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Research Article

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Expression and purification of human peptide transporter hPepT2(579-664)

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ABSTRACT

Human peptide transporter PepT2 contains 729 amino acids, 12-transmembrane domains. Essentially dipeptides, tripeptides and the similar peptide-like drugs can be transport by hPepT2 which plays an important role in nutrition and pharmacology. The fragment hPepT2(579-664) is located in the currently unknown function 10-12TMDs of hPepT2, which possibly contributes to important uptake and metabolism in vivo. So the BL21(DE3)-pET30a-hPepT2(579-664) the expression system was constructed, and the fragment protein was expressed under optimal condition, 300μM IPTG at 37 ˚C inducing 4 hours, and purified using Sephadex G-75 column to facilitate the further study on the structure and function of hPepT2.

Keywords: Human peptide transporter, hPepT2(579-664), Expression, Purification

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INTRODUCTION

Proton-coupled oligopeptide transporters (POTs) family is comprised by PepT1, PepT2, PHT1 and PHT2, responsible for the transport of most dipeptides, tripeptides and peptide-like drugs in human body [1-3]. Human oligopeptide transporter PepT2 (hPepT2) is widely expressed in a variety of tissues, with predominant expression in the kidney but also in brain, eye, mammary gland, etc. [4-7]. hPepT2 has been characterized as a high-affinity, low-capacity transporter containing 12 transmembrane domains (TMDs) and an extracellular loop between 9 TMD and 10 TMD [8-10]. The analysis of chimeric PepT1/PepT2 proteins indicates that the phenotypic characteristics of PepT2 are determined by 1-9 TMDs, the putative substrate binding domains in PepT2 lie in the region 7, 8, and 9 TMDs and the functions of 10-12 TMDs are unknown [11-13]. The fragment hPepT2(579-664) lies in the unknown region between 10 TMD and 11 TMD, whose properties will promote to understand the structure and function of PepT2. So the prokaryotic expression vector of hPepT2(579-664) was constructed and used to express the fragment protein which would be researched to supply groundwork for the further comprehensive understand on PepT2.

EXPERIMENTAL SECTION

Materials

hPepT2 cDNA and pET30a(+) plasmid were purchased from OriGene (Maryland, USA). Wizard SV Gel and PCR Clean-Up System were obtained from Promega (Madison, Wisconsin, USA). Restriction enzyme EcoRI, NdeI and BM201 Protein Maker Low Range (4.1-66kDa) were obtained from Bio Basic Inc (New York, USA). TIANprep Mini Plasmid Kit was obtained from TIANGEN (Beijing, China). Isopropyl-thio-β-D-thiogalactoside (IPTG) was supplied by Sangon (Shanghai, China). All primers were synthesized by Sangon (Shanghai, China). SephadexTM G-75 medium was obtained from Solarbio (Beijing, China). All other chemicals and reagents were purchased from TaKaRa (Dalian, China).

Cloning of the hPepT2 (579-664)

The primers covering the coding region of hPepT2 (579-664) were designed for PCR. The sense primer containing the endonuclease site of NdeI was 5′-GGTTCCATATGGCAGCATATCTGTTTGT-3′. The anti-sense primer

covering the endonuclease site of EcoRI was 5′-CCGGAATTCCTATGCCACAACAAGCACGAT-3′. PCR conditions were hot start at 95 ˚C for 5 min, followed by 35 cycles of denaturation at 94 ˚C for 1 min, annealing at 55 ˚C for 1 min and extension at 72 ˚C for 1 min, then followed by a final 8 min extension. The products were separated on a 1.5% agarose gel and purified by Wizard SV Gel and PCR Clean-Up System.

Construction of pET30a-hPepT2(579-664) recombinant plasmid

The pET30a(+) plasmid and hPepT2(579-664) PCR product were double digested using restriction enzyme EcoRI and NdeI at 37 ˚C for 16 h respectively and then linked by T4 DNA ligase at 16 ˚C for 18 h to obtain the recombinant plasmid pET30a-hPepT2(579-664). The recombinant plasmids were transformed into competence cells of *E.coil* DH5α and transformants were selected on LB (Luria-Bertani) agar plates containing kanamycin (50μg/ml) [14, 15]. Single colony was inoculated into 5 ml LB medium containing 50μg/ml kanamycin. The recombinant plasmids were extracted by using TIANprep Mini Plasmid Kit and sequenced by Sangon Biotech (Shanghai) Co., Ltd.

Optimization Expression of hPepT2(579-664)

For expression of hPepT2(579-664), the sequenced recombinant plasmids were transformed into *E.coil* BL21(DE3) cells. The expression strain was cultured on LB plating medium containing kanamycin (50 μg/ml) at 37 ˚C for 16-24 h. The single colony was selected and cultured in 5 ml LB medium containing 50 μg/ml kanamycin. The fresh overnight culture was diluted 1:50 with LB medium (50 μg/ml kanamycin) and grown at 37 ˚C to OD_{600} = 0.5 [16, 17]. Then the culture was induced at 37˚C under different concentration of IPTG (0.1, 0.3, 0.5, 0.7 and 1 mM) and different induced time (1, 2, 3 and 4 hours) in order to explore the optimal expression conditions. And the results were detected by 15% SDS-PAGE.

Purification of hPepT2 (579-664)

The *E.coil* BL21(DE3) strain containing recombinant plasmid pET30a-hPepT2(579-664) were inoculated into 24 ml LB medium (50 μg/ml kanamycin) and shaked overnight at 37 ˚C. This medium was added into 1.2 L LB medium (50 μg/ml kanamycin) and cultured at 37 ˚C to OD_{600} = 0.5, then induced by IPTG (0.3 mM) at 37 ˚C under continuous shaking for 4 h. The cells were harvested by centrifugation at 3000 rpm × 5 min, then suspended and washed twice using 100 ml of PBS lysis buffer (50 mM sodium phosphate buffer at pH 7.8). The cells were resuspended again with 30 ml PBS lysis buffer containing 1mM phenylmethylsulfonyl fluoride (PMSF) and 0.4 mg/ml of lysozyme, and incubated on ice for 30 min [16, 18, 19]. The lysate was disrupted by sonication at approximately 110 W for 20 × 30 s pulses with 1 min delay between pulses on ice, then centrifuged at 11000 rpm × 30 min to separate the insoluble substance and supernatant. The insoluble matter was resuspended with 20 ml PBS lysis buffer containing 0.5% Triton X-100 and incubated on ice for 15 min [20], and disrupted again by sonication at approximately 110 W for 20 × 30 s pulses with 1 min delay between pulses on ice. Then the precipitation was obtained by centrifuged at 11000 rpm × 15 min. The precipitation per gram was suspended by 9 ml buffer containing 8 M urea, 2 mM reduced glutathione and 0.2 mM oxidized glutathione and incubated at room temperature for 1h. Then this solution was diluted 1:9 with the same buffer and incubated at room temperature for 2 h. After centrifugation at 11000 rpm×15 min, the supernatant was dialyzed in sterile water for 24 h at 4 ˚C. The precipitation and supernatant in each step were detected by SDS-PAGE for understanding the location of target protein.

The supernatant obtained from the above was filtrated with 0.2 μm filter membrane and purified using Sephadex G-75 gel chromatography column. The purified protein was visualized on 15% SDS-P AGE.

RESULTS AND DISCUSSION

Construction of pET30a-hPepT2 (579-664) recombinant plasmid

To confirm the presence of recombinant plasmid pET30a-hPepT2(579-664), the ligation products were transformed into DH5α. The sequence of recombinant plasmid demonstrated that pET30a-hPepT2(579-664) was constructed successfully and could be used to express the fragment protein.

Expression and purification of hPepT2(579-664)

The 10-12 TMDs of hPepT2 are several unrecognized function region and contained relatively small rare codons which could affect its expression at a large extent in *E. Coil* [21], so pET30a-hPepT2(579-664) was transformed into E. coil BL21(DE3) competent cell, and several factors were changed to determine the optimal expression conditions of hPepT2(579-664). To evaluate the time course effect on the expression of hPepT2(579-664), bacterium were harvested every hour for 4 h following IPTG induction at 37˚C and total lysates were analyzed on SDS-PAGE [22, 23]. An expected band at the molecular masses of hPepT2(579-664) 9.34 kDa was detected and reached high level after 4 h induction (Fig. 1). Whereafter, the different IPTG dosage (0.1, 0.3, 0.5, 0.7 and 1 mM) were used to induce the expression of hPepT2(579-664) at 37˚C for 4 h. The expression of protein under 0.3 mM IPTG was a little better

than others according the detection of total lysates analyzed on SDS-PAGE (Fig. 2). So the optimal condition for the large-scale production of hPepT2 (579-664) was 0.3 mM IPTG inducing 4 h at 37˚C.

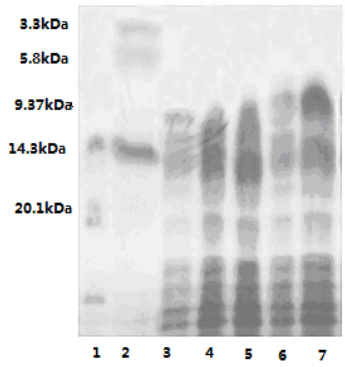

Fig.1 The expression of hPepT2(579-664) under different times
Lane 1 and 2, Protein Molecular Weight Marker (Low & ultra-low); lane 3, total lysate of BL21(DE3) before induced; lanes 4-7, total lysate of BL21(DE3) induced 1, 2, 3 and 4 h, respectively.

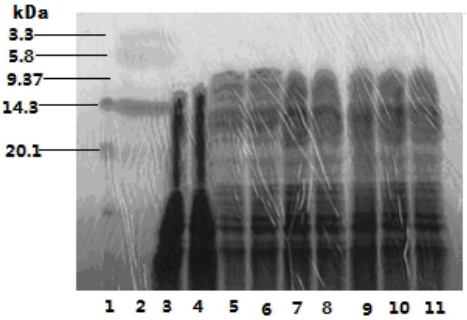

Fig. 2 The expression of hPepT2(579-664) under different IPTG dosage
Lane 1 and 2, Protein Molecular Weight Marker (Low& ultra-low); lane 3, BL21 (DE3) containing recombinant plasmid cultured 4 h without IPTG; lane 4, strain cultured 4h without IPTG; lane 5, BL21 (DE3) contain pET30a(+) plasmids culture 4 h after 35 μl of IPTG addition; lanes 6-11, total lysate of BL21 (DE3) culture 4 h after 5, 15, 25, 35, 50, and 100 μl of IPTG addition, respectively.

Gel chromatography with SephadexTM G-75 medium was used to purify target protein. The column was washed with buffer. The elution solution was collected as 1 ml/tube according the absorption at 260 nm which only corresponded to a main peak. The analysis results in Fig.3 indicated that the dialyzed supernatant mainly contained hPepT2(579-664), and most of hydridproteins were higher than 10 kDa and could be removed from column at the beginning of elution peak. Though some hPepT2(579-664) were lost with hybridprotein, which probably because of the high concentration of supernantant. The changes of separation condition may improve purification effect. In all Sephadex G-75 column could be used to purify the target protein hPepT2(579-664).

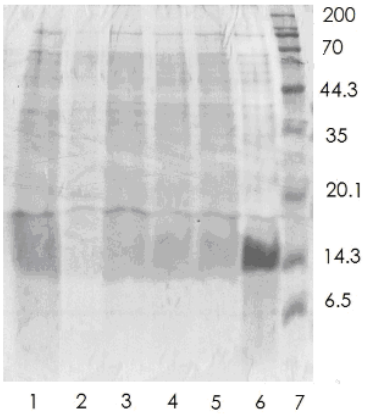

Fig. 3 SDS-PAGE of the hPepT2 (579-664) purification using G-75 column
Lane 1-6, the first to sixth ml of G-75 elution peak, respectively; Lane 7, Protein Molecular Weight Marker.

CONCLUSION

Though the structure and function of hPepT2(579-664) still are unknown, it acts as a important part of hPepT2 and probably has important scientific value for more comprehensive understanding PepT2. The BL21(DE3)-pET30a-hPepT2(579-664) expression system and Sephadex G-75 column can be used to expressed and purified the fragment protein hPepT2(579-664). The prokaryotic expressed hPepT2(579-664) will supply groundwork for study its function in vivo or vitro. And the further study of hPepT2(579-664) which interact with di/tri-peptides or peptide-like drugs in vivo or vitro is proceeding.

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