Asian Journal of Biochemistry

ISSN 1815-9923

Academic
Journals Inc.

www.academicjournals.com

Asian Journal of Biochemistry 1 (2): 162-168, 2006
ISSN 1815-9923

Cloning and Identification of a Novel Human Gene hSVOP Similar to Synaptic Vesicle Protein

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Abstract: SVOPs are synaptic vesicle proteins that contain 12 transmembrane regions. Herein, we have isolated a novel human homologue of SVOP gene from a human brain cDNA library. This gene contains an open reading frame of 1646 nucleotides, coding for a protein with 548 amino acids. hSVOP is highly conserved in evolution across different species from *C. elegans*, fruit fly, mouse, to human. Northern blot analysis showed that it is expressed specifically in human brain. Transient transfection of COS-7 cells with hSVOP-EGFP demonstrates that hSVOP is located on cytoplasmic membrane and may be involved in material transfer between the synaptic vesicle and cytoplasm.

Key words: Synaptic vesicle protein, SV2, transport protein, SVOP

Introduction

Synaptic vesicle is an important organelle in nerve cells. It plays an pivotal role in the neurotransmission process (Tanaka *et al*., 2001). Recently, two highly related proteins named SV2 and SVOP have been identified in synaptic vesicles. The SV2 is a glycoprotein weighting about ~80 kDa and contains an evolutionary conserved 12 transmembrane domain. Previous study demonstrated that SV2 is similar to bacterial sugar transporters that can use a proton gradient as driving force, which raised the possibility that SV2 may function as a novel transport protein in the transmission of specific substrate into synaptic and secretory vesicles (Bajjalieh *et al*., 1992). However, other studys also implicated that SV2 may act as a matrix protein for synaptic vesicles with a structural function. The SV2 related protein (SVOP) exhibits high similarity to SV2 (Janz *et al*., 1998). Contrary to SV2, the SVOP can not be glycosylated although it co-localize with SV2 in synaptic vesicle. Sequence analysis indicated that SVOP may represent an evolutionary precursor of SV2. However, the lack of extensive glycosylation in SVOP demonstrates that the function of it is distinct from SV2. Currently, the function of SVOP in synaptic vesicles is still waiting for further investigation (Logan *et al*., 2005).

In order to identify the function of SVOP protein in synaptic vesicles, we isolated a novel human synaptic vesicle protein that is highly related to SV2 named human SV2 related protein (hSVOP). Sequence comparison among its homologues demonstrates that it is the most conserved protein across *C. elegans*, fruit fly, mouse to human. Northern blotting analysis shows that a 3.6 kb transcript specifically expressed in adult brain. Transient transfection of COS-7 cells with hSVOP-EGFP demonstrates that hSVOP is located on cytoplasmic membrane. Collectively, these results suggest that

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hSVOP may play an important role in brain and neural tissues and may be involved in material transfer between the synaptic vesicle and cytoplasm.

Materials and Methods

Construction of cDNA library of Human Embryo Brain

The total RNA from 20-week human embryo brain was extracted using standard methods as described previously (Zeng *et al*., 2002). The RNA was pretreated with DNase I (RNase free) to eliminate DNA contamination. mRNA preparation and reverse transcription reaction were performed using a cDNA PCR Library Kit and cDNA Synthesis kit according to manufacturer's protocol (TAKARA). Briefly, 5 μg mRNA was purified from 500 μg total RNA using Rapid mRNA™ purification Kit (AMRESCO). Reverse transcription reactions were performed with the purified embryonic brain mRNA and Oligo dT-RA primer according to cDNA Synthesis kit protocol. After Cassette Adaptor Ligation reactions using cDNA PCR Library Kit, cDNA amplification reactions were performed with RA primer, CA primer and TaKaRa Ex Taq.

Blast Searching and Bioinformatics Analysis

BLASTn program was used to (http://www.ncbi.nlm.nih.gov) search human EST database. Blastn program was applied to identify the cytological locus of genes and to look for exons and introns. Amino acids analysis of the hSVOP sequence was performed by DNAstar software (Yi *et al*., 2004). Smart program (http://smart.embl-heidelberg.de/) was used to analysis the domain structure.

Isolation and Cloning of cDNA of this Novel Gene

According to the asembled sequence of this novel gene, we designed a pair of degenerated oligonucleotide primers (5'-TATAAGCTTTGGCGCAGCGCGGACATG-3' and 5'-TCGAAGCTTCAGTCCCCCATCGGTCAC -3'). PCR was performed on a PCRSPRINT reactor (Thermo Hybaid). Amplification was carried out at 94°C, 4 min; 94°C, 30 sec, 58°C, 30 sec, 72°C, 2 min for 35 cycles; then 72°C, 10 min. The amplification products were separated by agarose gel and the bands were cloned into pUCmT-vector (SANGON). The transformants were randomly chosen and sequenced with 377 DNA Sequencer (ABI PRISM) according to manufacturers procedures.

Northern Blot Hybridization

The full length cDNA of hSVOP was used as probe labeled with [a-^{32}P]dCTP. An adult human Multiple Tissue Northern blot (Clontech) was hybridized to the radio labeled probe. The Random Primer DNA labeling kit was purchased from TAKARA. Hybridization was carried out with 5×SSC, 5×Denhardt 10% dextran sulfate and denatured human DNA, at 42°C overnight. After hybridization, the blot was washed three times at 42°C in 2×SSC containing 0.1% SDS for 5 min and twice in 0.1×SSC and 0.1% SDS at 42°C for 15 min and then exposed to X-ray film at -80°C using an intensifying screen.

Construction of Plasmid Expression

In order to construct an expression vector, a sense primer 5'-TATCTCGAGTGGCGCAGCGCGGACATG- 3' and an antisense primer 5'-TCGAAGCTTCAGTCCCCCATCGGTCAC -3' was used to introduce an XhoI site at 5' terminal and a Hind III site at 3' terminal of the hSVOP ORF sequence by PCR. The PCR product was purified and then cloned in to pEGFP-N1.

Subcellular Localization

Hela cells were maintained in Dulbecco' s Modified Eagle' s sodium (DMEM), supplemented with 10% fetal calf serum at 37°C in a humidified atmosphere of 5% CO_2. Cells were transfected with pEGFPN1-hSVOP by Calcium Phosphate method according to the method described before (Huang *et al*., 2004). Forty eight hours after transfection, cells were fixed with 4% paraformaldehyde for 15 min at room temperature (pEGFPN1 was transfected as the control). The nucleus were stained with DAPI. Fluorescent images of cells were captured on a cooled charge-coupled device camera mounted on an Olympus inverted research microscope using Ultraview imaging software (Olympus®, Inc.)

All of the experiments mentioned above were conducted in The Center For Heart Development, College of Life Sciences, Hunan Normal University, in 2002-2005.

Results and Discussion

Cloning of hSVOP gene

In an effort to understand the role of novel transporter proteins in neural tissues, we screened the human EST database with a series of conserved transporter protein sequence. A number of ESTs representing the same novel gene were identified in our database search. Then, the partial cDNA sequence of this gene was assembled from ESTs DN989952, DA309695, AL563357, DA907901 and BP323150. To confirm the cDNA sequences identified from the database, one pair of gene-specific primers were designed based on the sequence of predict ORF assembled sequence for PCR. PCR reactions were performed using the brain library as template. A single 1600 bp fragment was obtained under standard PCR conditions and subcloned to T-vector. Sequence analysis indicated that the PCR products included the assembled partial cDNA sequence.

In order to obtain the full-length cDNA, 5' RACE and 3' RACE were performed as described previously (Huang *et al*., 2004). These procedures yielded a 500 bp DNA for 5' RACE fragment and 300 bp DNA for 3' RACE fragment. An analysis of these two fragments suggested that they were cDNA fragment from this novel human gene. Then, we assembled all these sequence fragments into a 3486 bp full-length cDNA of the human gene. A computer-assisted homology search showed that the amino acid sequence of this gene shares 94% identity with mouse gene SVOP so we named the human gene hSVOP for human SV2 related protein.

Sequence Analysis of the hSVOP Gene

hSVOP gene is consisted of 10 exons, located at 12q24.12 and spanning 28.09 kb on genome. It encodes a protein of 548 amino acids. The translation start code ATG is located at 173 bp and the flanking sequence is GAC**ATG**G, which is identical to Kozak sequence A/GNNATGG. The stop code is TAG, located at 1819 bp (Fig. 1).

Table 1: Amino acid sequences of 12 transmembrane regions in homolog SVOP

Transmembrane regions	Initial sequences	Ending sequences	Transmembrane regions	Initial sequences	Ending sequences
1	86	108	7	315	337
2	121	143	8	376	395
3	155	177	9	402	421
4	181	203	10	425	447
5	210	232	11	459	481
6	236	258	12	486	508

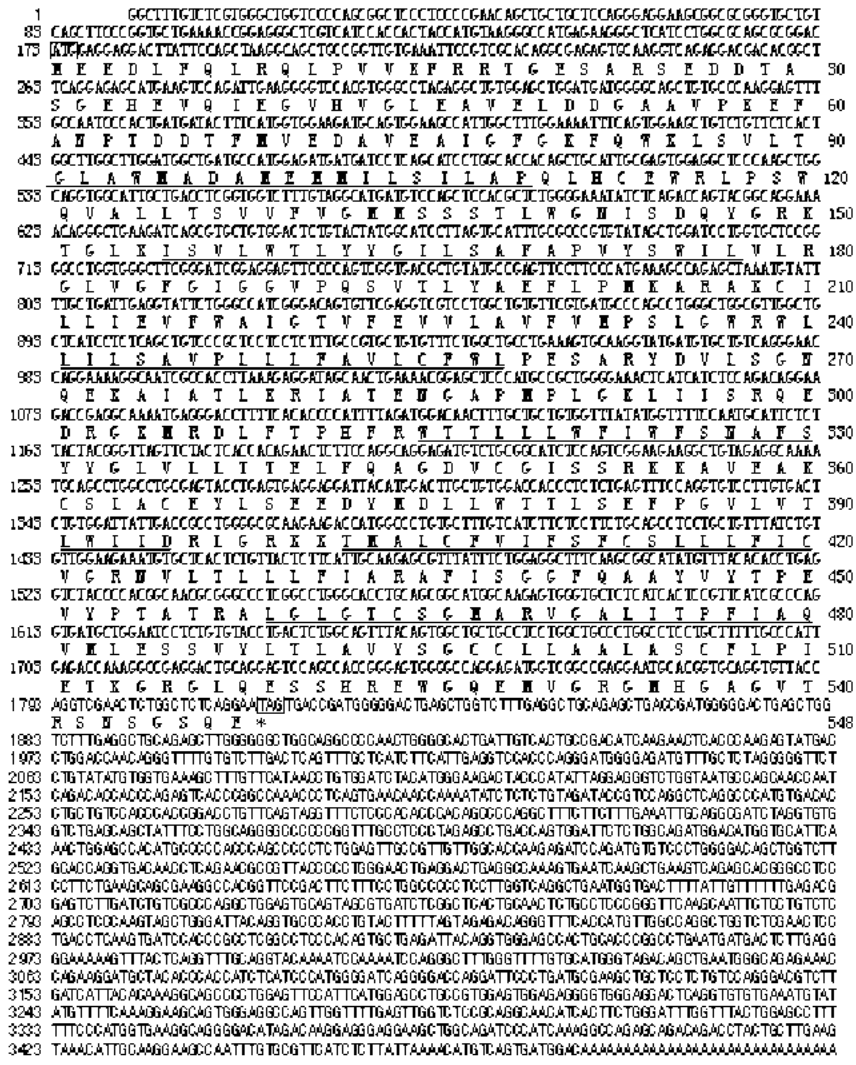

Fig. 1: The cDNA sequence and amino acid sequence of hSVOP. The initiation ATG and termination TAA codons are boxed. Both nucleotides and amino acids are numbered at the left side of each line, respectively. The twelve transmembrane regions are marked by underline

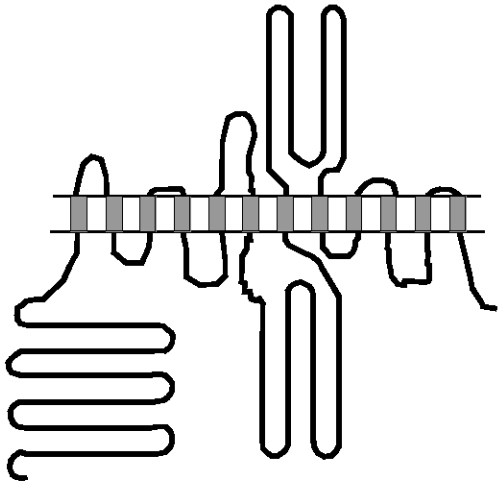

Fig. 2: The predicted structure of the transmembrane regions of hSVOP. The green boxes indicated the residues in the 12 transmembrane regions and the blue line indicates the residues in the lumen of synaptic vesicle and in cytoplasm

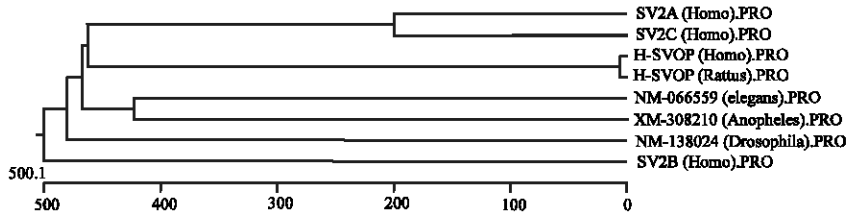

Fig. 3: Un-rooted phylogenetic tree analysis of hVSOP and other homologue proteins

The Structure of hSVOP Protein

The structure analysis with SMART program shows that hSVOP contains 12 highly hydrophobic transmembrane regions (Fig. 2 and Table 1). Furthermore, a sugar transporter motif has also been identified by comparing the sequence with the protein database. This motif existed in the members of the ATP-binding cassette (ABC) superfamily and the major facilitator superfamily (MFS). Moreover, recent studies show that it is not only involved in sugar uptaking but also in other important physiological processes such as drug efflux, Krebs cycle, phosphate exchange and so on.

hSVOP is Conserved During Evolution

BLAST searches using the sequence of hSVOP identified closely related sequences in *C. elegans*, fruit fly, mouse and rat. We then tried to analyze the evolutionary relationship between the hSVOP protein and the other homologue proteins with phylogenetic tree analysis (Fig. 3). Sequence alignment of these proteins demonstrates that hSVOP is one of the conserved proteins during evolution.

The Tissue Specific Expression of hSVOP

To characterize the transcript of hSVOP with respect to its size and expression distribution, Northern blotting with adult multiple tissue northern blot was performed. The result shows that the

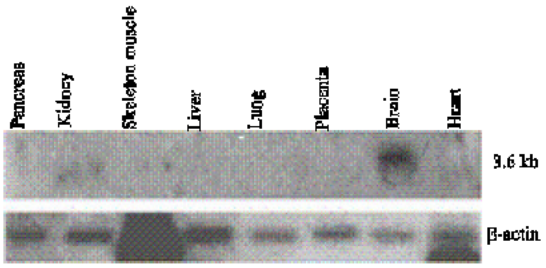

Fig. 4: The expression of hSVOP at adult stage. β-actin was used as control

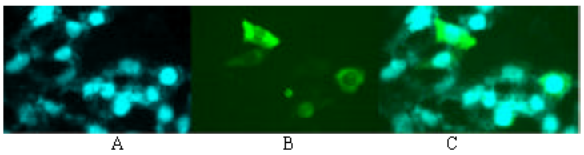

Fig. 5: The subcellular location of hSVOP. A) Hela cells were stained with DAPI. B) The location of fusion protein EGFP-N1-hSVOP in Hela cells. C) The merged image of A and B

transcript of hSVOP is about 3.6 kb and it is only expressed in brain at adult stage (Fig. 4). The highly expression in adult brain indicated that hSVOP play an important role in brain and neural tissues.

The Subcellular Location of hSVOP Protein

Since the hSVOP protein contains 12 transmembrane regions, we predicted that it is a membrane protein located on the membrane of synaptic vesicle. Therefore, Hela cells were transiently transfected by pEGFP-N1-hSVOP to express EGFP-N1-hSVOP fusion protein. Figure 5 shows that the fusion protein was located on nuclear membrane and cell membrane, while the EGFP-N1 protein was expressed in cytoplasm in control cells. This result suggested that hSVOP is located on cytoplasmic membrane and may be involved in material transfer between the synaptic vesicle and cytoplasm.

Currently, three subtypes of SV2 have been identified named SV2A, SV2B and SV2C (Bajjalieh *et al*., 1992, 1993, 1994). The SV2 protein contains highly conserved 12 transmember regions and may function as a transportation protein for neural transmitter (Bajjalieh *et al*., 1994). Besides, other studies suggested that SV2 may act as a matrix protein involved in construction of synaptic vesicles (El Far and Betz, 2002). Although SVOP and SV2 have the same transmembrane topology and both localize to synaptic vesicles, it can not be glycosylazed as SV2, which suggests that the function of SVOP is distinct from SV2.

In summary, we have cloned and characterized a human homologue of SVOP. Sequence analysis shows that this protein is highly related to SV2 protein and evolutionary conserved from *C. elegans*, fruit fly to mouse. Northern blotting indicated hSVOP expresses specifically in adult brain. The EGFP-N1-hSVOP fusion protein demonstrated that hSVOP is located on cytoplasmic membrane and may be involved in material transfer between the synaptic vesicle and cytoplasm. From these results, we can draw the conclusion that hSVOP probably functions as a sugar or ion transporter in synaptic vesicles and play an important role in adult brain.

Acknowledgements

We are grateful to all members of the Center for Heart Development, College of Life Sciences in Hunan Normal University for their excellent technical assistance and encouragement. This study was

supported in part by the National Natural Science Foundation of China (No. 90508004, 30470867, 30270722, 30570934, 30571048, 30570265), PCSIRT of Education Ministry of China (IRT0445), National Basic Research Program of China, 2005 CB522505 and the Foundation of Hunan Province (No. 04FJ2006).

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