

Short Sequence-Paper

Molecular cloning of human brain glutamate/aspartate transporter II

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Abstract

Glutamate transporters are membrane-bound proteins which are localized in glial cells and/or pre-synaptic glutamatergic nerve endings and are essential for the removal and termination of action of synaptic glutamate. Several cDNAs encoding glutamate transporters have been isolated from mammalian tissues, including human cerebellum. Here, we screened cDNA libraries derived from human brain stem and cerebellum, and isolated a novel cDNA that encodes for a glutamate transporter. This cDNA predicts a protein which contains 565 amino acids and is homologous to a rat brain Na⁺-dependent glutamate/aspartate transporter. The new cDNA is expressed in brain and is structurally distinct from the previously reported human glutamate transporter cDNA.

Key words: Glutamate transporter; cDNA; Human brain stem; Cloning; Sequence

Glutamate is known to serve as an excitatory neurotransmitter in mammalian CNS and has been implicated in neurodegenerative processes. Glutamate transport proteins are essential for the removal of synaptic glutamate via a high-affinity uptake process [1,2]. This process is shown to be altered in ischemia and some neuronal degenerations linked to excitotoxicity [3,4].

Because glutamate uptake depends on the co-transport of Na⁺ and counter-transport of K⁺, these proteins are called Na⁺/K⁺-dependent transporters [2]. Recent molecular biological studies led to the isolation of three distinct glutamate transporter cDNAs derived from rat brain [5,6] and rabbit small intestine [7]. We have also isolated a cDNA derived from human cerebellum which encodes for a glutamate transporter [8] and which is homologous to the rat transporter described by Storck et al. [5]. Since the human glutamate transporters are expected to exhibit multiplicity at the molecular level, we searched for additional carriers by screening a cDNA library constructed from human brain stem mRNA. A novel human cDNA, structurally

distinct from the previously reported human cerebellar transporter, was isolated and characterized. The new human cDNA is expressed in brain and is homologous to another rat brain glutamate/aspartate transporter described by Pines et al. [6]. The novel human cDNA may prove useful in understanding brain glutamatergic mechanisms and in the study of neurodegenerative disorders linked to glutamatergic dysfunction.

A λ Zap cDNA library derived from human brain stem (Stratagene, CA) was screened using a RT-PCR-generated DNA probe corresponding to the coding region of a rat brain glutamate/aspartate transporter [6]. To synthesize this probe, total RNA was isolated from rat brain by the CsCl method [9] and was reverse transcribed (RT) using a Gibco-BRL Superscript preamplification kit. Synthesized DNA was then amplified by the polymerase chain reaction (PCR) using *taq* DNA polymerase (Perkin Elmer) and oligonucleotide primers (5'TCGCCACTGTCTCCAGACGTG3'; 5'GAATTGGCTGAGAATCGGTC3') corresponding to the rat glutamate transporter [6]. Amplification was carried out as follows: denaturation at 94°C for 1 min; annealing at 55°C for 1 min; and extension at 72°C for 2 min, 30 cycles. Library screening was carried out using standard protocols [9]. Hybridizations were performed at 42°C in 50% formamide/6 × SSC and the final wash was done at 65°C in 0.1 × SSC. Sequencing

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The sequence data reported in this paper have been submitted to the GenBank/EMBL/DBDB under the accession number U01824.

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-178 //////////////////////////////////////////////////
CAGCTGCCGACTCCGCTGGACCTGGACCCCGACAGCTGGGAGGATGGGGGTGACCTAGTGTCTTCAGTAG

-106 //////////////////////////////////////////////////CATCA C GG
GGCAATCTCTGGACATCTTTATCTCCCCAGTACCTCTCCAGATCCTGAAGCCTGGGGCCAGGAGGAGGTTAGATGTGACAGTGG-----AAGGACAGTGGCCAACAAT

1 ATG CCC AAG CAG GTG GAA GTG CGA ATG CAC GAC AGT C C C A C CAG GAA CCC AAG CAC CGG CAC CTG GGC CTG CGC CTG 28
Met Pro Lys Gln Val Glu Val Arg Met His Asp Ser His Leu Gly Ser Glu Glu Pro Lys His Arg His Leu Gly Leu Arg Leu Met

85 TGT GAC AAG CTG GGG AAG AAT CTG CTG CTC ACC CTG CAG GTG TTT GGT GTC ATC CTG GGA TCA GTG TGT GGA GGG CTT CTT CGC 56
Cys Asp Lys Leu Gly Lys Asn Leu Leu Thr Ser Thr Val Phe Gly Val Ile Leu Gly Ser Val Cys Gly Gly Leu Leu Arg

169 TTG GCA TCT CCC ATC CAC CCT GAT GTG GTT ATG TTA ATA GCC TTC CCA GGG GAT ATA CTC ATG AGG ATG CTA AAA ATG CTC ATT 84
Leu Ala Ser Pro Ile His Pro Asp Val Val Met Leu Ile Ala Phe Pro Gly Asp Ile Leu Met Arg Met Leu Lys Met Leu Ile

253 CTC CCT CTA ATC ATC TCC AGC TTA ATC ACA GGG CTG TCA GGC CTG GAT GCT AAG C C T AGT GGC CGC TTG GGC ACG AGA GCC ATG 112
Leu Pro Leu Ile Ile Ser Ser Leu Ile Thr Gly Leu Ser Gly Leu Asp Ala Lys Ala Ser Gly Arg Leu Gly Thr Arg Ala Met

337 GTG TAT TAC ATG TCC ACG ACC ATC ATT GCT GCA GTA CTG GGG GTC ATT CTG GTC TTG GCT ATC CAT CCA GGC AAT CCC AAG CTC 140
Val Tyr Tyr Met Ser Thr Thr Ile Ile Ala Ala Val Leu Gly Val Ile Leu Val Leu Ala Ile His Pro Gly Asn Pro Lys Leu

421 AAG AAG CAG CTG GGG CCT GGG AAG AAG AAT GAT GAA GTG TCC AGC CTG GAT GCC TTC CTG GAC CTT ATT CGA AAT CTC TTC CCT 168
Lys Lys Gln Leu Gly Pro Gly Lys Lys Asn Asp Glu Val Ser Ser Leu Asp Ala Phe Leu Asp Leu Ile Arg Asn Leu Phe Pro

505 GAA AAC CTT GTC CAA GCC TGC TTT CAA CAG ATT CAA ACA GTG ACG AAG AAA GTC CTG GTT GCA CCA CCG CCG GAG GAG GGC 196
Glu Asn Leu Val Gln Ala Cys Phe Gln Gln Ile Gln Thr Val Thr Lys Lys Val Leu Val Ala Pro Pro Pro Asp Glu Glu Ala

589 AAC GCA ACC AGC GCT GTT GTC TCT CTG TTG AAC GAG ACT GTC ACT GAG GTC CCG GAG GAG ACT AAG ATG GTT ATC AAG AAG GGC 224
Asn Ala Thr Ser Ala Val Val Ser Leu Leu Asn Glu Thr Val Thr Glu Val Pro Glu Glu Thr Lys Met Val Ile Lys Lys Gly

673 CTG GAG TTC AAG GAT GGG ATG AAC GTC TTA GGT CTG ATA GGG TTT TCT ATT GCT TTT GGC ATC GCT ATG GGG AAG ATG GGA GAT 252
Leu Glu Phe Lys Asp Gly Met Asn Val Leu Gly Leu Ile Gly Phe Phe Ile Ala Phe Gly Ile Ala Met Gly Lys Met Gly Val

757 -CAG GCC AAG CTG ATG GTG GAT TTC TTC AAC ATT TTG AAT GAG ATT GTA ATG AAG TTA GTG ATC ATG ATC ATG TGG TAC TCT CCC 280
Gln Ala Lys Leu Met Val Asp Phe Phe Asn Ile Leu Asn Glu Ile Val Met Lys Leu Val Ile Met Ile Met Trp Tyr Ser Pro

841 CTG GGT ATC GCC TGC CTG ATC TGT GGA AAG ATC ATT GCA ATC AAG GAC TTA GAA GTG GTT GCT AGG CAA CTG GGG ATG TAC ATG 308
Leu Gly Ile Ala Cys Leu Ile Cys Gly Lys Ile Ile Ala Ile Lys Asp Leu Glu Val Val Ala Arg Gln Leu Gly Met Tyr Met

925 GTA ACA GTG ATC ATA GGC CTC ATC ATC CAC GGG GGC ATC TTT CTC CCC TTG ATT TAC TTT GTA GTG ACC AGG AAA AAC CCC TTC 336
Val Thr Val Ile Ile Gly Leu Ile Ile His Gly Gly Ile Phe Leu Pro Leu Ile Tyr Phe Val Val Thr Arg Lys Asn Pro Phe

1009 TCC TTT TTT GCT GGC ATT TTC CAA GCT TGG ATC ACT GCC CTG GGC ACC GCT TCC AGT GCT GGA ACT TTG CCT GTC ACC TTT CGT 364
Ser Phe Phe Ala Gly Ile Phe Gln Ala Trp Ile Thr Ala Leu Gly Thr Ala Ser Ser Ala Gly Thr Leu Pro Val Thr Phe Arg

1093 TGC CTG GAA GAA AAT CTG GGG ATT GAT AAG CGT GTG ACT AGA TTC GTC CTT CCT GGT GGA GCA ACC ATT AAC ATG GAT GGT ACA 392
Cys Leu Glu Glu Asn Leu Gly Ile Asp Lys Arg Val Thr Arg Phe Val Leu Pro Val Gly Ala Thr Ile Asn Met Asp Gly Thr

1177 GCC CTT TAT GAA GCG GTA GCC ATC CAC GGC ATC ATA GCC CAA ATG AAT GGT GTT GTC CTG GAT GGA GGA CAG ATT GTG ACT GTA AGC 420
Ala Leu Tyr Glu Ala Val Ala Ala Ile Phe Ile Ala Gln Met Asn Gly Val Val Leu Asp Gly Gly Gln Ile Val Thr Val Ser

1261 CTC ACA GCC ACC CTG GCA AGC GTC GGC GCG GCC AGT ATC CCC AGT GCC GGG CTG GTC ACC ATG CTC CTC ATT CTG ACA GCC GTG 448
Leu Thr Ala Thr Leu Ala Ser Val Gly Ala Ala Ser Ile Pro Ser Ala Gly Leu Val Thr Met Leu Leu Ile Leu Thr Ala Val

1345 GGC CTG CCA ACA GAG GAC ATC AGC CTG CTG GTG GCT GTG GAC TGG CTG CTG GAC T AGG ATG AGA ACT TCA GTC AAT GTT GTG GGT 476
Gly Leu Pro Thr Glu Asp Ile Ser Leu Leu Val Ala Val Asp Trp Leu Leu Asp Arg Met Arg Thr Ser Val Asn Val Val Gly

1429 GAC TCT TTT GGG GCT GGG ATA GTC TAT CAC CTC TCC AAG TCT GAG CTG GAT ACC ATT GAC TCC CAG CAT CGA GTG CAT GAA GAT 504
Asp Ser Phe Gly Ala Gly Ile Val Tyr His Leu Ser Lys Ser Glu Leu Asp Thr Ile Asp Ser Gln His Arg Val His Glu Asp

1513 ATT GAA ATG ACC AAG ACT CAA TCC ATT TAT GAT GAC ATG AAG AAC CAC AGG GAA AGC AAC TCT AAT CAA TGT GTC TAT GCT GCA 532
Ile Glu Met Thr Lys Thr Gln Ser Val Tyr Asp Asp Met Lys Asn His Arg Glu Ser Asn Ser Asn Gln Cys Val Tyr Ala Ala

1597 CAC AAC TCT GTC ATA GTA GAT GAA TGC AAG GTA ACT CTG GCA GCC AAT GGA AAG TCA GCC GAC TGC AGT GTT GAG GAA GAA CCT 560
His Asn Ser Val Ile Val Asp Glu Cys Lys Val Thr Leu Ala Ala Asn Gly Lys Ser Ala Asp Cys Ser Val Glu Glu Glu Pro

1681 TGG AAA CGT GAG AAA TAA GGATATGAGTCTCAGCAAACTCTTGAATAAACTCCCGAGCGTATCTATGGTAACTGATGATATAAACAAGCTTTCTTAAAAA 565
Trp Lys Arg Glu Lys *

1786 AAAAAA

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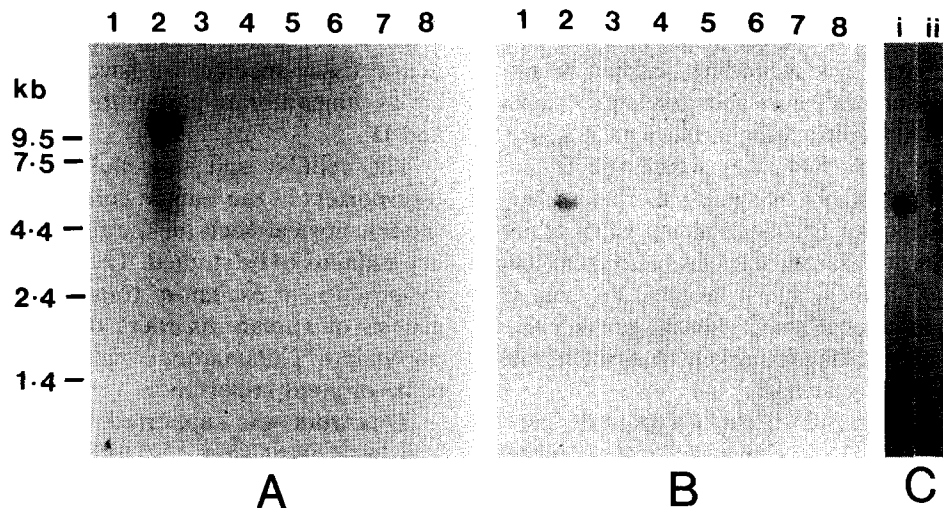


Fig. 2. Northern blot analysis of human tissue RNA. (A) Expression of human brain glutamate transporter II in human heart (lane 1), brain (lane 2), placenta (lane 3), lung (lane 4), liver (lane 5), skeletal muscle (lane 6), kidney (lane 7), and pancreas (lane 8). Poly(A)⁺ RNA (2 μ g), isolated from the above tissues, was hybridized with the cloned cDNA as a probe and exposed to the film for 1 day. No detection of positive signal was found for tissues other than the brain even after the blot had been exposed to the film for up to 10 days. (B) Expression of human brain glutamate transporter I in human tissues. Poly A⁺ RNA from the tissues described in B was probed with the previously described human glutamate transporter I [8]. For this, the blot from A was regenerated and hybridized with the transporter I cDNA. This blot was exposed to the film for 7 days. (C) Expression of human brain glutamate transporters in retinoblastoma cell lines. Total RNA (20 μ g) from human retinoblastoma cells was run in duplicates in an agarose gel, blotted and hybridized either with the cloned cDNA (human brain glutamate transporter II) (ii) or with the previously described human brain glutamate transporter I (i) [8]. These blots were exposed to the film for 5 days at -80°C . All hybridizations were done at 42°C (50% formamide, $5 \times$ SSPE, $5 \times$ Denhardt's solution, 2% SDS and 200 $\mu\text{g}/\text{ml}$ denatured salmon sperm DNA) over night. The stringency of the final wash conditions was 65°C in $0.1 \times$ SSC containing 0.1% SDS.

of the DNA was carried out on an Automated DNA Sequencer (Applied Biosystems, Model 373A) as well as manually by the dideoxy chain termination method [10] using the Sequenase kit (Version 2.0, US Biochemicals). For sequence confirmation, both strands of the DNA were sequenced. Analysis of the sequence data was done using Genetics Computer Group software.

The expression of the new human cDNA was studied by Northern blot analysis of various human tissues. Total RNA or poly(A)⁺ RNA (Clontech) was isolated from different tissues and run on an agarose gel (1.2%) containing formaldehyde according to standard techniques [9]. Hybridizations were done at 42°C (50% formamide, $5 \times$ SSPE, $5 \times$ Denhardt's solution, 2% SDS and 200 $\mu\text{g}/\text{ml}$ denatured salmon sperm DNA) overnight using the cloned cDNA (labeled with ^{32}P) as a probe. The stringency of the final wash conditions was 65°C in $0.1 \times$ SSC containing 0.1% SDS. For comparison, total RNA and poly(A)⁺ RNA blots, obtained

as above, were also hybridized with the previously described human brain glutamate transporter cDNA [8] using identical conditions.

RT-PCR generated a 829 bp probe corresponding to the coding region of the rat glutamate transporter described by Pines et al. [6]. Screening of the human brain stem library led to the isolation of several positive clones. Three were sequenced and specified the same cDNA (Fig. 1) showing an 88% nucleotide sequence identity with the rat aspartate/glutamate transporter described by Pines et al. [6]. In addition, using the cloned cDNA as a probe, we screened another cDNA library derived human cerebellum [8]. This led to the isolation of several positive clones two of which were sequenced and were also found to specify the same cDNA. Due to a 5-nucleotide deletion at the 5'UTR, as compared to the rat brain cDNA [6], which puts the predicted N-terminal methionine of the rat transporter out of frame, the first in-frame methionine (Met-1) of the human transporter corresponds to Met-

Fig. 1. Nucleotide and deduced amino acid sequence of the human glutamate transporter cDNA. Nucleotides corresponding to the rat glutamate transporter [6] are shown in italics directly above the human nucleotide sequence only where they are different. Also, predicted amino acids corresponding to the rat cDNA [6] are shown in italics directly below the human amino acid sequence only where they are different. Insertions are marked by relevant bases and deletions by dashes. The nucleotide number is indicated on the left and the amino acid number at the right of the sequences. Positive nucleotide numbering begins at the putative translation initiation codon ATG. The stop codon is indicated with an asterisk. Underlined are putative transmembrane regions; the potential N-glycosylation sites are overlined. Non-homologous regions are indicated by vertical lines.

10 of the rat protein (Fig. 1). Also, compared to the rat cDNA, a single base deletion after position 756 of the human transporter (the corresponding position in rat cDNA is 779) introduces a frame shift for amino acids 253–281 (Fig. 1). This shift is eliminated with a single base addition at position 844 after which the frame reading of the human cDNA is similar to that of the rat glutamate transporter [6]. Sequencing of 5 additional cDNA clones of different sizes, isolated from the brain stem library (3 clones) and from a cDNA library derived from human cerebellum (2 clones), gave identical sequencing results making it unlikely that this frame shift was due to a cloning artifact.

The amino acid sequence of the polypeptide predicted by the human cDNA shows an 89% identity with that derived from the rat brain cDNA [6]. Omitting the stretch of 28 discordant amino acids which results from the above frame shift raises the amino acid sequence identity to greater than 95%. In contrast, the homology of the new human brain stem cDNA to the previously reported human glutamate transporter derived from cerebellum [8] was low (66%), thus indicating the diverse genetic origin of the two human transporters. This was also true for the glutamate transporters described by Storck et al. [5] and Kanai and Hediger [7].

The protein predicted by the new human cDNA shows nine putative transmembrane regions and two N-glycosylation consensus motifs (Fig. 1). The region of these motifs is conserved in both human glutamate transporters and is probably used for N-glycosylation.

Northern blot analysis of human tissues revealed that the cloned cDNA is expressed in brain and in retinoblastoma cell lines (Fig. 2). No message was detected in heart, liver, lung, kidney, pancreas, placenta or skeletal muscle (Fig. 2). Also, human ovary and testis gave a non-detectable message (data not shown). The size of the new glutamate transporter mRNA (11 kb) was considerably larger than that of the previously described human glutamate transporter [8]

(Fig. 2). Since the two human glutamate transporters isolated thus far are expressed in brain and may be neural tissue-specific, we have designated these carriers as 'human brain glutamate/aspartate transporter I and II'.

The cellular and subcellular localization of these transporters in the human central nervous system, and the teleological need for the multiplicity of these carriers, remains to be studied. The role of these high-affinity proteins in excitatory transmission and the pathogenesis of human disorders that have been linked to presynaptic glutamatergic malfunction [3,4] may prove to be of great importance.

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