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RESEARCH PAPER

Expression and Activity Analysis of the Catalytic Domain of PTP1B

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Abstract: The amino acid sequence (1–301aa) coding the human PTP1B catalytic domain (PTP1Bc) was obtained from the GenBank. The PTP1Bc gene was constructed by overlapping PCR, then inserted into vector pET-22b (+) and expressed efficiently in *E. coli* BL21 (DE3) under the optimization condition after IPTG induction. The proteins were expressed mainly as inclusion bodies with the yield of more than 30% of total bacterial proteins. The expressed products were purified through Ni^{2+}-affinity chromatographic column. After purification, the purity of the proteins was more than 95%. The result of western blotting confirmed that the purified proteins could specially combine with anti-PTP1B antibody. The enzyme activities experiment showed that the protein had phosphatase activities. The gene construction, expression, and purification of PTP1Bc may provide basis for further study of its functions.

Keywords: PTP1Bc; expression; protein purification; activity analysis

Introduction

More than 100 million people worldwide suffer from Type II diabetes, which is closely linked to obesity, and about three-quarters of the obesity will lead to Type II diabetes. At present, the drugs used for the treatment of type II diabetes often lead to weight gain and some side effects; therefore, there is a need to search for safe and effective methods urgently for treating diabetes. Study on the mechanism of insulin signal transduction provided some new drug target for treatment of Type II diabetes. Among these, protein tyrosine phosphatase-1B (PTP1B) is the most promising one.

Tonks [1,2] and coworkers successfully purified PTP1B form human placenta for the first time in 1988. PTP1B, which is composed from 435 amino acids, is highly expressed in liver, muscle, and fat tissue, and its molecular weight is about 50 kD[3,4]. Recent study reported that PTP1B signal transduction pathway is involved in cancer and diabetes[5]. Zinker *et al*[6] found that antisense PTP1B oligonucleotide can accelerate insulin secretion and maintain blood glucose in normal level. PTP1B gene deficient mice were highly sensitive to insulin, and resistant to weight gain even when fed with fatty diet for a long-term, suggesting that PTP1B is a negative regulator in insulin signal transduction.

Hao *et al*[7] discovered that the enzyme activity of the catalytic domain of PTP (TC-PTP) in T cells is increased by 20–100 u than that of full-length protein, indicating that the activity of human full-length PTPs is lower than that of the catalytic domain. In our study, PTP1B catalytic domain gene was obtained by overlapping PCR, inserted into vector, and expressed efficiently in *E. coli.*

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1 Materials and methods

1.1 Materials

Plasmid pET-22b (+) were purchased from Novagen Company. The primers were synthesized by Shanghai Songon Company. The restriction endonucleases and T4 DNA ligase were purchased from NEB Company. The Plasmid Mini Extraction Kit and the Gel Extraction Kit were purchased from OMEGA Company. The Pfu DNA polymerase, DNA Marker, and Protein molecular weight standard were purchased from TaKaRa. Anti-mouse PTP1B was purchased from DaKeWe Bio-tech Company. Horseradish peroxidase (HRP) conjugated goat anti-mouse IgG was prepared by Xiamen boson bio-tech Company. The Ni^{2+}-HiTrap chelating HP resins were purchased from Amersham Pharmacia Bio-tech Company. pNPP was purchased from German E. Merk Company. The other reagents belong to analytically pure domestic products. *E. coli* BL21 (DE3) was prepared in our lab.

1.2 Methods

1.2.1 Construction of PTP1Bc: Nucleotide sequences of PTP1Bc were looked up from GenBank. Under the precondition of not affecting amino acid sequences, partial modification of the nucleotide sequences of PTP1Bc was done according to codon degeneracy and codon usage bias of *E. coli*. The modified sequence was divided into 16 primer segments. Under one reaction system, 16 primer segments were put in at the same time, synthesized into a complete PTP1Bc with the method of overlapping PCR, and the reaction conditions were as follows: 95°C for 4 min; 95°C for 30 s, 65°C for 2 h, 72°C for 10 min. With P17/P18 as primers, the first round PCR product was used as template to amplify the target gene. The final product was introduced into enzyme cutting sites of *Bam*H I and *Xho* I. The conditions used were 1 cycle at 95°C for 4 min followed by 35 cycles at 95°C for 40 s, at 55°C for 45 s, at 72°C for 1 min, and finally at 72°C for 10 min. The products were analyzed by 1.7% agarose gel electrophoresis.

Primers were designed as follows (from5' to 3'): P1: ATGGAGATGGAGAAAGAATTCGAACAGATCGACA AATCTGG; P2: ACGGAAAGTCGCTTGCCTCGTGAC GGATGTCTTGATAGATCGCTGCCCAAGAACCAGAT TTGTCGATCTGTTCGAATTCT;P3:GAGGCAAGCGAC TTTCCGTGTCGTGTGGCGAAATTACCGAAGAACAA AAACCGTAATCGCTACCGCGACGTTAGCCCATT; P4: TAATCAGGCTTGCGTTGATATAATCATTGTCCTCTT GGTGCAGCTTAATACGAGAATGGTCAAATGGGCTA ACGTCGCGG; P5: CAATGATTATATCAACGCAAG CCTGATTAAGATGGAAGAGGCACAACGTTCTTACA TCCTGACCCAAGGTCCATTACCAA; P6: ATGACAA CACCACGGCTCTTTTGCTCCCAGACCATTTCCCAGA AATGACCGCACGTATTTGGTAATGGACCTTGGGTC AG; P7: AAAGAGCCGTGGTGTTGTCATGCTGAAT CGTGTCATGGAAAAGGGCTCTCTGAAATGCGCACA ATACTGGCCGCAAAAAG; P8: TCCTCGCTAATTAA CGTCAGCTTCAGATTCGTGTCCTCAAAAATCATCTC TTTTTCTTCTTTTTGCGGCCAGTATTGTGC; P9: GAA GCTGACGTTAATTAGCGAGGACATTAAGAGCTACT ACACCGTCCGCCAATTAGAGTTAGAAAACCTGACC ACCCAGG; P10: CCGGGACGCCAAAGTCTGGCCA CGTCGTGTAGTGAAAGTGCAGGATCTCACGGGTTT CCTGGGTGGTCAGGTTTTCTAAC; P11: AGACTTTGG CGTCCCGGAATCTCCAGCCTCTTTTCTGAATTTTCT GTTTAAGGTGCGTGAGTCTGGTAGCCTGAGCCCGG; P12: GCTAAGCAGAACGTGCCGCTACGACCAATGC CCGCAGAGCAATGGACGACGACTGGGCCGTGTTCC GGGCTCAGGCTACC; P13: CGGCACGTTCTGCTT AGCAGATACGTGTTTACTGCTGATGGACAAGCG CA AAGACCCGTCTAGCGTTGACATCAAGAAGG; P14: TAACTGATCCGCCGTCTGGATTAAACCCATACGAA CTTGCGCATCTCTAACAGAACCTTCTTGATGTCAAC GCTAGACG; P15: ATCC AGACGGCGGATCAGTTAC GTTTCAGCTATCTGGCGGTGATTGAAGGCGCCAAG TTTATCATGGGTGACAGCAGCGT; P16: CGGCTCT AAGTCTTCATGAGATAACTCTTTCCATTGATCCTGA ACGCTGCTGTCACCCAT; P17: GACTGGATCCCATG GAGATGGAGAAAG; P18: CTGACTCGAGCGGCTCT AAGTCTTCAT

1.2.2 Construction, expression, and purification of the expression vector of PTP1Bc: PCR products and plasmid pET-22b (+) were digested respectively by *Bam*H I and *Xho* I. The PTP1Bc gene was cloned into plasmid pET-22b (+) to construct recombined plasmid pET-22b (+) / PTP1Bc with catalysis of T4 DNA Ligase, and then transformed into *E. coli* BL21 (DE3). Plasmid extraction from recombinants was identified by enzyme digestion. The positive plasmids were sent for sequencing. The correct recombinants were cultured overnight at 37°C, 1:100 diluted into LB, and cultured to the state of OD_{600} between 0.6 and 0.8; IPTG was added at the final concentration of 0.5 mmol/L for 4 h. The target protein was purified with Ni^{2+}-HiTrap chelating HP resins according to the method provided by Amersham Pharmacia Biotech. Purified protein was denatured using the gradient dialysis method.

1.2.3 Western blotting identification of the recombined protein[8]: The purified PTP1Bc was detected by Western blotting, and blank control was set at the same time. Protein extracts were separated using SDS-PAGE. Proteins were electrophoretically transferred to nitrocellulose membranes. Then, the membranes were incubated in phosphate buffered saline containing 5% skimmed milk. Anti-mouse PTP1B were added and incubated at 37°C for 60 min. After incubation with horseradish peroxidase-conjugated secondary antibodies (goat anti-mouse IgG) for 30 min at room temperature, protein bands were visualized by the DAB