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Expression and one-step ion-exchange purification of (AAR)IL-8 (human IL-8 receptor antagonist) ☆

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Abstract

Interleukin-8 (IL-8) is C-X-C chemokine, which is produced by a variety of cells. IL-8 plays an important role in the inflammatory response and may be a therapeutic target for some inflammatory diseases. To develop an IL-8 receptor antagonist, (AAR)IL-8 (IL-8 receptor antagonist) was constructed and successfully expressed in *Escherichia coli*. (AAR)IL-8 could be easily purified by one-step SP–Sepharose fast flow column after the lysate of recombinant bacterial cells was heated at 70 °C for 10 min. The purity of (AAR)IL-8 is more than 95%. This purification process resulted in final purified yields of 4.29 mg (AAR)IL-8/g cell paste. In addition, the purified (AAR)IL-8 can significantly inhibit the chemotaxis that was induced by human IL-8 in vitro and in vivo. These results showed that this purification process is very simple and effective. It could be easily amplified at a larger scale. (AAR)IL-8 might find use as a new therapeutic IL-8 receptor antagonist for some acute and chronic inflammatory diseases.

Keywords: IL-8; Antagonist; Expression; Purification; Inhibition

Interleukin-8 (IL-8)[1] belongs to the family of chemokines and has been shown to activate neutrophils in vitro and induce neutrophil accumulation in vivo when injected intradermally [1]. IL-8 is derived from a variety of cells and tissues. For instance, human endothelial cells could produce IL-8 after stimulation with TNF-α, IL-l or LPS [2]. IL-8 has been termed neutrophil-activating protein, neutrophil chemotactic factor, and T cell chemotactic factor [3].

IL-8 exhibits multiple bioactivities on neutrophils, such as induction of lysosomal enzyme releasing from neutrophils, expression of adhesion molecules on neutrophils, and adherence of neutrophils to unstimulated endothelial cells [4]. IL-8 levels in body fluids are increased in inflammatory diseases with neutrophil infiltration, including acute myocardial infarction, acute respiratory distress syndrome (ARDS), glomerulonephritis, inflammatory bowel diseases, and rheumatoid arthritis [5,6]. These suggest that IL-8 be involved in neutrophil-mediated inflammation and may be a target for therapeutic intervention in neutrophil-mediated inflammation.

Some studies indicated that the anti-IL-8 monoclonal antibody (mAb) markedly reduced endotoxin-induced neutrophil recruitment into the pleural space of rabbits [7], implicating IL-8 as a major mediator of LPS-induced neutrophil infiltration. Moreover, the anti-IL-8 mAb can prevent neutrophil infiltration and severe lung injury

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[1] *Abbreviations used:* IL-8, interleukin-8; ARDS, acute respiratory distress syndrome; mAb, monoclonal antibody; COPD, chronic obstructive pulmonary disease; IPTG, Isopropylthio-β-D-galactoside; CI, chemotactic index.

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caused by acid instillation [8]. It also was shown that the anti-IL-8 antibody could effectively prevent two models that are very relevant to clinical situations: endotoxemia-induced acute respiratory distress syndrome (ARDS)-like lung injury and cerebral reperfusion injury [9]. A clinical phase II study indicated that neutralization of IL-8 with anti-IL-8 mAb may improve dyspnea in patients with chronic obstructive pulmonary disease (COPD) [10].

Besides anti-IL-8 mAb, the receptor antagonists of IL-8 can also inhibit bioactivity of IL-8. (AAR)IL-8 with a molecular mass of approximately 7.5 kDa is a truncation analog of 72-amino acid IL-8: the first three amino acids of the N-terminal was eliminated and the ELR (Glu4-Leu5-Arg6) was substituted with AAR (Ala4-Ala5-Arg6). (AAR)IL-8 is one of the most potent receptor antagonists of IL-8 [11]. In this paper, we report the expression, two-step purification, and characterization of recombinant (AAR)IL-8. Our study lays the foundation of further investigation of this IL-8 receptor antagonist.

Materials and methods

The SP–Sepharose fast flow was purchased from Pharmacia Biotech. Restriction enzymes and T4 DNA ligase was purchased from Takara (Dalian, China). Isopropylthio-β-D-galactoside (IPTG) was purchased from Sigma. The expression vector pET-22b and its host strain BL21 (DE3) were purchased from Novagen. The plasmid pBV220/IL-8 containing the IL-8 cDNA was constructed in-house. The anti-IL-8 monoclonal antibody was purchased from R&D systems (USA). All other chemicals and reagents were obtained from other commercial sources and were of the highest purity available.

Construction of expression plasmid

The plasmid pBV220/IL-8 containing IL-8 cDNA was used as template DNA. PCR was performed using following primers: upstream, 5′AT**CATATG**GCCGCG CGCGTTGTCAGTGCATAAAG3′ (*Nde*I restriction site shown in bold) and downstream, 5′A**GGTCGAC**T TATGAGTTCTCAGCCCTCTT3′ (*Sal*I restriction site shown in bold). After denaturation of DNA for 5 min at 95 °C, amplification was performed for 30 cycles through a regime of 1 min at 94 °C, 1 min at 55 °C, and 1 min at 72 C. The products (229 bp) were purified using an agarose gel, digested with *Nde*I and *Sal*I, and inserted into the plasmid pET-22b. The resulting plasmid was named pET-22b/(AAR)IL-8. The construct was confirmed by DNA sequencing. *E. coli* BL21 (DE3) cells were transformed with the plasmid pET-22b/(AAR)IL-8.

Expression of the recombinant (AAR)IL-8

A single transformed BL21 (DE3) colony was used to inoculate 10 ml Luria–Bertani (LB) medium supplemented with ampicillin (100 μg/ml) grown with 200 rpm shaking overnight at 37 °C. Three milliliter culture was transferred to 300 ml fresh LB medium in a 500 ml shake flask. The culture was grown with 200 rpm shaking at 37 °C until the OD_{600} reached 0.5. 0.3 ml of 1 M IPTG was added for induction. One milliliter sample was collected at 4 h after induction. The pellet was resuspended in 100 μl of ddH_2O, mixed with 6× SDS loading buffer (0.35 M Tris–HCl, pH 6.8; 10.28% SDS; 36% glycerol; 5% β-mercaptoethanol, and 0.012% bromphenol blue), and heated at 95 °C for 10 min. The sample was centrifuged at 10,000*g* for 8 min and 10 μl supernatant was analyzed by Tricine–SDS–PAGE [12] and stained by Coomassie blue R-250.

Lysis of cells and purification of recombinant (AAR)IL-8

For shake flask production of (AAR)IL-8, 3000 ml LB medium was added to ten 500 ml shake flasks (300 ml per flask). Shake flasks were inoculated to a starting OD_{600} of 0.1 from an overnight culture grown in LB supplemented with ampicillin (100 μg/ml). Cultures were grown with 200 rpm shaking at 37 °C and then induced with 1 mM IPTG (final concentration) at the OD_{600} of 0.5 for 4 h. Cells were harvested by centrifugation at 10,000*g* for 15 min and stored at −20 °C.

Cell paste (2 g) was resuspended in 20 ml STE (50 mM Tris–HCl, pH 8.0; 1 mM EDTA; and 100 mM NaCl) and lysed with lysozyme (0.8 mg) and sonication (30 s per time, five times). The lysate was added to 10 tubes (2 ml per tube). Four tubes were heated, respectively, at 50, 60, 70, and 80 °C for 10 min. Another six tubes were heated at 70 °C for 10, 20, 30, 40, 50, and 60 min, respectively, and cooled to 0 °C rapidly. The supernatant was collected and analyzed by Tricine–SDS–PAGE.

Cell paste (10 g) was resuspended in 100 ml STE and lysed with lysozyme (8 mg) and sonication (30 s per time, five times). The lysate was heated at 70 °C for 10 min and cooled to 0 °C rapidly. The supernatant was collected after centrifuge at 10,000*g* for 15 min and dialyzed against 20 mM citrate buffer (pH 6.0) overnight. The suspension was centrifugated at 10,000*g* for 15 min at 4 °C and the supernatant was loaded in a SP–Sepharose fast flow column equilibrated with 20 mM citrate buffer (pH 6.0). The column was washed with the equilibrating buffer until the absorbance at 280 nm was below 0.01 and then eluted by using a linear gradient of 0–1 M NaCl in 20 mM citrate buffer (pH 6.0). The purified (AAR)IL-8 was collected and analyzed by Tricine–SDS–PAGE. The final product purity was judged by means of SEC-HPLC.