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Rapid report

Primary structure, functional characteristics and tissue expression pattern of human ATA2, a subtype of amino acid transport system A

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Abstract

We report here on the primary structure and functional characteristics of the protein responsible for the system A amino acid transport activity that is known to be expressed in most human tissues. This transporter, designated ATA2 for amino acid transporter A2, was cloned from the human hepatoma cell line HepG2. Human ATA2 (hATA2) consists of 506 amino acids and exhibits a high degree of homology to rat ATA2. hATA2-specific mRNA is ubiquitously expressed in human tissues. When expressed in mammalian cells, hATA2 mediates Na^+-dependent transport of α-(methylamino)isobutyric acid, a specific model substrate for system A. The transporter is specific for neutral amino acids. It is pH-sensitive and Li^+-intolerant. The Na^+:amino acid stoichiometry is 1:1.

Keywords: Amino acid transport; System A; Primary structure; Stoichiometry; Substrate specificity; Human

System A was one of the first amino acid transport systems to be described in mammalian cells [1,2]. The expression of transport system is widespread in mammalian tissues [3–5]. The unique characteristics of system A include the absolute requirement for Na^+ for transport activity, preference for short-chain neutral amino acids as substrates, stimulation of transport activity by alkaline pH of the extracellular medium and inhibition of transport activity by system A substrates on the *trans* side. α-(Methylamino)isobutyric acid (MeAIB) is considered the model substrate for system A. Na^+-dependent transport of MeAIB is usually taken as the functional activity mediated specifically by system A. An important feature of system A is its regulatory characteristics [6–8]. A variety of hormones, growth factors and mitogens are known to modulate the expression of this transporter in different organs including liver, adipose tissue, and skeletal muscle. In addition, physiological conditions such as starvation and pregnancy and pathological conditions such as diabetes have been shown to be associated with alterations in the transport activity of system A in different organs. Recently, we reported on the cloning of the protein from rat skeletal muscle that is responsible for system A activity in most tissues [9]. This protein, designated ATA2 for amino acid transporter A2, bears significant homology to the recently cloned rat glutamine transporter (GlnT) [10]. Since GlnT is capable of mediating Na^+-dependent transport of the sys-

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tem A-specific model substrate MeAIB, this transporter must be a subtype of system A. Therefore, we identify GlnT as ATA1 in this paper. Another transporter that shows significant similarity in amino acid sequence to ATA1 and ATA2 is the amino acid transport system N (SN1) [11]. These three amino acid transporters constitute the members of a gene family that is distinct from the previously known amino acid transporter gene families [12–14]. To date, ATA1, ATA2 and SN1 have been cloned only from rat or mouse tissues [9–11,15]. There have been no reports on the cloning of the human homolog of any of these three amino acid transporters. Here we report on the cloning of human ATA2, the first human homolog to be characterized at the structural and functional level that belongs to this most recently identified amino acid transporter gene family. We cloned this transporter (human ATA2) from a HepG2 human hepatoma cell line cDNA library. The cloned transporter is able to mediate the Na^+-dependent transport of MeAIB. The transport function is pH-sensitive, Li^+-intolerant, and is specific for short-chain neutral amino acids.

[^{14}C]MeAIB (specific radioactivity, 50 mCi/mmol) was purchased from American Radiolabeled Chemicals (St. Louis, MO, USA). Human retinal pigment epithelial (HRPE) cells were originally provided by Dr. Del Monte (University of Michigan, Ann Arbor, MI, USA) and have been in use in our laboratory for several years for heterologous expression of a variety of cloned transporters [16–18]. Cell culture media, TRIzol reagent, oligo(dT)-cellulose, and Lipofectin were from Life Technologies (Gaithersburg, MD, USA). Restriction enzymes were either from Promega or from New England Biolabs. Magna nylon transfer membranes used in the library screening were from Micron Separations, Inc. (Westboro, MA, USA).

The SuperScript plasmid system (Life Technologies, Rockville, MD, USA) was used to establish a unidirectional cDNA library with poly(A)$^+$ mRNA isolated from HepG2 cells (a human hepatoma cell line). Poly(A)$^+$ mRNA was prepared by subjecting total RNA twice to oligo(dT)-cellulose affinity chromatography prior to use in library construction. The cDNA products with sizes greater than 1 kb were separated by size fractionation and used for ligation at *Sal*I/*Not*I site in pSPORT1 vector.

A cDNA fragment of the recently cloned rat ATA2 [9] was used as the probe in the screening of the HepG2 cDNA library. HepG2 cDNA library was screened under low stringency conditions as described previously [16–18] using the rat ATA2-specific cDNA fragment as the probe. Positive clones were identified, and the colonies were purified by secondary screening. Both sense and antisense strands of the cDNA were sequenced using an automated Perkin-Elmer Applied Biosystems 377 Prism DNA sequencer. The sequence was analyzed using the GCG sequence analysis software package GCG version 10 (Genetics Computer Group, Inc., Madison, WI, USA).

The functional expression of the cloned human ATA2 cDNA was done in HRPE cells using the vaccinia virus expression system as described previously [16–18]. Transport measurements were made at 37°C for 15 min with [^{14}C]MeAIB as the substrate. The transport buffer was 25 mM Tris/HEPES (pH 8.0), containing 140 mM NaCl, 5.4 mM KCl, 1.8 mM $CaCl_2$, 0.8 mM $MgSO_4$, and 5 mM glucose. Endogenous transport was always determined in parallel using cells transfected with vector alone. This transport accounted for $< 10\%$ of the transport measured in cells that were transfected with the cDNA. cDNA-specific transport was calculated by adjusting for the endogenous activity. The kinetic parameters, Michaelis–Menten constant (K_t) and maximal velocity (V_{max}), were calculated by fitting the cDNA-specific transport data to the Michaelis–Menten equation describing a single saturable transport system. Analysis was done by non-linear regression and the resultant values for the kinetic parameters were confirmed by linear regression. Na^+ activation kinetics were analyzed by fitting the cDNA-specific transport data to the Hill equation and the Hill coefficient was calculated by non-linear regression as well as by linear regression.

A commercially available human multiple tissue blot (Origene, Rockville, MD, USA) was used to determine the tissue expression pattern of the cloned transporter. The blot was hybridized under high stringency conditions with the cloned cDNA as the probe.

Screening of the HepG2 cDNA library with the rat ATA2-specific cDNA probe resulted in the identification of a clone that was able to induce Na^+-depen-

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hATA2     1 MKKAEMGRFSISPDEDSSSYSSNSDFNYSYPTKQAALKSHYADVDPENQNFLLES
rATA2     1 MKKTEMGRFNISPDEDSSSYSSNGDFNYSYPTKQAALKSHYVDVDPENQNFLLES

hATA2    56 NLGKKKYETEFHPGTTSFGMSVFNLSNAIVGSGILGLSYAMANTGIALFIILLTF
rATA2    56 NLGKKKYETDFHPGTTSFGMSVFNLSNAIVGSGILGLSYAMANTGIALFIILLTF

hATA2   111 VSIFSLYSVHLLLKTANEGGSLLYEQLGYKAFGLVGKLAASGSITMQNIGAMSSY
rATA2   111 VSIFSLYSVHLLLKTANEGGSLLYEQLGHKAYGLAGKLAASGSITMQNIGAMSSY

hATA2   166 LFIVKYELPLVIQALTNIEDKTGLWYLNGNYLVLLVSLVVILPLSLFRNLGYLGY
rATA2   166 LFIVKYELPLVIKALMNIEDTNGLWYLNGDYLVLLVSFVLILPLSLLRNLGYLGY

hATA2   221 TSGLSLLCMVFFLIVVICKKFQVPCPVEAALIINETINTTLTQPTALVPALSHNV
rATA2   221 TSGLSLLCMIFFLIVVICKKFQIPCPVEVALMANETVNGTFTQ--VALAALASNS

hATA2   276 TENDSCRPHYFIFNSQTVYAVPILIFSFVCHPAVLPIYEELKDRSRRRMMNVSKI
rATA2   274 TAADTCRPRYFIFNSQTVYAVPILTFSFVCHPAVLPIYEELKSRSRRRMMNVSKI

hATA2   331 SFFAMFLMYLLAALFGYLTFYEHVESELLHTYSSILGTDILLLIVRLAVLMAVTL
rATA2   329 SFFAMFLMYLLAALFGYLTFYEHVESELLHTYSAIVGTDILLLVVRLAVLVAVTL

hATA2   386 TVPVVIFPIRSSVTHLLCASKDFSWWRHSLITVSILAFTNLLVIFVPTIRDIFGF
rATA2   384 TVPVVIFPIRSSVTHLLCPTKEFSWFRHSVITVTILAFTNLLVIFVPTIRDIFGF

hATA2   441 IGASAASMLIFILPSAFYIKLVKKEPMKSVQKIGALFFLLSGVLVMTGSMALIVL
rATA2   439 IGASAAAMLIFILPSAFYIKLVKKEPMRSVQKIGALCFLLSGVVVMIGSMGLIVL

hATA2   496 DWVHNAPGGGH
rATA2   494 DWVHDASAGGH
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Fig. 1. Primary structure of human ATA2. The amino acid sequence of human ATA2 (hATA2) is compared with that of rat ATA2 (rATA2). The regions of sequence conservation between the two proteins are boxed.

dent transport of MeAIB in HRPE cells (see below). Human ATA2 cDNA is 4479 bp long (GenBank accession number AF259799) and encodes a protein of 506 amino acids (Fig. 1). At the level of amino acid sequence, human ATA2 exhibits a high degree of homology to rat ATA2 (88% identity; 93% similarity). Rat ATA2 consists of 504 amino acids, two amino acids shorter than human ATA2. The extra two amino acids in human ATA2 are located in the sixth transmembrane domain. Except for this subtle difference, the predicted topology of human ATA2 is highly comparable to that of rat ATA2. Hydropathy analysis indicates that human as well as rat ATA2 possess 12 putative transmembrane domains.

The tissue distribution pattern of ATA2 mRNA in the human was studied by Northern blot (Fig. 2). All 12 tissues tested (brain, colon, heart, kidney, liver, lung, muscle, placenta, small intestine, spleen, stomach, and testis) were positive for ATA2 mRNA. The size of the hybridization-positive band was 4.5 kb. The hybridization signal was the weakest in colon and small intestine, but was easily detectable upon longer exposure of the autoradiogram. There was a

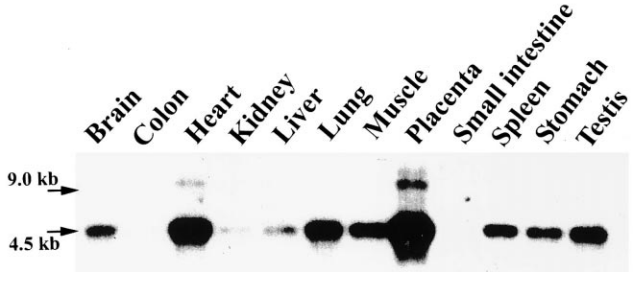

Fig. 2. Northern blot analysis of ATA2-specific mRNA in human tissues.

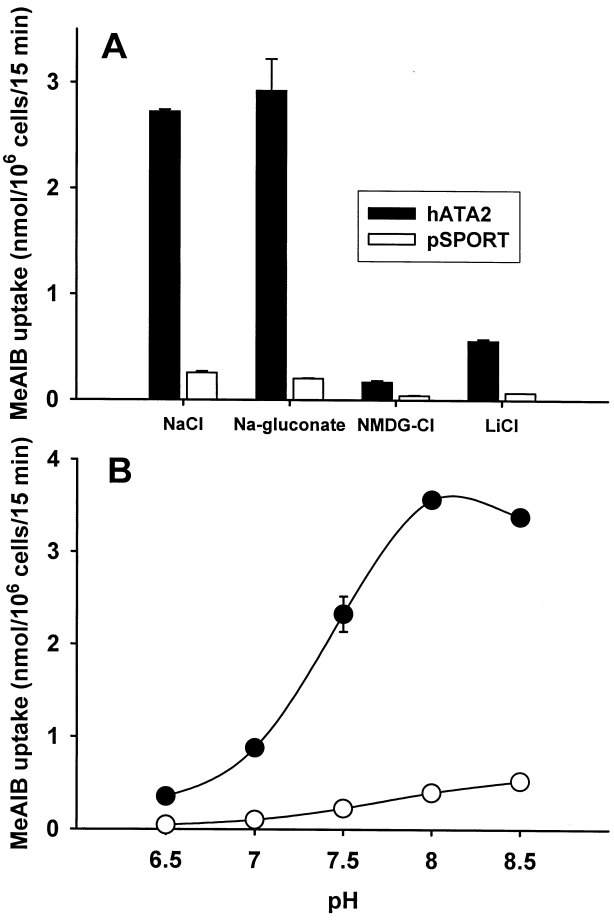

Fig. 3. (A) Ionic requirements of human ATA2 (hATA2). Transport of [^{14}C]MeAIB (16 μM) was measured in vector-transfected cells (open bar) and hATA2 cDNA-transfected cells (solid bar) at 37°C for 15 min in the presence of NaCl, sodium gluconate, NMDG chloride or LiCl under isoosmotic conditions. The pH of the transport buffer was 8.0 in all cases. Values are means ± S.E.M. from three determinations. (B) pH dependence of the transport function of hATA2. Transport of [^{14}C]MeAIB (16 μM) was measured in vector-transfected cells (○) and hATA2 cDNA-transfected cells (●) at 37°C for 15 min in the presence of NaCl. The pH of the transport buffer was varied by appropriately adjusting the concentrations of MES, HEPES, and Tris. Values are means ± S.E.M. from three determinations.

minor hybridization-positive band of 9 kb in size that was expressed primarily in the placenta and heart. This band was the result of a weak cross-hybridization of the ATA2-specific probe with ATA1 mRNA. We have cloned the human homolog of ATA1 from a human placental cDNA library (unpublished data). Northern blot analysis of the same human multiple tissue blot with an ATA1-specific cDNA probe has revealed the presence of a hybridization-positive band of 9 kb in size in placenta and heart. In addition, there was a minor hybridization-positive band of 4.5 kb in size in most tissues. This minor band was the result of a weak cross-hybridization of the ATA1-specific probe with ATA2 mRNA (unpublished data). This cross-hybridization was noted in spite of the use of high stringency conditions for the Northern blot analysis, indicating a high degree of homology between the two mRNA species at the nucleotide level.

Heterologous expression of human ATA2 in HRPE cells using the vaccinia virus expression technique led to the induction of MeAIB transport (Fig. 3A). Transport of MeAIB in the presence of NaCl was 11-fold higher in human ATA2 cDNA-transfected cells than in vector-transfected cells. The cDNA-specific transport was obligatorily dependent on the presence of Na^+ since replacement of Na^+

Table 1
Substrate specificity of human ATA2

Unlabeled amino acid	cDNA-specific [^{14}C]MeAIB transport in HRPE cells (pmol/10^6 cells/15 min)
Control	2631 ± 251 (100)
MeAIB	290 ± 7 (11)
Alanine	291 ± 28 (11)
Glycine	678 ± 124 (26)
Serine	578 ± 68 (22)
Proline	638 ± 60 (24)
Methionine	308 ± 34 (12)
Asparagine	490 ± 62 (19)
Glutamine	503 ± 78 (19)
Threonine	862 ± 73 (33)
Leucine	906 ± 98 (34)
Phenylalanine	1325 ± 203 (50)
Tryptophan	2696 ± 101 (102)
Arginine	3111 ± 428 (118)
Lysine	2429 ± 235 (92)
Aspartic acid	3010 ± 224 (114)
Glutamic acid	2508 ± 222 (95)

HRPE cells were transfected with either pSPORT alone or pSPORT-human ATA2 cDNA. Transport of [^{14}C]MeAIB (16 μM) was measured in these cells at 37°C for 15 min in the presence of NaCl (pH 8.0). When present, the concentration of unlabeled amino acids was 5 mM. cDNA-specific [^{14}C]MeAIB transport was calculated by subtracting the transport in vector-transfected cells from the transport in cDNA-transfected cells. Values in parentheses are percent of control transport measured in the absence of unlabeled amino acids. Values are means ± S.E.M. from four determinations.

with *N*-methyl-D-glucamine almost completely abolished the transport. The transport process was Li^+-intolerant as evidenced from the inability of Li^+ to substitute effectively for Na^+. Replacement of Cl^- with gluconate had no effect on the transport, suggesting that Cl^- ions are not involved in the transport process mediated by human ATA2. The transport function of human ATA2 was markedly pH-sensitive (Fig. 3B). The cDNA-specific transport was barely detectable at pH 6.0, but it increased dramatically when the pH was changed from 6.0 to 8.5 and the peak was at pH 8.0. There was a 10-fold increase in cDNA-specific transport when the pH of the extracellular medium was changed from 6.0 to 8.0. The cDNA-specific transport of MeAIB was saturable (Fig. 4A) and the transport values were found to fit very well to the Michaelis–Menten equation describing a single saturable system. The Michaelis–Menten constant for the transport process was 0.39 ± 0.05 mM. Fig. 4B describes the Na^+ activation kinetics of human ATA2. The relationship between cDNA-specific transport of MeAIB and Na^+ concentration was hyperbolic. When analyzed by fitting the data to the Hill equation, a value of 1.03 ± 0.19 was obtained for the Hill coefficient. The $K_{0.5}$ (i.e., the concentration of Na^+ necessary for half-maximal activation) was 9.6 ± 1.5 mM. These results show that the Na^+:MeAIB stoichiometry for human ATA2 is 1:1.

Competition experiments revealed that human ATA2 was able to recognize several neutral amino acids as substrates (Table 1). Alanine, glycine, serine, proline, methionine, asparagine, glutamine, threonine and leucine were very effective in competing with [^{14}C]MeAIB for the transport process mediated by human ATA2. Phenylalanine was comparatively less effective. Tryptophan, cationic amino acids (arginine and lysine), and anionic amino acids (aspartate and glutamate) were very weak in competing with human ATA2-mediated transport of [^{14}C]MeAIB.

Functionally, human ATA2 exhibits all of the characteristics that are known to be associated with system A in mammalian tissues. This includes the Na^+ dependence, pH sensitivity, Li^+ intolerance, and preference for short-chain neutral amino acids as substrates. There are no notable differences between human ATA2 and rat ATA2 at the functional

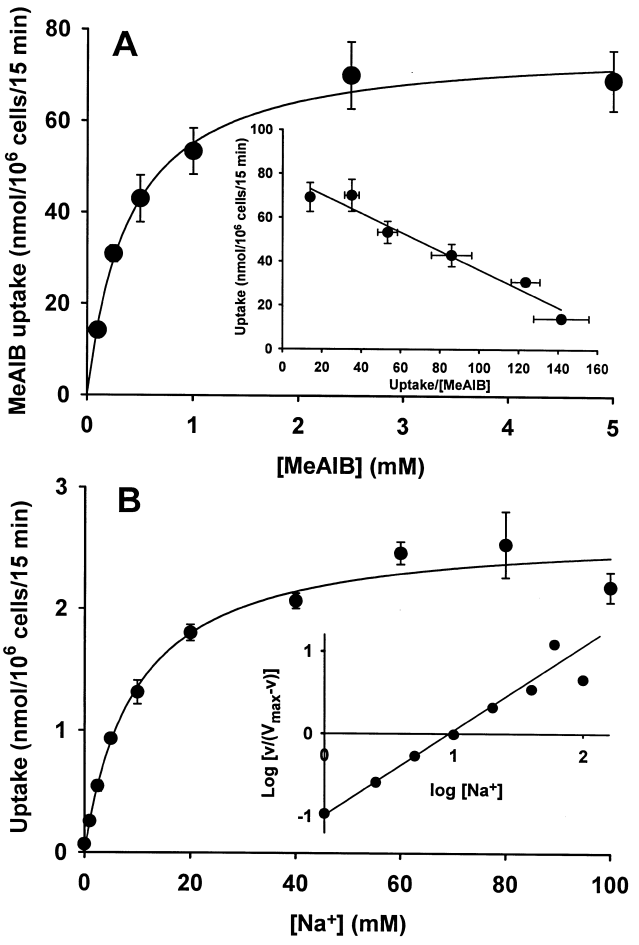

Fig. 4. (A) Saturation kinetics of MeAIB. Transport of MeAIB (0.1–5 mM) was measured in vector-transfected cells and human ATA2 cDNA-transfected cells at 37°C for 15 min in the presence of NaCl (pH 8.0). Values (means ± S.E.M.) represent only the cDNA-specific transport. (Inset) Eadie–Hofstee plot. (B) Na^+ activation kinetics. Transport of [^{14}C]MeAIB (16 μM) was measured in vector-transfected cells and human ATA2 cDNA-transfected cells at 37°C for 15 min. Concentration of Na^+ in the transport buffer (pH 8.0) was varied over the range of 0–100 mM. Values (means ± S.E.M.) represent only the cDNA-specific transport. (Inset) Hill plot.

level. One of the important features of system A is the regulation of its expression in a variety of physiological and pathological conditions. To date, all of these regulatory studies have been carried out primarily at the phenomenological level. Molecular studies of the regulation of this transporter have not been feasible because of the lack of the knowledge on the structure of the protein responsible for the transport function. The successful cloning of the rat and human ATA2 cDNAs ([9], present study) is

likely to lead to the generation of system A-specific cDNA probes and antibodies that can be used in future investigations of the regulation of the expression of the transporter at the level of mRNA and protein.

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