微球蛋白基因克隆及其在大肠杆菌中的高效表达

何贤辉  徐丽慧  刘毅  曾耀英

（组织移植与免疫教育部重点实验室（暨南大学），广州430052）
（暨南大学生物工程研究所，广州430052）
（郑州大学第一附属医院皮肤科，郑州450063）

摘 要

1. 微球蛋白（β2-m）是主要组织相容性复合体（EFG）类分子的轻链部分，为制备EFG类分子四聚体的必要成分。根据已报道的序列设计特异引物，利用H81IGH方法从人白细胞中克隆了β2-m基因，并构建了成熟β2-m的原核表达载体，在大肠杆菌中得到高效表达。表达的β2-m大部分在包涵体中，经洗涤、变性和复性，并以强阴离子交换柱层析纯化，获得JJK1IL7I纯的人重组β2-m。印迹法分析表明该蛋白具有与抗人天然β2-m抗体反应的特性。此工作为制备EFG类分子四聚体奠定基础。

关键词

微球蛋白，基因克隆，重组蛋白，包涵体

材料和方法

1.1 材料

β2-m、β2-m cDNA、E. coli、MHC I、RT-PCR、MHC I

1.2 方法

1.2.1 RNA、cDNA、3 ml、IPTG
1.2.2 PCR β2m 5'-ATATCCATATGCTCGCTCGGCGCTTAG-3' 5'-AACTAGGATCCATATGCTCGATCCCGAC-3' β2m PCR 50 μL 94℃ 2 min 94℃ 30 s 55℃ 30 s 72℃ 1 min 35 37℃ 10 min PCR Nde I + BamH I QIAquick Gel Extraction Kit pET-3c E. coli DH5α PCR β2m Nde I + BamH I QIAprep Spin Miniprep Kit ABI377 TaKaRa Taq

1.2.3 β2m G/C 5'-ATATCCATATGCTCGCTCGGCGCTTAG-3' 5'-AACTAGGATCCATATGCTCGATCCCGAC-3' β2m PCR β2m Nde I + BamH I pET-3c DH5α

1.2.4 β2m E. coli pET-β2m BL2(DE3)pLysS LB 37℃ 1:100 50 mg/L 30 mg/L LB 37℃ Aco 0.6 ~ 0.8 M IPTG 37℃ 4 h 50 mmol/L Tris-HCl pH 8.0 EDTA 50 mmol/L PMSF 10 mmol/L DTT

Fig. 1 Cloning of β2m gene from human leukocytes and construction of recombinant expression vector for mature β2m 1 bp 200 bp DNA ladder 2 RT-PCR product 3 pET-3c/Nde I + BamH I 4 expression vector for mature β2m/Nde I + BamH I 5 recombinant plasmid containing β2m gene/Nde I + BamH I
2

2.1 \( \beta_2 \) m

Parker (198) \( \beta_2 \) m \( \beta_2 \) m \( \beta_2 \) m \( \beta_2 \) m \( \beta_2 \) m \( \beta_2 \) m \( \beta_2 \) m \( \beta_2 \) m \( \beta_2 \) m

2.2 \( \beta_2 \) m

E. coli \( \beta_2 \) m \( \beta_2 \) m \( \beta_2 \) m \( \beta_2 \) m \( \beta_2 \) m \( \beta_2 \) m

2.3 \( \beta_2 \) m E. coli

SDS-PAGE Western Blot

Fig. 2 SDS-PAGE and Western blotting analyses of mature \( \beta_2 \) m expressed in E. coli

BL21(DE3)pLysS strain BL21

1. pre-stained protein molecular weight marker 2. BL21 pET-\( \beta_2 \) m 3. pET-\( \beta_2 \) m induced with IPTG for 4 h 5. BL21 pET-\( \beta_2 \) m supernatant 6. BL21 pET-\( \beta_2 \) m pellet 7. washed pellet 8. inclusion body 9. purified \( \beta_2 \) m 10. purified \( \beta_2 \) m

2.4 Western

pET-\( \beta_2 \) m

12 kD

Fig. 3 Purification of recombinant \( \beta_2 \) m with Q-Sepharose column chromatography

The column was equilibrated with 10 mmol/L Tris-HCl pH 7.0. The sample was loaded on the column and eluted with 0-100 mmol/L NaCl linear gradient at a low rate of 1 mL/min. Fractions of 3 mL were collected. Bar indicates the pooled fractions.
REFERENCES


Cloning of Human $\beta_2$-microglobulin Gene and Its High Expression in Escherichia coli

HE Xian-Hui1, XU Li-Hui1,2, LIU Yi1,2, ZENG Yao-Ying1 *

1 Key Laboratory of Tissue Transplantation and Immunology1 Jinan University1 Ministry of Education1 Guangzhou 5106321China
2 Institute of Bioengineering1 Jinan University1 No1 Huangpu Road West1 Guangzhou 5106321China
3 Department of Dermatology1 the First Affiliated Hospital1 Zhengzhou University1 Zhengzhou 4500521China

Abstract Human $\beta_2$-microglobulin ($\beta_2$m) is the light chain of major histocompatibility complex MHC class I molecule. High-yield production of this protein is a prerequisite to the preparation of MHC class I tetramer. The present study aims to obtain recombinant human $\beta_2$m expressed in Escherichia coli E. coli for the purpose of preparing MHC class I tetramers. For cloning of human $\beta_2$m gene, a pair of specific primers was designed based on the published sequence of this gene and the cDNA of full coding region for $\beta_2$m precursor was obtained by RT-PCR from the total RNA of human leukocytes. The amplified cDNA was subsequently cloned and its sequence was confirmed by DNA sequencing analysis. The sequence has been deposited in GenBank with accession number of AY187687. The prokaryotic expression vector containing a gene encoding mature $\beta_2$m was constructed by inserting the DNA fragment which was generated by PCR reaction with the cloned $\beta_2$m gene as template into an IPTG-inducible expression vector pET-3c plasmid. The first eight codons for N terminal amino acid residues of $\beta_2$m were optimized for its expression in E. coli. The complete sequence of $\beta_2$m gene in the expression vector was verified by DNA sequencing analysis. High-yield expression of $\beta_2$m was achieved in E. coli transformed with the expression vector and most of the recombinant $\beta_2$m existed in the inclusion body after IPTG induction. The inclusion body was washed extensively and $\beta_2$m in the inclusion body was solubilized with 8 mol/L urea. The $\beta_2$m was refolded by dialysis and purified by ion-exchange chromatography Q-Sepharose. Western blotting assay indicated that the polyclonal antibody against human native $\beta_2$m could react specifically with the recombinant protein. The purified protein appeared as a single band on both SDS-PAGE and Western blotting indicating that it was chemical and antigenic pure. This work establishes a convenient approach for renaturation and purification of large quantity of recombinant $\beta_2$m which is identical to the native protein without any tags fused except for a methionine residue at the amino terminus. This provides the basis for the preparation of MHC tetramers.

Key words $\beta_2$-microglobulin gene cloning recombinant protein inclusion body