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Increased soluble heterologous expression of a rat brain 3-*O*-sulfotransferase 1 – A key enzyme for heparin biosynthesis

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ABSTRACT

Heparan sulfate (HS), is a glycosaminoglycan (GAG) involved in various biological processes, including blood coagulation, wound healing and embryonic development. HS 3-*O*-sulfotransferases (3-OST), which transfer the sulfo group to the 3-hydroxyl group of certain glucosamine residues, is a key enzyme in the biosynthesis of a number of biologically important HS chains. The 3-OST-1 isoform is one of the 7 known 3-OST isoforms and is important for the biosynthesis of anticoagulant HS chains. In this study, we cloned 3-OST-1 from the rat brain by reverse transcription-polymerase chain reaction (RT-PCR). After codon optimization and removal of the signal peptide, the recombinant plasmid was transformed into *Escherichia coli* BL21 (DE3) to obtain a His tagged-3-OST-1 fusion protein. SDS-PAGE analysis showed that the expressed 3-OST-1 was mainly found in inclusion bodies. The 3-OST-1 was purified by Ni affinity column and refolded by dialysis. The activity of obtained 3-OST-1 was 0.04 U/mL with a specific activity of 0.55 U/mg after renaturation. Furthermore, a co-expressed recombinant plasmid pET-28a-3-OST-1 with the chaperone expression system (pGro7) was constructed and transferred to *E. coli* BL21 (DE3) to co-express recombinant strain *E. coli* BL21 (DE3)/pET-28a-3-OST-1 + pGro7. The soluble expression of 3-OST-1 was significantly improved in the co-expressed recombinant strain, with enzyme activity reaching 0.06 U/mL and having a specific activity of 0.83 U/mg. *N*-sulfo, *N*-acetylheparosan (NSNAH) was modified by the recombinant expressed 3-OST-1 and the product was confirmed by ^{1}H NMR showing the sulfo group was successfully transferred to NSNAH.

1. Introduction

Heparan sulfate (HS) plays a key role in a variety of important biological processes including virus infection, regulation of blood coagulation, embryonic development, inflammation, tumor growth inhibition [1–5]. HS and heparin is made up of repeating disaccharide units of consisting of glucosamine and glucuronic acid residues. In biosynthesis, heparosan is formed by alternately adding glucosamine and glucuronic acid to the non-reducing end of the chain [6]. Subsequently, this high-molecular heparosan is modified by a series of enzymatic reactions, including *N*-deacetylation, *N*-sulfation [7], glucuronic acid C5 epimerization, and *O*-sulfation at different positions and to different levels [8]. Due to the incompleteness of these modifications, the synthesized HS polysaccharides also have different structures. The interactions of HS with different proteins depend on the sites and *O*-sulfation levels of the polysaccharide chains [9]. In recent years, many studies have shown that HS and its derivatives play important roles in virus infection [10,11]. A HS chain containing a 3-*O*-sulfo-D-glucosamine residue was found to bind to viral glycoprotein D protein and block the interaction between HSV-1 virus and cell surface proteoglycans [12]. In addition, 3-*O*-sulfo group containing HS chain can bind to antithrombin III, HSV-1 glycoprotein D, fibroblast growth factor/receptor (FGF/FGFR) [13,14] and has a role in ovulation [15].

HS 3-*O*-sulfotransferases (3-OST) catalyzes the transfer of sulfo groups to the 3-hydroxyl group of certain glucosamine residues. The 3-OST gene has been cloned from endothelial cells of newborn mice and human umbilical vein endothelial cells, and the 3-OST enzyme was heterologously expressed [16]. The structure of HS is altered spatiotemporally for regulating plenty of biological activities in the developing brain including the proliferation of neuronal progenitors, extension of axons and formation of dendrites [17]. These sulfotransferases including 3-OST were important in the signaling of several HS-binding

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proteins in the mouse brain [17]. There is no report on the heterologously expression of 3-OST gene from cells of rat brain. Although the genome of mice is more closely related to human genome than rat's, research on heterologous expression of 3-OST gene from rat brain cell would enrich the knowledge on structure and functions of sulfotransferases from diverse source. Additionally, the 3-OST from rat brain cell may supply one more potential selection to be a tool enzyme for heparin preparation from heparosan. Among the 7 isoforms of 3-OST [18], 3-OST-1 is the rate-limiting enzyme, controlling cellular production of the critical active structure [19]. Therefore, 3-OST-1 was selected for cloning and expressing in *E. coli* in our current study. Codon optimization was performed for the 3-OST-1 gene and the signal peptide was removed to achieve efficient heterologous expression of the target protein. Codon selection and distribution is one of the factors that affect the efficient heterologously expression of genes. Optimizing the codon sequence on exogenous genes could improve heterologous protein expression. While signal peptides of eukaryotes are expressed in prokaryotes, most of these do not have secretive function, and are always fused with the target protein, affecting the correct folding and function of the target protein [20].

In this paper, to achieve effectively heterologous expression of the exogenous protein in *E. coli*, the 3-OST-1 gene was inserted into the His-tagged pET-28a expression plasmid, which contains a strong promoter of T7 Lac capable of rapidly and stably transcribing downstream genes under the action of the host bacterial with T7 RNA polymerase. At the same time, the His-tag carried by the vector helps purify the His-tag fused target protein. Finally, the enzyme activity, of the protein obtained, was measured using a 3′-phosphoadenosine-5′-phosphosulfate (PAPS) regeneration system [21]. ^{1}H NMR spectrum was conducted to confirm that 3-OST-1 could successfully transfer a sulfo group to the specific site of the substrate *N*-sulfo, *N*-acetylheparosan (NSNAH).

2. Materials and methods

2.1. Animals, strains, plasmids, enzymes and reagents

The Wistar Rats were purchased from the China National Laboratory Animal Resource Center (Shanghai, China).

The *E. coli* DH5α, *E. coli* TransB (DE3) and *E. coli* BL21 (DE3) were purchased from TransGen Biotech. The pMD-19-T vector and pMAL-c2X were obtained from TAKARA and Beijing Dingguo Biotechnology (China), respectively. The plasmid pET-28a and pet 20 b were obtained from Sangon Biotech (Shanghai, China) and the plasmid pGro7 was obtained from TAKARA.

The reagents for PCR were purchased from Sigma (St. Louis, MO). The oligonucleotide primers were synthesized from Sangon Biotech (Shanghai, China). Ni-NTA Sefinose™ Resin Kit was purchased from Sangon Biotech. The NSNAH were prepared in our laboratory.

2.2. RT-PCR

The HS3-OST-1 sequence was obtained from Genebank (the accession number was **NM_053391.1**). Based on the sequence, two specific primers, which contained *Bam* H I and *Hin* d III restriction enzyme sites, were synthesized. The total RNA of the Wistar rat brain was extracted, and cDNA was synthetized as described previously. 3-OST-1 from the Wistar rat brain was cloned by RT-PCR.

The PCR products were connected to the pMD-19-T vector. *E. coli* DH5α was used for the propagation of recombinant plasmids. The competent DH5α cells were transformed with cloning vector pMD-19-T by white and blue screening. Recombinants were sequenced by Introversion (Shanghai) to verify the correct nucleotide sequencing.

2.3. Codon optimization

After sequencing, the rare codons of the sequence were analyzed and the codons were optimized by Synbio Technology (Suzhou).

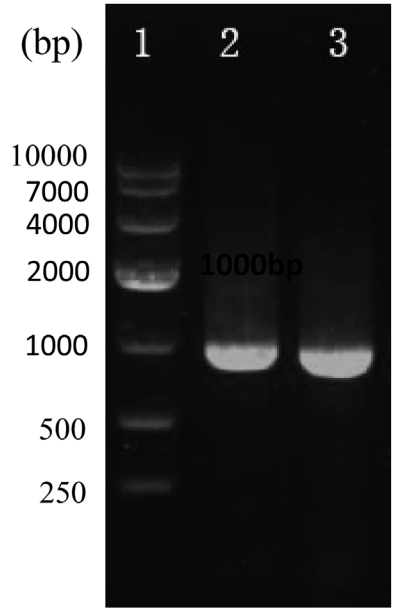

Fig. 1. PCR product of rat 3-OST-1. Lane 1, DNA marker; Lane 2 and 3, PCR fragment.

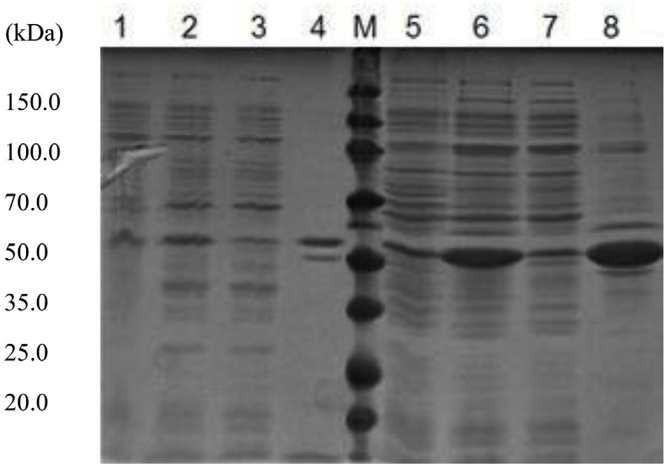

Fig. 2. Expression of 3-OST-1 via *E. coli* TransB (DE3)/pET-20b-3-OST-1 (Lane 1-4) and *E. coli* BL21 (DE3)/pET-28a-3-OST-1 (Lane 5–8). M: Protein markers; Lane 1 and 5: Un-induced total cell protein; Lane 2 and 6:Total cell protein induced by 1 mM IPTG; Lane 3 and 7: Supernatant induced by 1 mM IPTG; Lane 4 and 8: Precipitate induced by 1 mM IPTG.

2.4. Expression of 3-OST-1 gene

The gene with optimized codons was inserted into the pET-28a vector. The recombinant vector was transferred into *E. coli* BL21 (DE3) using heat transfer method. The transformant colony, *E. coli* BL21 (DE3)/pET-28a-3-OST-1, was screened on medium containing 50 mg/L kanamycin. The transformant was inoculated into 50 mL LB medium containing 50 mg/L kanamycin and incubated at 37 °C and 180 rpm. After overnight culture, the broth was inoculated into fresh LB medium containing 50 mg/L kanamycin. When the OD_{600} of the broth reached 0.6, 1 mM IPTG was added to induce the express of 3-OST-1 by the recombinant strain cultured at 22 °C and 180 rpm.

The induced host cells were harvested by centrifugation at 8000 rpm for 20 min at 4 °C. After washing, binding buffer (25 mM Tris-base, 500 mM NaCl and 10 mM imidazole, pH 8.0) was used to suspend