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Expressions and purification of a mature form of recombinant human Chemerin in *Escherichia coli*

Di Xiang [a], Jing Zhang [a], Yizhe Chen [a], Yiping Guo [a], Adrian Schalow [b], Zhonghui Zhang [b], Xiaojia Hu [c], Hongjing Yu [a], Mei Zhao [a], Shunying Zhu [a], Huili Lu [a], Mingyuan Wu [a], Yan Yu [d], Anja Moldenhauer [b], Wei Han [a,*]

[a] *Laboratory of Regeneromics, School of Pharmacy, Shanghai Jiaotong University, Shanghai 200240, PR China*
[b] *Institute for Transfusion Medicine, Charite-Universitaetsmedizin Berlin, Campus Virchow-Clinic, Augustenburger Platz 1, 13353 Berlin, Germany*
[c] *Laboratory of Chemistry and Bioactivity of TCM, School of Pharmacy, Shanghai Jiaotong University, Shanghai 200240, PR China*
[d] *Shanghai Municipality Key Laboratory of Veterinary Biotechnology, School of Agriculture and Biology, Shanghai Jiaotong University, Shanghai 200240, PR China*

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ABSTRACT

Chemerin is a novel chemokine that binds to the G protein-coupled receptor (GPCR) ChemR23, also known as chemokine-like receptor 1 (CMKLR1). It is secreted as a precursor and executes pro-inflammatory functions when the last six amino acids are removed from its C-terminus by serine proteases. After maturation, Chemerin attracts dendritic cells and macrophages through binding to ChemR23. We report a new method for expression and purification of mature recombinant human Chemerin (rhChemerin) using a prokaryotic system. After being expressed in bacteria, rhChemerin in inclusion bodies was denatured using 6 M guanidine chloride. Soluble rhChemerin was prepared by the protein-specific renaturation solution under defined conditions. It was subsequently purified using ion-exchange columns to more than 95% purity with endotoxin level <1.0 EU/μg. We further demonstrated its biological activities for attracting migration of human dendritic cells and murine macrophages *in vitro* using established chemotaxis assays.

Introduction

Chemerin is first identified as an inducible gene by *tig*-2 (Tazarotene-induced gene 2) in the non-affected skin of psoriatric patients [1]. Chemerin is secreted as a precursor, and the last six amino acids (aa) need to be proteolytically cleaved at its C-terminus to be active [2]. Strong activators of Chemerin include the serine proteases factor XIIa and plasmin, cathepsin G (CG), human leukocyte elastase (HLE), and mast cell tryptase [3,4]. After maturation, the activated form of Chemerin attracts dendritic cells (DC) and macrophages, which both express the Chemerin receptor ChemR23 (CMKLR1, also known as Dez in mice) [2,5,6]. Chemerin–ChemR23 interaction plays an important role in the recruitment of blood $CD56^{low}CD16^{+}$ natural killer (NK) cells, but not $CD56^{high}CD16^{-}$ NK cells in a dose-dependent manner, consistent with their ChemR23 expression. Chemerin may thereby participate in leukocyte recruitment during inflammation and autoimmune diseases [7]. High level expression of Chemerin is reported during the differentiation of adipocytes. It potentiates insulin-stimulated glucose uptake and insulin signaling in 3T3-L1 adipocytes, which identifies Chemerin as a novel adipokine [8]. Along with ChemR23, chemokine (CC motif) receptor-like 2 (CCRL2), another G protein-coupled receptor (GPCR) has been identified as a receptor for mature Chemerin. The N-terminus of Chemerin binds to CCRL2 on murine and human mast cells. The receptor binding induces no signals, but concentrates Chemerin on the surface of CCRL2 expressing cells, and this might contribute to the effects of Chemerin on adjacent ChemR23-expressing cells [9].

The post-secretion processing of this protein limits the study of Chemerin in animal models. In this report, we present a new method for expression and purification of active rhChemerin[1] in its mature form. Using *Escherichia coli* expression system, we constructed a pET28a vector containing the targeted gene excluding nuclear acids corresponding to the signal peptide and the last six amino acids of pro-Chemerin. Using anion and cation ion-exchange columns, highly purified rhChemerin (>95%) was prepared with low endotoxin level (<1.0 EU/μg). To determine the bio-activity of this

* Corresponding author. Address: School of Pharmacy, Shanghai Jiaotong University, Room 6-202, 800 Dongchuan Road, Minhang District, Shanghai 200240, People's Republic of China. Fax: +86 21 34204750.
E-mail address: weihan@sjtu.edu.cn (W. Han).

[1] *Abbreviations used:* rhChemerin, recombinant human Chemerin; hCMKLR1, human chemokine-like receptor 1, also named as ChemR23, and known as Dez in mice; CCRL2, chemokine (CC motif) receptor-like 2; GPCR, G protein-coupled receptor.

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protein, we evaluated its chemoattraction on ChemR23-expressing human dendritic cells generated from cord blood derived hematopoietic progenitors and murine peritoneal macrophages, using *in vitro* transmembrane chemotaxis assays.

Materials and methods

Construction of the rhChemerin expressing vector

The full length of human Chemerin gene (*hChemerin*, GeneBank Accession No. NM_002889) is 734 base pairs (bp) long with a coding sequence (CDS) of 492 bp. The first 60 bp of CDS encode a 20-aa signal peptide while the last 21 bp encode a 6-aa peptide which needs to be removed from the immature protein. The fragment of interest was amplified by polymerase chain reaction (PCR) from human liver cDNA library using sense 5′-CATGCCATGGAGCTCAC GGAAGCCCAGCGCCG-3′ and antisense 5′-CCGGAATTCTTAGGAGAA GGCGAACTGTCCAG-3′ primers (Sangon, China), in which the underlined nucleotides were digestion sites for the restriction enzymes NcoI and EcoRI, respectively. PCR was performed in conditions as follows: 95 °C for 5 min (m); 94 °C for 30 s (s), 63 °C for 30 s, 72 °C for 30 s, 30 cycles; 72 °C for 10 m. The resulting 500 bp fragment was cloned into pTA2 (Toyobo, Japan) and sequenced (Invitrogen, USA). pTA2 vector containing the gene of interest was extracted and digested by NcoI and EcoRI (Fermentas, Lithuania) and then subcloned into a pET28a vector (Novagen, Germany) prepared by NcoI and EcoRI digestion. The recombinant pET28a vector containing *hChemerin* gene was chemically transformed into the competent *E. coli* BL21 (DE3) bacteria (Novagen, Germany). The engineered strain was identified by digesting the plasmid pET28a-*hChemerin* with NcoI and EcoRI simultaneously.

Expression of rhChemerin

The rhChemerin protein has the sequence of MELTEAQRRGLQ-VALEEFHKHPPVQWAFQETSVESAVDTPFPAGIFVRLEFKLQQTSCRKRD WKKPECKVRPNGRKRKCLACIKLGSEDKVLGRLVHCPIETQVLREAEEH QETQCLRVQRAGEDPHSFYFPGQFAFS, which corresponds to aa 21–157 in the precursor form of human Chemerin except that the underlined methionine is not native. As reported previously [2], this form will be considered as mature form.

The signal peptide and the 6-aa peptide KALPRS at the C-terminus were removed on purpose. The protein was expressed by inoculating successfully transformed bacteria into LB medium (1% NaCl, 1% tryptone, 0.5% yeast extract; pH 7.0) containing 100 μg/ml kanamycin at 37 °C with vigorous shaking. After the medium reached an optical density (OD) of 0.6–0.8 at 600 nm, isopropyl-β-D-thiogalactopyranoside (IPTG) was added into the medium to a final concentration of 1 mM to induce the bacterial production of rhChemerin. The incubation continued for another 4 h (h) at 42 °C, after which the cell pellets were spun down at 5000 rpm for 15 m at 4 °C and washed with phosphate-buffered saline (1× PBS: 137 mM NaCl, 2.7 mM KCl, 4.3 mM Na_2HPO_4, 1.4 mM KH_2PO_4; pH 7.4).

Isolation and refolding of rhChemerin

rhChemerin existed in bacterial inclusion bodies. After fermentation, bacteria were harvested by centrifugation and 1 g of wet bacteria was resuspended in 10 ml of ultrasonication buffer (1× PBS, 1 mM EDTA; pH 7.4). The bacterial inclusion bodies were collected by centrifugation after ultrasonication at set conditions of 3 s sonication and 3 s resting on ice for a cycle for 300 cycles (Scientz JY92-2D, China). After being washed once in PBS by centrifugation, 100 mg of inclusion bodies were resuspended in 1 ml of denaturing buffer (6 M guanidine chloride, 1 mM EDTA, 50 mM NaCl, 50 mM Tris–HCl; pH 8.0). The approximate protein concentration of solubilized inclusion bodies before renaturing was 12 mg/ml. The protein was refolded by slow dilution of the denaturing buffer into 100 volumes of renaturing buffer (1 mM reduced glutathione (GSH), 0.1 mM oxidized glutathione (GSSG), 0.5 M guanidine chloride, 0.4 M sucrose, 0.1 M Tris–HCl; pH 9.5), and concentrated eightfold using the tangential flow concentrator with a 10 kDa cutoff filter (Minimate TFF System; Pall, USA). The protein solution was further diluted into 10 volumes of dilution buffer (1 mM GSH, 0.1 mM GSSG, 0.1 M Tris; pH 9.5) and the pH was adjusted to 7.5 with 1 M HCl. After centrifugation at 15,000 rpm for 30 m, the supernatant was applied to fast protein liquid chromatography (FPLC; GE Healthcare, USA).

Purification of rhChemerin

The calculated isoelectric point (p*I*) of rhChemerin is 8.57. Accordingly, rhChemerin was purified using anion-exchange (Q Sepharose FF) and cation-exchange (S-Sepharose FF) columns sequentially (GE Healthcare, USA). The protein in the dilution buffer was loaded onto a Q Sepharose FF column equilibrated with wash buffer 1 (20 mM Tris–HCl, 1 mM EDTA, 25 mM NaCl; pH 7.5) at a speed of 5 ml/min. The flow-through was collected and adjusted with glacial acetic acid to pH 4.5. After clarification by centrifugation, the supernatant was loaded onto an S-Sepharose FF column equilibrated with wash buffer 2 (50 mM NaAc–HAc buffer, 1 mM EDTA, 25 mM NaCl; pH 4.5) at a speed of 5 ml/min. The protein was eluted using a 0.3–1.0 M NaCl gradient in wash buffer 2 (1%/min) at a speed of 1 ml/min. Fractions were collected according to their UV absorption peaks and conductivity curve. The protein concentration and purity were determined by the Bradford method using bovine serum albumin (BSA) as standard [10], Coomassie brilliant blue R-250 or silver staining (Beyotime, China) after sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE), reversed phase-high performance liquid chromatography (RP-HPLC) and high performance liquid chromatography–size exclusion chromatography (HPLC–SEC).

SDS–PAGE, RP-HPLC, and HPLC–SEC assays

SDS–PAGE was performed using a 15% running gel on the PowerPac Basic (Bio-Rad, USA). Both reducing and non-reducing SDS–PAGE were run to determine the purity and dimer status of rhChemerin. The proteins on the gel were visualized by staining with Coomassie brilliant blue R-250 or silver, and quantified for their purity using the color density scanning software (Tanon, China).

RP-HPLC was performed to determine the purity of rhChemerin using an ODS C18 column (4.6 × 250 mm, 5 μm) on Shimadzu LC 2010A HT (Shimadzu, Japan). Thirty microliters of rhChemerin at 1 mg/ml in PBS was loaded onto the column. The protein was eluted with acetonitrile solution for 20 m at a speed of 1 ml/min using an isocratic program at a constant 70% acetonitrile. Purity of the protein was analyzed by integrating the area of OD 280 peaks in the elution curve between 7.5 and 20 m.

HPLC–SEC was performed to determine the purity and dimer status of rhChemerin using TSK-GEL G2000SWXL (Tosoh, Japan) on ACME 9000 (Younglin, Korea). 20 μl of rhChemerin at 0.5 mg/ml in PBS was loaded onto the column. The protein was eluted with phosphate buffer (1.27 mM Na_2HPO_4, 1.5 mM KH_2PO_4, 0.4 M NaCl; unadjusted pH) at a speed of 0.8 ml/min. OD 280 was used to monitor the protein elution profile.

Western blotting

Western blotting was performed as previously described [11]. Monoclonal anti-human Chemerin antibody (R&D Systems, USA)

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was used as primary antibody at 100 ng/ml and peroxidase conjugated goat anti-mouse IgG (Proteintech Group Inc., USA) was used as secondary antibody at 1:1000 dilution. HRP mediated color reaction was performed using the enzyme substrate provided in the DAB kit as described (Boster, China).

Endotoxin (Pyrogen) assay

The endotoxin level of rhChemerin solution was determined quantitatively using the Chromogenic Tachypleus Amebocyte Lysate (TAL) endpoint assay kit as described by the manufacturer (Chinese Horseshoe Crab Reagent Manufactory Co., Ltd., China). The assay was the pharmaceutical industry standard procedure for quality control of biologics. Procedures followed Chinese Pharmacopeia 2005.

rhChemerin chemotaxis assay with human dendritic cells in vitro

Dendritic cells were generated and chemotaxis experiment was performed as previously described [12]. Briefly, dendritic cells from $CD34^+$ hematopoietic progenitors isolated from cord blood were cultured in DC conditioning medium based on StemSpan (Stem Cell Technologies, Canada) containing 5% pooled human serum, glutamine (2 mM), gentamicin (50 μg/ml; Gemini Bio-Products Inc., USA), 100 ng/ml granulocyte–macrophage colony-stimulating factor (GM-CSF; specific activity 2.5×10^7 U/mg; Peprotech, USA), 20 ng/ml c-kit ligand (stem cell factor; specific activity 5×10^6 U/mg; Amgen, USA), 100 ng/ml tumor necrosis factor-α (TNF-α; specific activity 1×10^8 U/mg; Peprotech, USA), and 20 ng/ml interleukin-4 (specific activity 1×10^7 U/mg; Peprotech, USA). The peak DC frequency was achieved as defined by CD83 and HLA-DR's positive status. After 14 days of culture, $4–5 \times 10^5$ DCs were harvested for chemotaxis assays. $1.5–2 \times 10^5$ DCs were suspended in 100 μl serum-free medium (X-Vivo 15; BioWhittaker Inc., USA) and placed on top of an 8-mm microporous transmembrane in a 24-well plate (Boyden chamber; Corning, USA). One hundred nanogram per milliliter MIP-3α (Peprotech, USA) and different concentrations of rhChemerin were added to the lower chamber to establish chemotactic gradients. Serum-free medium with 0.1% BSA in PBS was used as a negative control. After 4 h of incubation at 37 °C, cells from both chambers were harvested and manually counted. The results of chemoattractant activity of rhChemerin on human dendritic cells were calculated by determining the number of migrated cells as percent of input cells.

rhChemerin chemotaxis assay with murine peritoneal macrophages in Vitro

Chemotaxis assay was performed using Transwell systems (Costar 3422, 8 μm pore; Corning, USA). After Balb/c mice were sacrificed, the peritoneal macrophages were isolated and suspended in RPMI 1640 cell culture medium (Gibco, USA) containing 10% FBS (HyClone, USA). Cells were incubated overnight (37 °C, 5% CO_2). On the following day, the non-adherent cells were washed off gently. Adherent cells were collected as peritoneal macrophages by repeated washes with RPMI 1640. Cells ($6–15 \times 10^4$) in 200 μl RPMI 1640 were added to the top chamber of the 24-well transmembrane plate. Different concentrations of rhChemerin were added into the lower chamber in 600 μl RPMI 1640. The chambers were then incubated for 3–4 h (37 °C, 5% CO_2). The cells on the upper surface of the filter membrane were wiped off and the transmembrane was carefully placed on a glass slide and stained with Wright's staining solution. The cells that migrated through and attached to the membrane were counted in five random fields for three wells per test condition using a light microscope (Nikon, Japan). The results of chemoattractant activity of rhChemerin on murine peritoneal macrophage were calculated by determining the number of migrated cells as percent of input cells [13].

Results

The mature form of rhChemerin expressed in E. coli

Human Chemerin gene (*hChemerin*) encodes the secreted protein in an immature form. The functional protein is generated after removal of the last 6 aa at the C-terminus [2]. We propose to create a bioactive form of rhChemerin by expressing the protein without its native signal peptide and the last 6 aa using prokaryotic expression system.

The *hChemerin* cDNA encoding the mature form of rhChemerin was prepared from a human liver cDNA library by PCR. The fragment was verified by its size (500 bp) after electrophoresis in agarose gel (Fig. 1A), and subcloned into *E. coli* expression vector pET28a. The insert fragment was confirmed by DNA sequencing (Fig. 1B). *E. coli* chemically transformed with pET28a-*hChemerin* vector was cultured and induced with 1 mM IPTG for rhChemerin expression. Bacteria samples were collected and analyzed with SDS–PAGE (Fig. 2A). As indicated in lane 3, a protein band at about 16 kDa was detected after IPTG induction, which matched the predicted molecular weight of rhChemerin.

The mature form of rhChemerin purified by ion-exchange chromatography

After IPTG induction, bacteria were lysed by ultrasonication and followed by SDS–PAGE analysis. The majority of rhChemerin protein existed in the form of inclusion bodies (Fig. 2B, lane 3). rhChemerin was denatured by guanidine chloride and renatured in the renaturing buffer by dilution at pH 9.5 (Fig. 2B, lanes 4 and 6). As indicated in Fig. 2B, lane 5, denaturing buffer dissolved most of the rhChemerin. Concentration of rhChemerin in denaturing buffer was 12 mg/ml and in renaturing buffer before loading to Q Sepharose FF it was decreased to about 20 μg/ml.

According to its p*I* 8.57, rhChemerin was purified using anion-exchange (Q Sepharose FF at pH 7.5) and cation-exchange (S-Sepharose FF at pH 4.5) columns sequentially. The Q Sepharose FF column was used mainly to remove bacterial endotoxin which

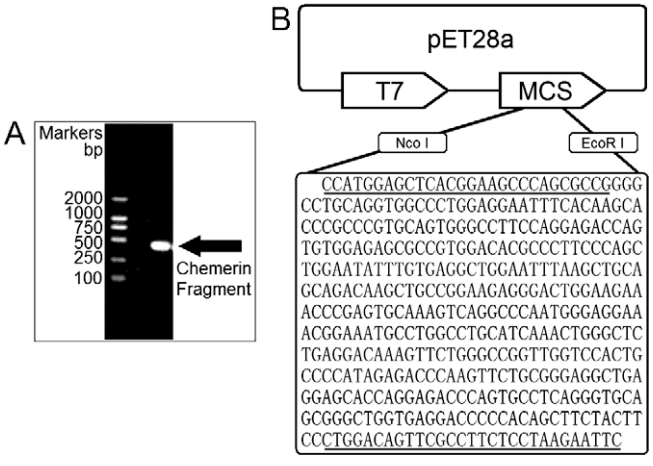

Fig. 1. The construction of rhChemerin expressing vector. (A) The human Chemerin gene cloned from human liver cDNA library by PCR amplification. A fragment corresponding to the *hChemerin* gene of correct size was detected. (B) The plasmid map of pET28a–*hChemerin* and the confirmation by sequencing. PCR primers were underlined. The forward primer included an NcoI site and the reverse primer included an EcoRI site and a "TAA" stop codon.

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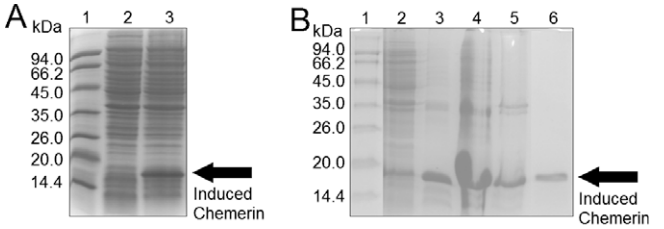

Fig. 2. *Escherichia coli* expressing rhChemerin induced by IPTG. Samples were applied on SDS–PAGE and stained with Coomassie brilliant blue R-250. (A) Lane 1, protein molecular weight (MW) markers; lane 2, bacteria extracts without IPTG induction; lane 3, bacteria extracts with IPTG induction. Induced rhChemerin was visualized in lane 3 at a molecular weight of about 16 kDa. (B) Lane 1, MW markers; lane 2, supernatant of bacteria after ultrasonication; lane 3, inclusion bodies; lane 4, denatured rhChemerin; lane 5, undissolved rhChemerin in denaturing buffer; lane 6, refolded rhChemerin.

bound to the column, and the protein at pH 7.5 appeared in the flow-through. rhChemerin was purified using the S-Sepharose FF column at pH 4.5 in the sodium acetate buffer. According to the OD 280 absorption peaks and conductivity curve, the eluted fractions at conductivity of 70 mS/cm were collected (Fig. 3A). The protein solution was dialyzed, concentrated, and formulated into PBS at 1 mg/ml.

The protein was characterized by RP-HPLC and silver staining after SDS–PAGE for its purity. One major peak in RP-HPLC (Fig. 3B) and one band at about 16 kDa in the silver stained gel (Fig. 4A, lane 2) were observed, which demonstrated the purity of rhChemerin was above 95%.

To further characterize the protein in solution, the protein in reducing and non-reducing conditions was analyzed by SDS–PAGE followed by silver staining. A band at about 30 kDa (4.7% by density) was detected in the non-reducing condition (Fig. 4A, lane 3),

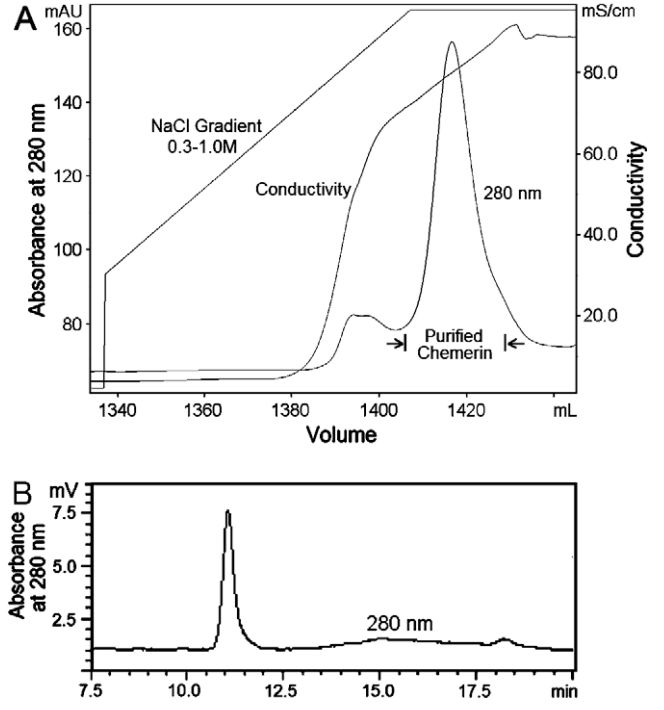

Fig. 3. The purification of rhChemerin by FPLC and the purity determination by RP-HPLC. (A) Ion-exchange chromatography purification of rhChemerin over S-Sepharose column. Eluted fractions containing "purified rhChemerin" at the indicated OD 280 peak and conductivity were collected and analyzed for the rhChemerin purity. (B) Purity determination of rhChemerin by RP-HPLC. Loading volume was 30 μl of rhChemerin at 1 mg/ml in PBS. One major peak was observed, and the calculated purity of rhChemerin was above 95%.

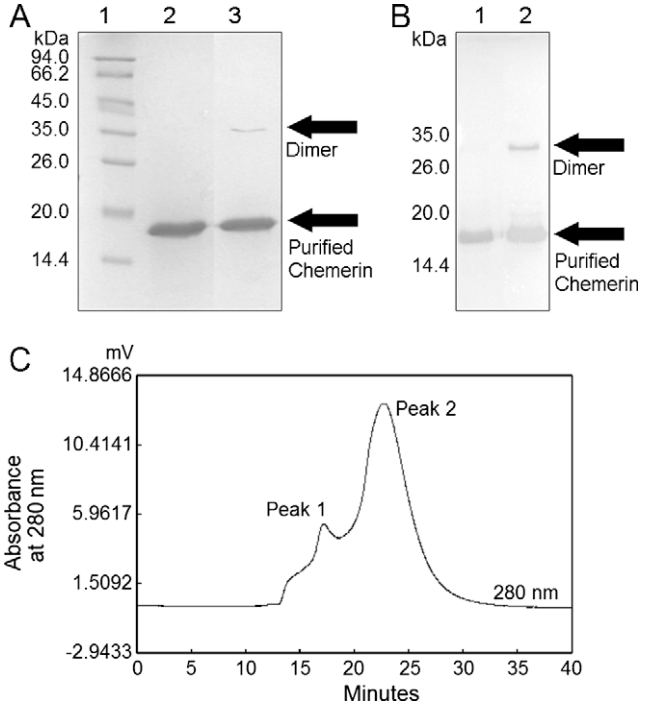

Fig. 4. The purity and two forms of rhChemerin determined by reducing and non-reducing SDS–PAGE, Western blotting and HPLC–SEC. (A) Reducing and non-reducing SDS–PAGE followed by silver staining. Loading volume of rhChemerin was 3 μg. Lane 1, MW markers; lane 2, reducing SDS–PAGE of purified rhChemerin. Only one band was observed, which indicated that the purity of rhChemerin was above 99%; Lane 3, non-reducing SDS–PAGE of purified rhChemerin. A possible dimer was observed at about 30 kDa. (B) Western blotting. Loading volume of rhChemerin was 3 μg. Lane 1, reducing SDS–PAGE of purified rhChemerin; lane 2, non-reducing SDS–PAGE of rhChemerin. The new observed band at about 30 kDa was detected by the antibody in addition to the rhChemerin monomer at about 16 kDa. (C) HPLC–SEC. Loading volume was 20 μl of rhChemerin at 0.5 mg/ml in PBS. Two peaks were observed at minutes 17.2 and 22.6.

which was also detected by the Western blotting with the monoclonal antibody against human Chemerin (Fig. 4B, lane 2). Moreover, a small protein peak ahead of the major one was detected by HPLC–SEC (Fig. 4C), in which the proteins in their native forms migrate according to their size and conformation. Taken together, the data strongly suggested that the purified rhChemerin existed in two forms, a minor form of dimer and a major form of monomer, due to the inter-molecular formation of disulfide bond.

The protein yield from a representative batch is presented in Table 1. The results were calculated from 800 ml cultures of *E. coli* expressing rhChemerin. The final yield was 14% with the most loss at the renaturing step, followed by the S-Sepharose FF purification step. Only 23% of denatured rhChemerin was successfully recovered. A single laboratory scale purification of 800 ml bacteria culture produced 5 mg rhChemerin which was sufficient for the biological characterization of the protein *in vitro* and *in vivo* in mice.

We tested endotoxin levels from three different batches of the purified rhChemerin and the results were below 1.0 EU/μg, which was considered acceptable compared to commercially available proteins.

The mature form of rhChemerin is biological active

The biological activity of rhChemerin was measured by chemotaxis assays on human dendritic cells and murine peritoneal macrophages. The chemotaxis activity of the protein was defined by the numbers of migrated cells compared to the number of input cells (Fig. 5A and B). Human cord blood $CD34^+$ cell derived dendritic cells responded to rhChemerin at concentrations between

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Table 1
The purification of rhChemerin using the *E. coli* expression system.

	Total protein (mg)	rhChemerin purity (%)	Total rhChemerin (mg)	rhChemerin yield (%)
Cell lysate by ultrasonication	203	19	39.4	100
Denature	51.4	75	38.6	98
Renature/Q-Sepharose loading	9.25	94	8.70	22
S-Sepharose loading	8.48	95	8.06	20
Purified rhChemerin	5.50	99	5.44	14

Results were derived from 800 ml cultures of *E. coli* expressing the recombinant protein (weight of wet bacteria: 1.91 g; weight of inclusion bodies: 0.208 g). Total protein was estimated by the method of Bradford (BSA was used as a standard). The yield after cell ultrasonication represents the baseline of 100%. A representative batch out of three with similar results is shown.

10 and 1000 ng/ml, which was comparable to the positive control MIP-3α at 100 ng/ml (Fig. 5A). Furthermore, the migration of murine peritoneal macrophages across the membrane was also significantly enhanced by rhChemerin at concentrations between 1 and 1000 ng/ml, and peaked at 30 ng/ml with maximal of sevenfold higher than the negative control (Fig. 5B). A positive dose–response was observed for rhChemerin at 1–30 ng/ml range, and a negative one at higher concentration (>30 ng/ml). Thus the mature form of rhChemerin was biological active and behaved as a typical chemokine with cell-type specific chemotaxis activities *in vitro* [11].

Discussion

Chemerin, a newly discovered protein as the ligand of ChemR23, is synthesized as a precursor and needs to be processed to show full biological activity. As reported previously, the recombinant form of the protein was prepared using several systems. Wittamer et al. reported the production of the Chemerin precursor in mammalian cells, and generation of the mature form by trypsin digestion [2]. Zabel et al. produced the protein in fusion with tags using Baculovirus as well as bacterial system [4,6,9]. Meder et al. used yeast expression system and purified the protein with six steps of HPLC [14]. Chemerin produced by these methods is sufficient for conducting biochemical and cellular function studies *in vitro*.

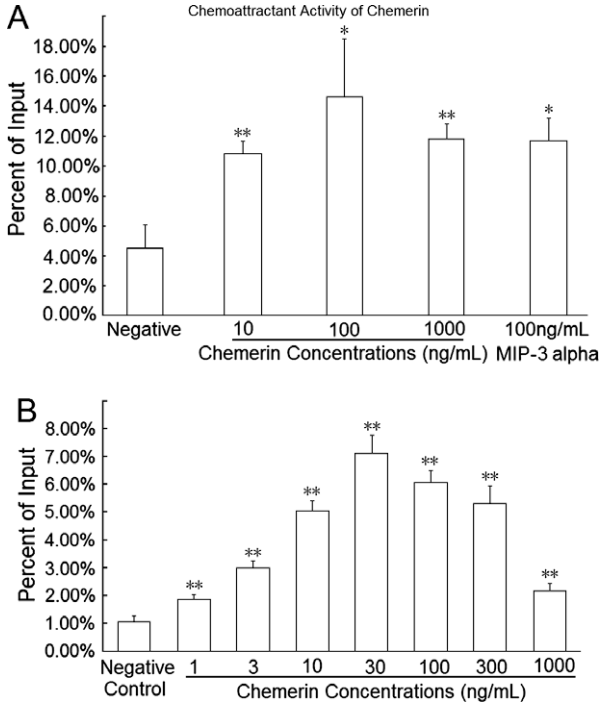

Fig. 5. Chemoattractant activity of rhChemerin on human dendritic cells and murine peritoneal macrophages. The activity of rhChemerin was shown as percentage of migrated cells per input. The data in all conditions with rhChemerin were significantly higher than that of the negative control ($^{*}p < 0.05$, $^{**}p < 0.01$) by two-tailed Student's *t* test. (A) Human dendritic cells derived from human cord blood $CD34^{+}$ cells. MIP-3α (100 ng/ml) was used as positive control. Results were shown as means ± SEM of three independent experiments. (B) Murine peritoneal macrophages. Results were shown as means ± SD of triplicate wells. A representative assay out of three with similar results was shown.

We report for the first time a new method to produce rhChemerin in its mature form without tags, and with the quality control and bio-activity assay for final products. The final preparation of rhChemerin is successfully formulated in PBS at neutral pH without toxic chemicals like dithiothreitol (DTT) or phenylmethanesulfonyl fluoride (PMSF) utilization in the process, which meets the requirements for *in vivo* use. The optimized denaturing and renaturing conditions followed by two ion-exchange chromatography produce sufficient material at laboratory scale for conducting experiments in animal disease models. In addition, we provide strong evidence that two forms of rhChemerin may present in solution, a major form of monomer and a minor form of dimer. At present, the contribution of the bio-activity of the two forms in the chemotaxis assay is not known.

The most difficult step in the process development is to refold the denatured protein properly, and GSH–GSSG redox system in alkaline buffer seems to be necessary. Using the method presented here, rhChemerin can be purified with high purity, acceptable yield and bio-activity. The chemoattractant activity of rhChemerin on murine macrophages is similar to other reports using mouse F4/$80^{lo}CD11b^{+}$ macrophages and mouse peritoneal mast cells [9,15]. In chemotaxis experiments using human DCs, rhChemerin shows similar bio-activity compared to previous reports on human DCs and human CMKLR1 (ChemR23) transfectant [2,4–6,16]. Pro-Chemerin can be activated by serum [4]. We found the addition of 10% serum in our chemotaxis assays did not affect the chemoattractant activity of rhChemerin, which indicated that the mature form of Chemerin (rhChemerin) did not need to be processed by the protease in serum. In fact, pro-Chemerin shows a very low chemoattractant activity [4,16]. Thus, rhChemerin produced by our method possesses full biological activity without further treatment.

In conclusion, the method producing mature form of rhChemerin described in this report is simple and efficient with lab-scale (milligram) production. The recombinant protein matches the native mature human Chemerin in sequence, except for one methionine at the N-terminus, which is required by prokaryotic gene expression. It also matches the quality of commercialized protein. Furthermore, it is biologically active on murine peritoneal macrophages and human dendritic cells, demonstrating a cross-species bio-activity. The easy access of rhChemerin and its characteristic cross-species bio-activity should facilitate the study of physiological and pathological roles of rhChemerin in murine models.

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