Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Biotechnology 118 (2005) 246–256
Journal of BIOTECHNOLOGY
www.elsevier.com/locate/jbiotec

High level expression of functionally active human lactoferrin in silkworm larvae

Tao Liu [a,b], Yao-Zhou Zhang [b,*], Xiang-Fu Wu [c]

[a] *Institute of Biochemistry, College of Life Sciences, Zhejiang University, Hangzhou 310029, China*
[b] *Institute of Biochemistry, Zhejiang Sci-Tec University, Hangzhou 310018, China*
[c] *Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences, Shanghai 200031, China*

Received 12 January 2005; received in revised form 21 April 2005; accepted 2 May 2005

Abstract

In this paper, recombinant human lactoferrin (rhLf) was expressed very well using *Bombyx mori* nuclear polyhedrosis baculovirus expression system. Infection of silkworm larvae with recombinant virus, vBm-hLf, the rhLf was efficiently secreted into larvae hemolymph and the concentration of product purified was about 65 μg/ml. The isolated rhLf molecular mass was ~78 kDa, lower than that of the human lactoferrin (hLf) standards, which may be due to incomplete glycosylation or protein degradation. Furthermore, the rhLf was characterized and its biological activities were evaluated by in vivo bioassay using dextran sodium sulfate (DSS)-induced colitis mouse model that mimics some characteristics of colitis disease in human. We conclude that silkworm expression system can be used successfully to express functional human lactoferrin.

Keywords: Human lactoferrin; Silkworm larvae; Recombinant baculovirus; Colitis

1. Introduction

Lactoferrin (LF) is an ~80 kDa iron-binding glycoprotein that is found predominantly in the milk of most mammals. LF is a major protein component of secreted fluids, such as saliva, gastric juice, bile and other exocrine secretions of mammals. Lactoferrin is also released from the specific, secondary granules of neutrophils during inflammatory responses (Kabilan et al., 2003; Hidefumi et al., 2001). Lactoferrin exhibits bacteriostatic activity against a wide range of Gram-negative and Gram-positive bacteria due to its ability to chelate iron, which is essential for microbial growth. In addition, lactoferrin exhibits non-iron-dependent antibacterial, antifungal, antiviral, antitumor, anti-inflammatory and immunoregulatory activities (Lonnerdal and Iyer, 1995; Andersson et al., 2000; Iyer and Lonnerdal, 1993; Bezault et al., 1994; Ikeda et al., 1998). Lactoferrin constitutes an important component of the innate immune system and

* Corresponding author. Tel.: +86 571 86843190; fax: +86 571 86843198.
E-mail address: liuniu73@yahoo.com.cn (Y.-Z. Zhang).

0168-1656/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.jbiotec.2005.05.005

may represent a novel therapeutic with broadspectrum potential. Recently, therapeutic utilization of human lactoferrin (hLf) for treatment of inflammatory bowel disease has been extensively studied, and its application to medicine is highly anticipated (Jun-Ichi et al., 2002). As large amounts of hLf are required for such applications, an efficient and economical way of producing hLf must be established.

Although prokaryotes expression systems have been widely used for low-molecular-mass recombinant proteins, they are not always successful: the proper disulfides are not formed and the protein is often present in inclusion bodies. Moreover, glycosylation does not occur in prokaryotic cells. Much attention has recently been paid to the easy-to-handle baculovirus eukaryotic expression system using the *Bombyx mori* nuclear polyhedrosis virus (BmNPV), which provided a powerful promoter and posttranslational modifications of heterologous proteins. Furthermore, the BmNPV expression system is the high level of foreign protein production in silkworm larvae ranging from 20 to 500-fold higher than that in established cell lines (Miyajima et al., 1987) and more economic.

In this study, we described the expression, purification and characterization of recombinant human lactoferrin (rhLf) in silkworm larvae. Furthermore, we studied the potential ability of rhLf to attenuate colitis using a dextran sodium sulfate (DSS)-induced colitis model in mice that mimics some characteristics of colitis disease in human.

2. Materials and methods

2.1. Cell lines and silkworm

The *B. mori*-derived cell line BmN (conserved by our lab) was cultured at 27 °C with TC-100 medium (GIBCO, Grand Island, NY) supplemented with 10% (v/v) fetal calf serum.

A hybrid strain of silkworm (commercial name: Qingsong × Haoyue, purchased from ZheJiang Chinagene Biomedical Co.) was used in this study. The larvae were reared with mulberry leaves at 23–25 °C.

2.2. Reverse transcription-PCR

Total RNA from mammary gland (kindly provided by Xin Qiao hospital) was isolated by Trizol reagent (Invitrogen) following the manufacturer's protocols. And cDNA was synthesized using 2 μg of total RNA by SuperScript II transcriptase (Invitrogen) according to protocols of SuperScript first strand synthesis system for RT-PCR (Invitrogen). For amplification of cDNA, primers for hLf (upstream primer, 5-TACGGATCC-ATGAAACTTGTCTTCCTC-3; downstream primer, 5-TAGCTCGAGTTACTTCCTGAGGAATTCAC-3) were used with a 5′ *Bam*HI site and a 3′ *Xho*I site introduced, which were synthesized by Sangon. PCRs were performed in a Mastercycler personal (Eppendorf) at adequate conditions. In all, 30 cycles (94 °C, 1 min; 55 °C, 1 min, 30 s and 72 °C, 2 min). The double digestion was carried out in a total 50 μl reaction mixture (1 μg of PCR products, 10 units of *Bam*HI and *Xho*I, respectively, 5 μl 10 × K universal buffer and add distilled H_2O to 50 μl). PCR products (∼2.1 kb) were separated on 2% agarose gel and visualized with ethidium bromide staining and sequenced using a 3100-Avant Genetic Analyzer (ABI PRISM). The sequences were confirmed using NCBI BLAST software.

2.3. Construction of recombinant baculovirus transfer vector and recombinant baculovirus

All standard recombinant DNA protocols were followed as described (Ausubel et al., 1993; Sambrook et al., 1989). The PCR products were digested with *Bam*HI (TaKaRa) and *Xho*I (TaKaRa) to release the hLf fragment. The same set of enzymes was used to digest the *B. mori* nuclear polyhedrosis virus polyhedrin based transfer vector $pBacPAK_8$. Then the hLF gene was subcloned into $pBacPAK_8$ to obtain the recombinant plasmid pBacPAK-hLf. And the gene clone of hLf was under the control of the BmNPV polyhedrin promoter.

Recombinant baculovirus, named vBm-hLf, was constructed by cotransfection. The purified recombinant baculovirus vector pBacPAK-hLf and linearized *Bsu 36I*-digested $BacPAK_6$ viral DNA were used for coinfection of BmN insect cells (1×10^6 cells) described by Palhan et al. (1995). Four days after transfection, culture supernatants were diluted to 10^{-4} to 10^{-6} and 200 μl of aliquots were added to each well of 96-well tissue culture plates (Becton Dickinson) together with 1×10^4 BmN cells. After 10–14 days, the viral progeny was visually screened for the presence of