-dependent dielectric of 4 was employed, with an 8-Å cutoff for nonbonded interactions. Aasp (P8) and Arg (P9) were assigned charges of −1 and +1, respectively. During the simulation, the SHAKE algorithm (32) was used for nonpolar hydrogen atoms using a 100-fs interval with a dynamics calculation interval of 1 fs. The temperature was raised from 0 K to 350 K in seven equal steps of 200 fs, with coupling to the temperature bath every 10 fs. The simulation was continued for a further 40 ps, with coordinates, temperatures, and potential energies being recorded at 200-fs intervals. During this simulation, both the “local temperatures” of the backbone and

2 Synthetic peptides are aligned with mEGF-(33–42), and residue positions are numbered in the text starting from the N-terminal cysteine 33. For example, the positions for mEGF-(33–42) are as follows: Cys (P1), Val (P2), Ile (P3), Gly (P4), Tyr (P5), Ser (P6), Gly (P7), Asp (P8), Arg (P9), Cys (P10).

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side chain atoms and also the root mean square deviation of the C-α atoms. The starting mEGF-like conformation were monitored. A conformer from late in the simulation was chosen as being representative and was refined by minimization to give the final mEGF-(33–42) fold.

**Modeling of Lam.B1-(925–933)** Using Molecular Dynamics at 350 K—For the laminin B1 nonapeptide, the mEGF-(33–42) structure (S0) was used as a starting point. The C-terminal cysteine-(S-Acm)-amide residue at P10 was removed, and the exposed arginyl residue (P9) was capped with an amide. Since this laminin nonapeptide, unlike mEGF-(33–42), was not N-terminally acetylated, the N-terminal acetyl capping group was replaced with an ammonium group. The rest of the residues were then “mutated” to reflect the required laminin sequence on a residue-by-residue basis with the proline geometry being adjusted by the software. This structure was minimized without charges and subjected to a dynamics simulation, extended to 51.4 ps, with identical conditions to the mEGF-(33–42) decapeptide.

**Modeling of Random mEGF-(33–42)** Using Molecular Dynamics at 350 K—In order to generate a possible structure for the random peptide, the same starting molecular conformation (S0) of mEGF-(33–42) was used as for the generation of the previous structures. This was mutated to reflect the desired sequence, and the S-ACM groups were added as before, with manual adjustment of the side chain torsion angles. The starting conformation was then minimized and subjected to the same dynamics simulation as the other two structures. The simulation extended to 41.4 ps.

**RESULTS**

**Laminin Receptor Assay**—Laminin receptor assays were carried out on live cells in suspension at 4°C to prevent possible interference from laminin-induced receptor up-regulation or temperature-dependent laminin polymerization (16, 33).

Both the Lam.B1-(925–933) and mEGF-(33–42) peptides were found to be approximately equipotent with unlabeled native laminin in their respective abilities to compete with binding of 125I-labeled laminin to receptors on the human breast cancer cell line T-47D, and the immortalized human endothelial cell line SK HEP-1. For example, Lam.B1-(925–933), mEGF-(33–42), and laminin displaced the radiolabeled ligand from T-47D cells with respective IC50 values of 1 nM, 3 nM, and 1 nM (see Fig. 1A, Table I). In SK HEP-1 cells, the two synthetic peptides exhibited receptor binding activity close to that of native laminin (IC50 value range = 1.5–2 nM, Table I). This similarity in binding affinities was confirmed in receptor assays of early passage BRCE cells, where laminin, Lam.B1-(925–933), and mEGF-(33–42) were equally active with IC50 values in the range 3–4.3 nM (Table I).

In contrast, both neuromedin B and random mEGF-(33–42) failed to bind to the laminin receptor on any of the three cell types (Fig. 1A, Table I).

**Laminin Attachment Assay**—Preliminary investigations determined that cell binding to solid-phase laminin becomes increasingly irreversible with time. After addition to the laminin substratum, cells rapidly become resistant to detachment by subsequently added peptides, such that after 15 min of adsorption, additions of mEGF-(33–42) or Lam.B1-(925–933) are essentially without effect (not shown). EC50 values were 2-fold higher if cells and peptides were added simultaneously to the laminin substratum, compared with cells pre-equilibrated with the competing peptides. Binding by cell suspensions of mEGF-(33–42) or Lam.B1-(925–933) reached equilibrium by 40 min, as assessed by subsequent laminin attachment assays, and remained steady for up to 4 h of preincubation (not shown).

In the final assay configuration (described under “Materials and Methods”), treatment of the T-47D human breast cancer cells with solutions of laminin, Lam.B1-(925–933), or mEGF-(33–42) all resulted in inhibition of cell attachment to the laminin-coated wells. However, in contrast to the results obtained in liquid phase laminin receptor assays, binding to solid phase laminin was more resistant to displacement by the two synthetic peptides compared with displacement by native laminin (Fig. 1B, Table I). In all cell lines tested, concentrations of soluble laminin required for 50% inhibition of attachment (EC50 values) were in the range 0.2–0.3 nM, which is approximately 1000-fold lower than the EC50 values of Lam.B1-(925–933) (230–330 nM) or mEGF-(33–42) (269–380 nM). Similar results were obtained with both established human cell lines (T-47D, SK HEP-1) and primary cultures of endothelial cells (BRCE and avian embryonic endothelial cells).

Neither neuromedin B (an unrelated decapeptide included as a negative control) nor the random mEGF-(33–42) peptide had any effect on cell attachment to the laminin substratum when each was tested up to concentrations of 1 mM (Fig. 1B, Table I). Attachment of cells to laminin was found to be blocked by the anti-67-LR antibody (anti-peptide Pro-20-Ala), but was unaf-
profiles are not shown in the interests of brevity. Results are presented as IC50 (nM) "sweep" displays of the obtained at 350 K for mEGF-(33–42), Lam.B1-(925–933), and square deviation values (Table III). Stable structures were cases, neither of these control peptides caused inhibition up to concentrations of 1 μM.

In the modeling studies, both active peptides adopted a right-handed helical conformation of similar geometry, making just over a complete turn (Table III, Fig. 4). This was stabilized by a hydrogen bond (not shown) between the P2 carbonyl oxygen and the P5 amide proton of the second through sixth residues with less stability at the ends of the molecules (Fig. 3). The representative minimized structures (obtained at 22.6, 36.6, and 32.4 ps for mEGF-(33–42), Lam.B1-(925–933), and the random peptide, respectively) are shown in Fig. 4.

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Lam.B1-(925–933) was better than the mEGF-(33–42)/mEGF comparison (Table III), and unlike the synthetic peptides, the Tyr side chain is not solvent-exposed in native mEGF (Table III; Ref. 35).

The random peptide adopted a significantly tighter helical conformation, stabilized by three backbone hydrogen bonds between residues at positions P3–P9 in a typical α-helical pattern. In addition, unlike the active peptides, this compact helical conformation continued beyond P6 up to the penultimate residue and resulted in a much smaller Tyr (P5) to Arg (P9) distance (Table III). The termini were drawn together by a hydrogen bond (not shown) between the carbonyl oxygen of the N-terminal acetyl groups and a C-terminal amide proton.

**DISCUSSION**

The results presented show that the antiangiogenic activities of mEGF-(33–42) are mediated by the YIGSR-specific 67-kDa laminin receptor. Both mEGF-(33–42) and Lam.B1-(925–933) have affinities similar to that of native laminin in whole cell receptor binding assays (Fig. 1A, Table I), results being similar in the three cell lines tested. The IC_{50} values for laminin, Lam.B1-(925–933), and mEGF-(33–42) (range ~1–5 nM) are in good agreement with laminin receptor affinities previously reported for the whole laminin molecule (K_{D} = 1–2 nM) (36, 37). To our knowledge, this is the first report of laminin receptor radioligand displacement assays using these synthetic peptides.

Treatment of cells with solutions of laminin, Lam.B1-(925–933), or mEGF-(33–42) inhibits cell attachment to solid-phase laminin substrata (Fig. 1B, Table II). Unlike 4°C receptor assays, however, where all three ligands are found to be equipotent, attachment assays reveal large differences in the effective concentrations required to half-maximally inhibit adhesion to solid-phase laminin. The EC_{50} values for the synthetic peptides were approximately 1000-fold higher than the EC_{50} values for native laminin, and the peptides’ EC_{50} values were approximately 100-fold higher than their IC_{50} values obtained from the receptor assays (Tables I and II).

In the case of the small synthetic ligands, the concentrations required to inhibit attachment suggest that the laminin receptors must be almost saturated in order to prevent even 50% of maximal attachment. By contrast, laminin (in solution) is relatively more efficient in inhibiting cell binding to solid-phase laminin when compared with its ability to displace 125I-labeled laminin binding to suspended cells (EC_{50} for attachment is approximately 10-fold lower than the IC_{50}). This suggests that soluble laminin need only occupy a minority of the cells’ complement of receptors to inhibit binding to solid-phase laminin. The inhibitory effect of the peptides on cell binding to solid-phase laminin is both time- and concentration-dependent. Thus, the peptides are less effective when added simultaneously with the cells at the time of plating onto the laminin substratum, and cells that are allowed to bind to solid-phase laminin become increasingly resistant to detachment by subsequent treatment with mEGF-(33–42) or Lam.B1-(925–933). This suggests that binding to solid-phase laminin is not a simple equilibrium binding process but instead becomes increasingly irreversible with time. This may be a consequence of ancillary binding by the attached cell to multiple sites on native laminin that are distinct from the 67-LR binding domain; laminin contains a variety of both integrin and nonintegrin binding sites (1). Initially the process of formation of these supplementary bonds could be interfered with by co-incubation with native laminin in solution, but the small peptides, which only contain the 67-LR binding domain, would be ineffective. Despite the presence of ancillary binding sites on laminin, saturation of the YIGSR-specific high affinity sites alone (by pretreatment with either Lam.B1-(925–933) or mEGF-(33–42)) nevertheless renders the cells incapable of attaching to solid-phase laminin. This suggests that binding to these ancillary sites on the solid-phase laminin molecules is dependent upon, and consequent to, binding to 67-LR. The results also suggest that these supplementary bonds are not made during 4°C incubations with 125I-laminin in solution.

Although fully attached cells become resistant to detachment, their motility is affected by the peptides; 500 nM Lam.B1-(925–933) produces stimulation of motility approximately equal to that elicited by 5 nM laminin, and 50–500 nM mEGF-(33–42) fully antagonizes that stimulus (5). The fact that the relative potency of mEGF-(33–42) in motility assays is in good agreement with attachment assay results suggests that near saturation of 67-LR by mEGF-(33–42) is sufficient to account for its observed ability to block laminin-stimulated motility.

The respective inactivities of neuromedin B (an unrelated decapeptide) and a random synthetic peptide containing all of the residues found in mEGF-(33–42) demonstrate that the inhibitory effects of mEGF-(33–42) are not merely a non-specific perturbation, nor are they manifested by a random array of its residues; but they are likely to be sequence-specific. In final confirmation that the effects of mEGF-(33–42) are mediated by the 67-LR, we found that solid-phase mEGF-(33–42) was able to induce attachment of T-47D cells and that this attachment could be completely blocked by preincubating cells with the anti-67-LR antibody (Fig. 2). In contrast, the R1 monoclonal antibody (24), directed against the EGF receptor, had no effect on cell adhesion to mEGF-(33–42).

The fact that the small linear peptides have receptor affinities equal to that of laminin would suggest that all three ligands for the 67-LR adopt a common conformation. Using unconstrained molecular dynamics, we have obtained struc-
tures for mEGF-(33–42), Lam.B1-(925–933), and the random peptide, which were all different from the native EGF fold, used as a starting conformation. Our minimized Lam.B1-(925–933) structure is in broad agreement with previous molecular modeling studies of Lam.B1-(925–933) and YIGSR-NH₂ (38, 39), both of which predicted a right-handed helix, and proposed this to be important for bioactivity. Our corresponding structure for mEGF-(33–42) shows some differences from Lam.B1-

![Diagram showing Cα sweep diagrams obtained during the dynamics simulation. Conformers are shown for mEGF-(33–42) (A), human Lam.B1-(925–933) (B), and the random peptide (C) (see “Materials and Methods” for modeling procedure). In these diagrams the yellow “sheaves” represent sequential conformations (0.2 ps) of the Cα atoms for each of the peptides during a 10-ps window in the simulation. For each peptide a representative conformation was chosen; this is shown with its side chain atoms colored by atom type. Each molecule is presented in an approximately equivalent view, with the N terminus to the left and the P5 tyrosyl side chain to the upper right. The simulation windows are as follows (the time value in parentheses indicates the temporal position of the representative molecule in each case): 20–30 (22.6) ps (A), 33–43 (36.6) ps (B), and 30–40 (32.4) ps (C).]
FIG. 4. Stereo view of the minimized model structures after dynamics together with native mEGF. A, mEGF-(33–42); B, human Lam.B1-(925–933); C, the random peptide (see “Materials and Methods” for modeling procedure) are shown, together with the NMR-defined structure of mEGF, entry 1EPI from the Brookhaven Protein Data Bank (D). In these diagrams of the peptides, the complete side chains are shown, superimposed on the C-α trace. Residues of high backbone structural homology between the different peptide molecules are highlighted by coloring the trace in green (P3–P6). For the native mEGF molecule, the region outside the sequence 33–42 is cyan, and the rest is in red except for residues 35–37, which, having a similar fold to the modeled mEGF-(33–42) peptide, are colored green. The disulfide bridge between residues 33 and 42 in the native mEGF is also highlighted by being displayed in yellow. Each molecule is presented with the N terminus to the left.
EFG-(33–42) and Laminin Receptor

(925–933) at the N and C termini, but the overall fold is similar. The inactive random peptide, on the other hand, adopts a much tighter and more regular helical fold (Table III).

The elliptical fold adopted by mEFG-(33–42) and Lam.B1-(925–933) (Fig. 4, A and B) results in conserved residue pairs (Gly (P4), Tyr (P5), and Gly (P7)) being superimposable and places the aromatic ring of Tyr (P5) some 11–16 Å from the guanidino group of Arg (P9) in the two structures, more than twice the distance found in the inactive random mEFG-(33–42) (Table III). The requirement for a tyrosyl and guanidino group on the same face of a helical fold is a feature of the active Lam.B1-(925–933) structures derived from NMR studies by Ostheimer, et al. (40). The side chains of the adjacent nonconserved residue pairs Ile/Ser (P6) and Ser/Asp (P8) also occupy an equivalent position to that found in the synthetic analogues (Table III, Fig. 4, A and B). Thus, the primary sequence homology is insufficient for receptor recognition; rather, we propose that the consensus sequence GXGXR must adopt an open right-handed helical fold, presenting the aromatic side chain of a tyrosyl residue (P5) and the guanidino group of the arginy1 residue (P9) on the same side of the fold.

Various studies have demonstrated that Lam.B1-(925–933) analogues exert antimetastatic effects via inhibition of attachment to basement membranes. The results presented here show that mEFG-(33–42) has a similar antimetastatic potential. As an antiangiogenic agent it has the advantage of being a pure antagonist of laminin in human and chick cells, whereas Lam.B1-(925–933) acts as an agonist or partial agonist (5). mEFG-(33–42) does not bind to the EGF-receptor and may therefore be a useful probe for studies of the incompletely characterized YIGSR-specific receptor. Further studies to investigate the role of individual residues in the mEFG-(33–42) sequence are under way.

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