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# Chemerin and its receptors in leukocyte trafficking, inflammation and metabolism

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ABSTRACT

Chemerin was isolated as the natural ligand of the G protein-coupled receptor ChemR23. Chemerin acts as a chemotactic factor for leukocyte populations expressing ChemR23, particularly immature plasmacytoid dendritic cells, but also immature myeloid DCs, macrophages and natural killer cells. Chemerin is expressed by epithelial and non-epithelial cells as an inactive precursor, present at nanomolar concentrations in plasma. Processing of the precursor C-terminus is required for generating bioactive forms of chemerin. Various proteases mediate this processing, including neutrophil serine proteases and proteases from coagulation and fibrinolytic cascades. ChemR23-expressing cells are recruited in human inflammatory diseases, such as psoriasis and lupus. In animal models, both pro-inflammatory and anti-inflammatory roles of chemerin have been reported. Recently, two other receptors for chemerin were described, GPR1 and CCRL2, but their functional relevance is largely unknown. Both chemerin and ChemR23 are also expressed by adipocytes, and the emerging role of chemerin as an adipokine regulating lipid and carbohydrate metabolism is an area of intense research.


ChemR23, also known as CMKLR1, was cloned as an orphan G protein-coupled receptor (GPCR) structurally related to receptors for chemoattractants, such as bacterial and mitochondrial formyl peptides (FPR1, FPR2 and FPR3), complement fragments (C5a and C3a) and prostaglandin D2 ($DP_2$, previously known as CRTH2 or GPR44) [1,2]. Chemerin was later identified as the natural ligand of ChemR23 [3]. A cell line expressing ChemR23 was used to search for bioactive molecules in fractions from human inflammatory fluids and tissue extracts, using a calcium-mobilization assay based on the luminescence of aequorin. Bioactivity was detected in an ascitic fluid secondary to an ovarian carcinoma, and following purification of this activity through HPLC steps, mass spectrometry analysis resulted in the identification of a protein derived from Tazarotene-induced gene 2 (Tig2). Tig2 was previously described, following a subtraction hybridization approach, as a gene up-regulated by retinoic acid and its synthetic analog tazarotene in skin raft cultures [4]. Provided its activity on the ChemR23 receptor, the active form of the Tig2 gene product was named chemerin.

## 1. Structure of chemerin

Chemerin structure is unrelated to that of chemokines or other chemoattractant factors for leukocytes. It is predicted to share a so-called cystatin fold with a set of extracellular proteins, which include cystatins type 2 (cysteine protease inhibitors), cathelicidins (precursors of bactericidal peptides) and kininogen (precursor of bradykinin). Extracellular proteins sharing the cystatin-like fold have in common 4 conserved cysteines, which were shown to stabilize the structure by forming two disulfide bonds (crystal structures available in PDB include chicken cystatin, 1CEW; human cystatin D, 1ROA; porcine protegrin 3, 1PFP). The chemerin primary structure is relatively divergent from other cystatin-like folds and contains six cysteines instead of four, suggesting the existence of an additional disulfide bond (Fig. 1). In addition to the cysteine pattern, the number and location of introns is highly conserved within the gene structure of chemerin, cathelicidins, cystatins and kininogen, further demonstrating their evolutionary relationship. Indeed, among the five introns of the chemerin gene, three split the coding sequence (each time between full codons), and the location of these introns relative to codons and cysteines is similar in the other genes encoding cystatin-like folds (Fig. 1). The classical cystatin fold includes an N-terminal α-helix followed by a 4-stranded antiparallel β sheet. An NMR assignment of $^{15}N$ and $^{13}C$-labeled human chemerin expressed in *Escherichia coli* was reported [5]. The tri-dimensional structure of the protein was not described, but only two of the four β-strands of the cystatin fold,

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**Fig. 1.** Alignment of preprochemerin amino acid sequence with other proteins sharing the cystatin fold. Human preprochemerin sequence is aligned with human and mouse cathelicidin precursors (CAP-18 and CRAM respectively), the three cystatin-like domains of human high-molecular-weight kininogen, and human cystatin 3. The signal peptides are displayed as italic lowercase gray letters. The cysteines stabilizing the cystatin core structure are represented in red. Chemerin contains two additional cysteines that presumably form an additional disulphide bond. The location of the introns splitting the coding sequence in the corresponding genes are indicated by red triangles, and the amino acids in green correspond to the split codon, or to the two successive codons between which the intron is located. The sequences in blue correspond to the pro-sequence of chemerin, removed by proteolytic activation of the precursor, the bactericidal peptides released from cathelicidin precursors, and bradykinin, released by proteolysis from kininogen.

and a long additional C-terminal α-helix were proposed, suggesting divergence from the classical cystatin fold.

Noteworthy, cathelicidins and kininogens require proteolytic processing in order to generate the peptides displaying bioactivity. Cathelicidins are secreted as propeptides that generate by proteolytic cleavage a C-terminal peptide displaying bactericidal properties. High-molecular-weight kininogen is composed of three cystatin-like modules, and C-terminal proteolysis of this precursor generates bradykinin, which acts through members of the GPCR family ($B_1$ and $B_2$). Similarly, chemerin was shown to be secreted as an inactive precursor, prochemerin, which is 143 amino acid long in human, following removal of the 20 amino acid long signal peptide. Prochemerin has very low affinity for ChemR23, and activates very poorly the receptor. In order to acquire its bioactivity, prochemerin needs to be processed by proteolytic enzymes within its C-terminal domain, which lies outside the cystatin-like fold. The requirement for proteolytic processing was made clear from the purification of bioactive material from natural sources, and the expression of (pro)chemerin in recombinant systems. The main form purified from ascitic fluid lacked the last 6 amino acids from prochemerin and bioactive material purified following expression or prochemerin in CHO-K1 cells had the same structure [3]. In contrast, purification of the intact precursor by a monoclonal antibody recognizing specifically the prochemerin C-terminus led to very little activity on ChemR23 ($EC_{50} > 400$ nM).

## 2. Chemerin-derived peptides

The requirement for C-terminal processing of prochemerin pinpointed the role of this domain for the activation of ChemR23. By analogy, all chemokines share a structure, different from that of chemerin, composed of a disordered N-terminus essential for bioactivity, a three stranded β-sheet and a C-terminal α-helix. Chemokines are known to bind their respective receptors through interactions of their core structure with the extracellular N-terminal domain and loops of the receptor, while the unfolded N-terminus interacts with a second binding site located in the transmembrane helix bundle, triggering the conformational change and leading to G protein activation. The functional role of chemokine N-terminal peptides is illustrated by the important changes in bioactivity resulting from proteolytic trimming of this domain or other post-translational modifications such as citrullination [6]. In most cases however, peptides derived from chemokine N-terminus display very poor binding and functional properties. In contrast, short peptides derived from chemerin C-terminus were shown to display significant bioactivity [7]. Indeed, chemerin could be trimmed down to a C-terminal nonapeptide ($Y^{149}$FPGQFAFS$^{157}$, named chemerin-9), while keeping an $EC_{50}$ of 5 nM for ChemR23 (compared to 0.1–0.2 nM for full size chemerin). In mouse, the corresponding nonapeptide $L^{148}$FPGQFAFS$^{156}$ displays similar properties [8]. It appears therefore that chemerin interacts, similarly to chemokines, through two distinct domains with its receptor. The cystatin-like domain interacts presumably with the N-terminus and loops of ChemR23, although this has not been experimentally confirmed, while the C-terminus triggers activation, presumably through an interaction involving the helix bundle. Despite its cystatin-like structure, chemerin does not appear to inhibit cysteine proteases [9].

The use of C-terminal peptides also allowed to delineate how precise the C-terminal proteolytic processing needs to be, and what are structural determinants necessary for activation of ChemR23. It was shown that addition of a single amino acid or removal of two amino acids, relative to chemerin-9, modified the potency by four orders of magnitude [7]. Besides, $Y^{149}$, $F^{150}$, $G^{152}$, $F^{154}$, $F^{156}$ and the terminal carboxyl group were shown as essential moieties for the bioactivity of the peptides. On this basis, binding tracers were developed, such as $^{125}$I-YHSFFPGQFAFS, allowing the pharmacological characterization of ChemR23. Chemerin-derived peptides constitute interesting tools for studying the pharmacology of the receptor *in vitro*, but there is no evidence that such peptides are generated *in vivo*, and these peptides are short lived in plasma and other biological media, such as cell cultures, as a result of proteolytic degradation. The half-life of chemerin-9 in biological media was