The C-terminal Nonapeptide of Mature Chemerin Activates the Chemerin Receptor with Low Nanomolar Potency*

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Valérie Wittamer‡§, Françoise Grégoire¶, Patrick Robberecht†, Gilbert Vassart‡, David Communi‡, and Marc Parmentier‡**

From the ²Institut de Recherche Interdisciplinaire en Biologie Humaine et Moléculaire et the ³Laboratoire de Chimie Biologique et de la Nutrition, Université Libre de Bruxelles, Campus Erasme, Route de Lennik 808, B-1070, Brussels, Belgium

Chemerin is a novel protein identified as the natural ligand of ChemR23 (chemerinR), a previously orphan G protein-coupled receptor expressed in immature dendritic cells and macrophages. Chemerin is synthesized as a secreted precursor, prochemerin, which is poorly active, but converted into a full agonist of chemerinR by proteolytic removal of the last six amino acids. In the present work, we have synthesized a number of peptides derived from the C-terminal domain of human prochemerin and have investigated their functional properties as agonists or antagonists of human chemerinR. We found that the nonapeptide YHSFFFPGQFAFS (chemerin-9), corresponding to the C terminus of processed chemerin, retained most of the activity of the full-size protein, with regard to agonism toward the chemerinR. Extension of this peptide at its N terminus did not increase the activity, whereas further truncations rapidly resulted in inactive compounds. The C-terminal end of the peptide appeared crucial for its activity, as addition of a single amino acid or removal of two amino acids modified the potency by four orders of magnitude. Alanine-scanning mutagenesis identified residues Tyr156, Phe156, Gly152, Phe153, and Phe156 as the key positions for chemerinR activation. A modified peptide (YHSFFFPGQFAFS) was synthesized and iodinated, and a radioligand binding assay was established. It was found that the ability of the various peptides to activate the chemerin receptor was strictly correlated with their affinity in the binding assay. These results confirm that a precise C-terminal processing is required for the generation of a chemerinR agonist. The possibility to restrict a medium sized protein to a nonapeptide, while keeping a low nanomolar affinity for its receptor is unusual among G protein-coupled receptors ligands. The identification of these short bioactive peptides will considerably accelerate the pharmacological analysis of chemerin-chemerinR interactions.

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‡ Supported by a FIRST-Entreprise fellowship of the Walloon Region.
§ Chercheur Qualifié of the Belgian Fonds National de la Recherche Scientifique.
¶ To whom correspondence should be addressed. Tel.: 32-2-555-4171; Fax: 32-2-555-4655; E-mail: mparmen@ulb.ac.be.

G protein-coupled receptors (GPCRs)1 constitute the largest family of membrane receptors, and members of this family represent the ultimate targets of over half the biologically active molecules used presently as therapeutic agents (1). The GPCR family still includes a large number of orphan receptors, which have been made available through various cloning procedures, such as PCR amplification using degenerate oligonucleotides and systematic sequencing of cDNA libraries and genomes. Some of these orphan receptors have allowed over the past few years to uncover novel extracellular signaling pathways involved in various physiological or pathological processes (2, 3). Such new ligands were purified on the basis of their activity on an orphan receptor and subsequently characterized through mass spectrometry and sequencing analysis, a process referred to as reverse pharmacology. These new ligands include, among others, nociceptin/orphanin FQ (4, 5), orexins (6), ghrelin (7), mestatins/kisspeptins (8, 9), and apelin (10). The involvement of some of these new systems in important physiological processes has already been amply demonstrated, such as the role of orexins in the control of feeding and sleep (6). As a consequence, a number of these newly identified receptors constitute promising targets for future drug development.

ChemerinR was identified as a human orphan G protein-coupled receptor, originally cloned from genomic DNA by low stringency PCR (11). The mouse ortholog, sharing 80% identity at the amino acid level, was described in parallel as Dez (12). We have recently identified the natural ligand of this receptor, following the identification of a specific biological activity in a human ascitic fluid secondary to an ovary carcinoma (13). This biological activity was purified, and the ligand was characterized by mass spectrometry as deriving from the tazarotene-induced gene (Tig)-2. However, the Tig-2 gene encodes a secreted protein, prochemerin, which is poorly active on chemerinR. Prochemerin requires the removal of six amino acids at its C-terminal end, to become a high affinity agonist of chemerinR. The extracellular protease, which is responsible for this activation, is presently not known, but a number of cell lines, including CHO-K1 and COS-7 cells, were shown to process prochemerin adequately. Prochemerin belongs to the structural cathelicidin/cystatin family of proteins, which includes precursors of bactericidal peptides (cathelicidins), precursors of mediators active on leukocytes through G protein-coupled receptors (prokininogen, cathelicidin precursors) as well as cysteine protease inhibitors (cystatins) (14–16). ChemerinR is expressed primarily on immature dendritic cells and macrophages, suggesting a role in the early steps of the mounting of an immune response. Chemerin was indeed shown to promote...
Intracellular calcium release in monocyte-derived macrophages and dendritic cells and to induce chemotaxis of these cell populations in an in vitro setting.

The production of bioactive chemerin in bacteria, yeast, or mammalian cells is a tedious process, which has impaired the study of its function so far. In this work, we have tested the functional activity of peptides derived from the C-terminal domain of prochemerin and have determined that a nonapeptide could mimic functional properties of chemerin onto its receptor with high potency (EC_{50} of 7 nM). The structure-activity relationships of this peptide are analyzed as well as its pharmacological properties on chemerinR-expressing cell lines.

**EXPERIMENTAL PROCEDURES**

**Cell Culture**—CHO-K1 cell lines expressing the human recombinant chemerin receptor have been described previously (13). Briefly, a bis-tronic eukaryotic expression vector encoding human chemerinR was used to generate stable transfectants in a CHO-K1 cell line co-expressing a mitochondria-targeted form of aequorin and Gr_{66} in WTA11-ChemerinR cell line. Cells were maintained in Ham’s F-12 medium supplemented with 10% fetal calf serum (Invitrogen), 100 units/ml penicillin, 100 μg/ml streptomycin (Invitrogen), and 400 μg/ml G418.

**Peptide Synthesis**—All the peptides were synthesized by solid phase methodology using the Fmoc (9-fluorenyl-methoxycarbonyl) strategy with an automated Symphony apparatus. The peptides were cleaved, precipitated with 10 volumes of cold ether and purified on reverse phase and ion exchange chromatographies to reach our purity criteria (over 97%). The peptide purity was assessed by capillary electrophoresis, and the conformation was verified by electrospray mass spectrometry.

**Functional Assay**—The functional response of chemerinR to peptides was analyzed by measuring the aequorin luminescence in the WTA11-ChemerinR cell line, as described (17). Briefly, the cells were collected from plates with phosphate-buffered saline supplemented with 5 mM EDTA, pelleted for 2 min at 1000 × g, resuspended in Dulbecco’s modified Eagle’s medium at a density of 5 × 10^5 cells/ml and incubated for 3 h in the dark in the presence of coelenterazine H (Molecular Probes) at a final concentration of 5 μM. Cells were diluted 5-fold before use. Peptides in a volume of 50 μl were added to 50 μl of cell suspension, and luminescence was measured for 20 s in a Packard lumimeter. Functional parameters were determined with the program Prism (Graphpad Software) using nonlinear regression applied to a sigmoidal dose-response model.

**Binding Assays**—The YHSSFFPQGQAFS peptide derived from chemerin 146–157 was radioiodinated on the tyrosine by the IODO-GEN technique and separated from unincorporated radioiodine using a C18 Sep-Pak cartridge. The specific activity of this tracer was estimated to 1250 Ci/mmol. Transfected CHO-K1 cells stably expressing chemerinR were collected from plates with phosphate-buffered saline supplemented with 5 mM EDTA, gently pelleted for 2 min at 1000 × g, and resuspended in binding buffer (50 mM Hepes, pH 7.4, 1 mM CaCl_{2}, 5 mM MgCl_{2}, 0.5% bovine serum albumin). A mixture of protease inhibitors (one Complete™ tablet/50 ml, Roche Diagnostics) was added before use. Binding assays were performed in Minisorb Tubes (Nunc), using 800,000 cells in a final volume of 200 μl. For saturation binding assays, increasing concentrations of tracer were used. Total binding was measured in the absence of competitor, and nonspecific binding was measured with an excess (1 μM) of unlabelled ligand. For competition binding assays, 0.5 nM tracer (about 100,000 cpm) was used with variable concentrations of competitors. Samples were incubated for 20 min at 27 °C, then bound tracer was separated by filtration through GF/B filters presoaked in 1% bovine serum albumin. The filters were washed four times with ice-cold binding buffer supplemented with 500 mM NaCl and counted in a scintillation counter. Data resulting from competition binding assays were analyzed for the nonspecific binding (0%) and the specific binding in the absence of competitor (100%). Binding parameters were determined with Prism using nonlinear regression applied to a one-site competition binding model.

**RESULTS**

**Peptides Derived from the C Terminus of Processed Chemerin Are Potent Agonists of the Chemerin Receptor**—Previous work has demonstrated that the proteolytic processing of prochemerin into chemerin is essential for the biological activity of the protein (13). This processing affects the C-terminal end of the protein, located after the last cysteine involved in the disulfide bonds that presumably stabilize the cystatin fold that characterizes this secreted protein. We therefore reasoned that the C-terminus of the protein might constitute an unstructured flexible domain and that peptides derived from this domain could retain part of the biological activity of the full-size protein on the chemerin receptor. To test this hypothesis, we synthesized a first set of peptides derived from the C-terminal end of prochemerin and chemerin. Chemerin 139–157 represents the last 19 amino acids of the active processed protein, starting at Gln_{139}^, four amino acids after the last cysteine (see Table I for sequences). As a control, the corresponding peptide prochemerin 139–163 was synthesized, which contains the C-terminal extension the six amino acids of the precursor, which are cleaved off during its proteolytic processing. Prochemerin 158–163, the six amino acids peptide removed during processing, was also synthesized. These three peptides were tested for their ability to trigger intracellular calcium release through the chemerinR, using an aequorin-based assay. For this purpose, a CHO-K1 cell line expressing the human recombinant chemerin receptor, aequorin and Gr_{66} was used, as previously described (13). Human recombinant chemerin and prochemerin, expressed in CHO-K1 cells and purified from conditioned media by affinity chromatography and high pressure liquid chromatography (13), were used as references. As shown in Fig. 1A and Table I, prochemerin 139–163 was devoid of any biological activity up to concentrations of 10 μM, whereas chemerin 139–157, lacking the last six amino acids of the former, activated the chemerin receptor with high potency (EC_{50} of 16.7 ± 3.2 nM). The activity of this peptide is only slightly less potent as compared with recombinant human chemerin (EC_{50} of 4.5 ± 0.7 nM). As described previously, recombinant prochemerin was poorly active (EC_{50} of 393 ± 116 nM), and this preparation is most probably contaminated by trace amounts of the processed protein, resulting in an overestimation of its actual potency. As expected, no activity was displayed by prochemerin 158–163 (data not shown). On one hand, these results are consistent with our previous data on full-size proteins, which had highlighted the functional importance of the C-terminal proteolytic processing of prochemerin, transforming a poorly active precursor into a high affinity agonist. On the other hand, these results were surprising, because they suggest that chemerin can be down-sized to an unstructured C-terminal peptide of 19 amino acids, without significant loss of biological activity.

**Accurate Processing of the Prochemerin C Terminus Is Critical for Bioactivity**—Following the observation that chemerin-derived peptides retain a strong biological activity onto chemerinR, we used additional synthetic peptides to determine the structural determinants that are critical for the bioactivity of chemerin. We first investigated the requirement for accurate processing of prochemerin at its C terminus, by adding or removing single amino acids at this end of prochemerin 139–157 (Table I). As shown in Fig. 2A and Table I, addition of a single amino acid (prochemerin 139–158) strongly affected the potency of the peptide, because no activation of chemerinR was observed up to concentrations of 10 μM. A similar loss of potency was obtained after removal of two or three amino acids (chemerin 139–155 and 139–154). However, removal of a single amino acid resulted in a peptide able to activate the receptor but with a 6-fold drop of potency (chemerin 139–156, EC_{50} of 97 ± 13 nM). These data demonstrate that an accurate processing of prochemerin C terminus is required, to generate potent chemerinR agonists.

**Identification of the Nonapeptide YFPGQGQAFS as the Shortest Peptide Retaining High Potency**—After specifying the opti-
The sequence of all peptides tested in the present study are displayed, as well as the functional (pEC50) and binding (pIC50) parameters of their activity on chemerinR-expressing CHO-K1 cells. Data are given as mean ± S.E. and were calculated from at least three independent experiments.

<table>
<thead>
<tr>
<th>Peptide nomenclature</th>
<th>Peptide sequence</th>
<th>pEC50</th>
<th>pIC50</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recombinant prochemerin</td>
<td>1–163</td>
<td>6.45 ± 0.15</td>
<td>ND</td>
</tr>
<tr>
<td>Recombinant chemerin</td>
<td>1–157</td>
<td>8.38 ± 0.07</td>
<td>8.18 ± 0.27</td>
</tr>
<tr>
<td>Prochemerin 139–163</td>
<td>QRAGEDPSFYFPGQAFSKALPRS</td>
<td>≤5</td>
<td>ND</td>
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<tr>
<td>Chemerin 139–157</td>
<td>QRAGEDPSFYFPGQAFS</td>
<td>7.79 ± 0.78</td>
<td>7.70 ± 0.07</td>
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<td>Prochemerin 158–163</td>
<td>KALPRS</td>
<td>≤5</td>
<td>ND</td>
</tr>
<tr>
<td>Chemerin 139–158</td>
<td>QRAGEDPSFYFPGQAFSK</td>
<td>≤5</td>
<td>≤6</td>
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<tr>
<td>Chemerin 139–156</td>
<td>QRAGEDPSFYFPGQAF</td>
<td>7.02 ± 0.06</td>
<td>7.16 ± 0.09</td>
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<tr>
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<tr>
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<td>QRAGEDPSFYFPGQF</td>
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<td>Chemerin 139–153</td>
<td>QRAGEDPSFYFPGQ</td>
<td>ND</td>
<td>ND</td>
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<tr>
<td>Chemerin 151–157</td>
<td>PFGQAFS</td>
<td>≤5</td>
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<tr>
<td>Chemerin 150–157</td>
<td>FPGQAFS</td>
<td>5.84 ± 0.16</td>
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<td>Chemerin 149–157 (named chemerin-9)</td>
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<td>Chemerin 148–157</td>
<td>YFPQGQAFAFS</td>
<td>8.11 ± 0.12</td>
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<tr>
<td>Chemerin 146–157</td>
<td>HSFPQGQAFS</td>
<td>7.94 ± 0.12</td>
<td>ND</td>
</tr>
<tr>
<td>Chemerin 145–157</td>
<td>PHSFPQGQAFS</td>
<td>7.85 ± 0.01</td>
<td>ND</td>
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<tr>
<td>[Ala149] chemerin-9</td>
<td>AFGQGQAFAFS</td>
<td>6.32 ± 0.08</td>
<td>6.65 ± 0.14</td>
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<td>[Ala150] chemerin-9</td>
<td>YAPGQGQAFAFS</td>
<td>6.84 ± 0.12</td>
<td>6.60 ± 0.13</td>
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<td>[Ala151] chemerin-9</td>
<td>YFAGQGQAFAFS</td>
<td>7.38 ± 0.08</td>
<td>6.89 ± 0.14</td>
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<td>[Ala153] chemerin-9</td>
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<td>7.59 ± 0.11</td>
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<td>≤6</td>
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<td>[Ala156] chemerin-9</td>
<td>YFPQGQFAFS</td>
<td>7.32 ± 0.06</td>
<td>7.23 ± 0.09</td>
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<td>[Ala157] chemerin-9</td>
<td>YFPAGGQQAFAFS</td>
<td>6.82 ± 0.10</td>
<td>ND</td>
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<td>Tyr[Ser141] chemerin 146–157</td>
<td>YHSFFPQGQAFS</td>
<td>7.76 ± 0.11</td>
<td>7.72 ± 0.05</td>
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Fig. 1. Biological activity of prochemerin, chemerin, and peptides derived from their C terminus on chemerinR. The biological activity of recombinant prochemerin (○), recombinant processed chemerin (■), the 25-amino acid C-terminal peptide of prochemerin (prochemerin 139–163, □), and the corresponding 19-amino acid C-terminal peptide of processed chemerin (chemerin 139–157, ▼), using the aequorin-based intracellular Ca2+ release assay. The curves represent the mean ± S.E. of duplicate data points and are representative of at least three independent experiments.

Fig. 2. Effects of C-terminal modifications of chemerin-derived peptides. A, functional aequorin-based intracellular Ca2+ release assay and B, competition binding assay using chemerin-derived peptides differing at their C-terminal end. Chemerin 139–157 (●), prochemerin 139–158 (○), chemerin 139–156 (△), and chemerin 139–155 (■) were used in both assays. The curves represent the mean ± S.E. of duplicate data points and are representative of at least three independent experiments.

As compared with the full-size protein. This minimal C-terminal nonapeptide, that represents a new reference peptide for chemerinR pharmacology was renamed chemerin-9. The rapid loss of potency following further truncation suggests an impor-
Peptide Agonists of the Chemerin Receptor

Aromatic Residues in Chemerin-9 Are Critical for Receptor Activation—Within the minimal nonapeptide active on the chemerin receptor, we next investigated the relative contribution of each amino acid by using an L-alanine-scanning mutagenesis approach. Eight alanine-substituted chemerin-9 analogues (Ala155 was not mutated) were synthesized and tested for their ability to promote functional activation of chemerinR, as compared with the reference peptide. As shown in Fig. 5, the substitution by serine affected significantly the potency of the analogs (Fig. 6). A dissociation constant ($K_d$) of 23,801 nM and a binding capacity ($B_{max}$) of 19 ± 5 nM. These results indicate that it is the aromatic ring of tyrosine, and not the hydroxyl group, that is important for the bioactivity of the peptide. They also validated the use of the Phe substitute as a substrate for radioiodination. Using the IODO-GEN technique, a tracer with a specific activity of 1250 Ci/mmol was obtained, and this tracer was used in a saturation binding assay, using chemerinR-expressing CHO-K1 cells. Saturation binding data were compatible with a single binding site for $^{125}$I-Tyr-[Phe149]-chemerin-9 (Fig. 6). A dissociation constant ($K_d$) of 23.9 ± 9.4 nM and a binding capacity ($B_{max}$) of 23,801 ± 401 binding sites per cell were derived from a competitive binding assay using the unlabeled peptide as competitor. In the conditions used, nonspecific binding represented about 30% of the total binding at the $K_d$ concentration of tracer, and no specific binding was observed on untransfected CHO-K1 cells.

Residues Involved in Bioactivity Are Also Necessary for Receptor Binding—With the aim of complementing the pharmacological characterization of chemerin-derived peptides, we used the binding assay to explore the ability of these peptides first tested whether this tyrosine could be substituted either for Phe or Ser. We selected the dodecapeptide chemerin 146–157 as the template for these modifications, and we added in parallel a tyrosine at the N terminus of this peptide, a position expected to be neutral for bioactivity, even after addition of the bulky iodine atom (Table I). These two analogs were tested for their ability to promote functional activation of chemerinR, as compared with the reference peptide. As shown in Fig. 5, the substitution by serine affected significantly the potency of the analogs (Fig. 6). A dissociation constant ($K_d$) of 23,801 nM and a binding capacity ($B_{max}$) of 19 ± 5 nM. These results indicate that it is the aromatic ring of tyrosine, and not the hydroxyl group, that is important for the bioactivity of the peptide. They also validated the use of the Phe substitute as a substrate for radioiodination. Using the IODO-GEN technique, a tracer with a specific activity of 1250 Ci/mmol was obtained, and this tracer was used in a saturation binding assay, using chemerinR-expressing CHO-K1 cells. Saturation binding data were compatible with a single binding site for $^{125}$I-Tyr-[Phe149]-chemerin-9 (Fig. 6). A dissociation constant ($K_d$) of 23.9 ± 9.4 nM and a binding capacity ($B_{max}$) of 23,801 ± 401 binding sites per cell were derived from a competitive binding assay using the unlabeled peptide as competitor. In the conditions used, nonspecific binding represented about 30% of the total binding at the $K_d$ concentration of tracer, and no specific binding was observed on untransfected CHO-K1 cells.

Development of a Radioligand for the Chemerin Receptor—From the results of the alanine-scanning mutagenesis, it appeared that the single tyrosine residue present in full-size chemerin (Tyr149) plays a critical role in receptor activation. This observation is consistent with the fact that all previous attempts to develop a binding assay based on the iodination of this tyrosine in full-size chemerin were unsuccessful. To obtain a bioactive peptide that would be suitable for iodination, we

![Fig. 3. Effects of N-terminal modifications of chemerin-derived peptides. A, functional aequorin-based intracellular Ca$^{2+}$ release assay of chemerin 139–157 (●) and N-terminally truncated peptides: chemerin 151–157 (△), chemerin 150–157 (▼), chemerin 149–157 (●), chemerin 148–157 (○), and chemerin 146–157 (□). B, competition binding assay for some of these peptides (the same symbols apply), using a chemerinR-expressing CHO-K1 cell line and $^{125}$I-Tyr-[Phe149]-chemerin 146–157 as tracer. The curves represent the mean ± S.E. of duplicate data points and are representative of at least three independent experiments.

![Fig. 4. Alanine scanning of the chemerin-9 peptide. A, functional aequorin-based assay and B, competition binding assay of peptides representing an ala-scan of chemerin-9 (chemerin 149–157). Chemerin-9 (●), [Ala150]-chemerin-9 (▲), [Ala151]-chemerin-9 (▼), [Ala153]-chemerin-9 (○), [Ala153]-chemerin-9 (□), and [Ala157]-chemerin-9 (△) were tested in both assays. The curves represent the mean ± S.E. of duplicate data points, and are representative of at least three independent experiments.](http://www.jbc.org/)

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![Image](http://www.jbc.org/)
Peptide Agonists of the Chemerin Receptor

Chemerin is a protein of 163 amino acids that we have recently identified as the natural ligand of the previously orphan receptor ChemR23, renamed as the chemerin receptor. Chemerin is able to recruit immature dendritic cells and macrophages, which are the major expression sites of the receptor. Studying the pharmacology and function of this new system has been impaired so far by the difficulty of producing functionally active recombinant chemerin. We have previously demonstrated that chemerin is secreted as a precursor of low biological activity, which, upon proteolytic cleavage of the last six amino acids, is converted into a potent agonist of the receptor. The length of the C-terminal end of chemerin appeared therefore to be critical for receptor activation. In this study, we have investigated the structure/function relationships of chemerin, and in particular, the possibility that synthetic peptides derived from the C terminus of chemerin might share some of the functional properties of the natural full-size protein on its receptor.

The situation of chemerin is reminiscent of what prevails for most chemokines, a major class of leukocyte chemoattractant molecules. Chemokines are small proteins (7–10 kDa) that are composed of a core domain structured by two disulfide bonds, and a flexible N-terminal domain (18). This N-terminal domain has been shown in all chemokines investigated so far to contribute much to the activation of their receptors. Indeed, truncation of the N terminus of chemokines frequently affects their potency (and sometimes their binding affinity as well) (19–21), and proteolytic processing of this domain has been shown in many cases to regulate the bioactivity of these molecules (20, 22–24). Moreover, synthetic peptides derived from chemokine N termini have been shown in some instances to display binding and/or functional activity on the cognate receptors (25, 26).

By analogy, we postulated that the C terminus of chemerin might play a similar role as the N terminus of chemokines. Given its homology with cystatins and cathelicidin precursors, as well as the similar organization of their genes, it is likely that chemerin adopts the so-called cystatin fold (13). This fold is presumably stabilized by two disulfide bonds common to cystatins, and an additional bond specific to chemerin, but compatible with the cystatin fold (data not shown). We therefore synthesized peptides derived from the C-terminal end of chemerin but starting after the last cysteine involved in the putative disulfide bonds. The first peptides synthesized were a 19-amino acid peptide corresponding to the C-terminal end of active chemerin (chemerin 139–157), and the corresponding 25-amino acid peptide corresponding to the C-terminal end of prochemerin, before proteolytic processing (prochemerin 139–163). Chemerin 139–157 was found to display high potency toward the chemerin receptor, whereas prochemerin 139–163 was totally inactive. These results confirmed the importance of the C-terminal processing of prochemerin. Testing longer and shorter peptides demonstrated that precise processing is essential for biological activity, because addition or removal of a single amino acid at the C terminus resulted in a strong reduction of biological activity. This observation is important, because mass spectrometry analysis of bioactive chemerin purified from natural sources (human ascitic fluid) of from CHO-K1 cells transfected with a prochemerin construct, has identified chemerin variants that lack one or two amino acids at their C terminus (data not shown). Our data demonstrate that these variants are poorly active and do not contribute much to the overall biological activity. The nature of the specific protease involved in chemerin processing remains to be determined.

N-terminal truncation of the initial active peptide allowed to identify the nonapeptide YFPGQFAFS (chemerin-9) as the
shortest molecule displaying strong agonist activity onto the chemerin receptor (Fig. 7). Alanine scanning mutagenesis identified Gly\textsuperscript{152}, Phe\textsuperscript{154}, and Phe\textsuperscript{156} and to a lower extent Tyr\textsuperscript{149} and Phe\textsuperscript{150} as critical for the bioactivity of the chemerin-derived peptides and likely of natural full-size chemerin as well. The replacement of Tyr\textsuperscript{149} by Phe was well tolerated, whereas a Ser at that position decreased the peptide potency, pointing toward a role of the aromatic ring of the tyrosine. The iodinated tracer that was designed on the basis of these data allowed us to demonstrate that the residues identified as being important in the functional assay are also required for the binding of peptides to the chemerin receptor. Disruption of binding by an amino acid substitution can indicate either a direct interaction of the residue side chain with the receptor or a modification of the peptide conformation required for binding. We postulate that the aromatic side chains of Tyr\textsuperscript{149}, Phe\textsuperscript{150}, Phe\textsuperscript{154}, and Phe\textsuperscript{156} are involved in hydrophobic interactions with the receptor, and the extracellular loops of the chemerin receptor contains indeed a number of aromatic amino acids that constitute potential contact points with the ligand. Further studies will be required to confirm the importance of these aromatic side chains and how they contribute to the stable interaction with the receptor. Alanine substitution of Gly\textsuperscript{152} leads to an almost inactive compound. Glycine residues are unlikely to contribute directly to binding, but are known to provide conformational flexibility to the peptide backbones. We postulate therefore that Gly\textsuperscript{152} allows the C terminus of chemerin to adopt the specific conformation required for interaction with the chemerin receptor binding pocket, while the more bulky side chain of alanine impairs the peptide flexibility.

Examples of short synthetic peptides derived from natural protein ligands and reported to retain strong biological activities are rare. In most cases, the affinity displayed by these peptides is much lower than that of the full-size proteins (27). The situation of chemerin appears therefore as unusual. We have mentioned above the proposed analogy between the C terminus of chemerin and the N terminus of chemokines, in terms of their importance for receptor activation, and the regulation of their activity by proteolytic processing. This analogy has initially led to the design of the first synthetic chemerin-derived peptides. The overall outcome of this study, however, greatly differentiates chemerin from the family of chemokines, in the way they interact with their cognate receptors. Indeed, the interaction of chemokines with their receptors involves a two-site model: their binding involves first a high affinity binding site between the core of the chemokine and the N terminus and loops of the receptor, whereas activation of the receptor usually requires a second interaction between the flexible N terminus of the chemokine and the transmembrane helix bundle of the receptor (18). As stated above, the structure of the N terminus of chemokines is essential for receptor activation, and truncation of this domain frequently leads to partial or full antagonists. However, synthetic peptides derived from this domain have usually low binding and functional activities (19). N-terminal peptides of SDF-1α were shown to bind and activate CXCR4, and to inhibit human immunodeficiency virus entry, but at concentrations in the micromolar range (25, 26). For other chemokines, peptides derived from the N terminus did not display any biological activity. The situation with chemerin is strikingly different, because a synthetic C-terminal nonapeptide acts as a full agonist and displays a binding affinity that is similar to that of the natural full-size protein. In the case of other chemoattractant proteins, such as the anaphylatoxins C5α or C3α, the flexible tail is also located at the C terminus of a structured core region (28). Synthetic peptides consisting in the last eight amino acids of C5α or the last five amino acids of C3α were identified as agonists on their respective receptors, but with potencies 2 to 3 orders of magnitude lower than the natural factor (29–31).

The structured cystatin-like N-terminal domain of chemerin might also display functions totally independent from the action on chemerinR investigated here. Indeed, cystatins are known for their inhibitory action on cysteine proteases. Cathepsidins have also been described as displaying independent actions according to the domain of the protein considered: the C-terminal peptide released by proteolytic processing acts as a bacterialic peptide, whereas the structured N-terminal domain recruits leukocyte populations. A dual role of chemerin cannot therefore be excluded, and specific actions of the cystatin-like module are presently being analyzed. The N-terminal domain of chemerin might also interact with proteoglycans, similarly to the core domain of chemokines, and this property could be essential for promoting leukocyte migration in vivo (32, 33).

In conclusion, we have characterized the pharmacology of the human chemerin receptor using chemerin-derived analogues and identified key residues contributing to the pharmacological activity of this novel ligand. Our results showed that the C-terminal nonapeptide of mature chemerin behaves in vitro as a high affinity ligand and a potent agonist of the chemerinR. These peptide ligands will significantly facilitate in vitro pharmacological studies, because the production of recombinant chemerin proved to be a tedious process. The aromatic residues identified as necessary for the activity of the peptide will also ease the rational design of peptide mimics displaying higher stability in vivo and will help in understanding how these peptides interact with the chemerinR.

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The C-terminal Nonapeptide of Mature Chemerin Activates the Chemerin Receptor with Low Nanomolar Potency
Valérie Wittamer, Françoise Grégoire, Patrick Robberecht, Gilbert Vassart, David Communi and Marc Parmentier

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