Cloning of an Amino Acid Transporter with Functional Characteristics and Tissue Expression Pattern Identical to That of System A*

Mitsuru Sugawara‡, Takeo Nakanishi‡, You-Jun Fei‡, Wei Huang‡, Malliga E. Ganapathy§, Frederick H. Leibach‡ and Vadivel Ganapathy‡¶

From the Departments of ‡Biochemistry and Molecular Biology and §Medicine, Medical College of Georgia, Augusta, Georgia 30912

*This work was supported by National Institutes of Health grants DA10045 and HD33347

¶Corresponding author
Vadivel Ganapathy, Ph.D.
Department of Biochemistry and Molecular Biology
Medical College of Georgia
Augusta, GA 30912
Tel: 706-721-7652
Fax: 706-721-6608
Email: vganapat@mail.mcg.edu

Running title: Cloning of the amino acid transport system A
Keywords: Amino acid transport
System A
Primary structure
Electrogenicity
Stoichiometry
Substrate specificity
Skeletal muscle

Abbreviations used are: ATA, amino acid transporter A; G1nT, glutamine transporter; SN, system N; rATA, rat ATA; rGlnT, rat GlnT; rSN, rat SN; MeAIB, \(\alpha\)-(methylamino)isobutyric acid; HRPE, human retinal pigment epithelial; kbp, kilobase pair(s)
ABSTRACT

We report here on the cloning and functional characteristics of the protein responsible for the system A amino acid transport activity that is known to be expressed in most mammalian tissues. This transporter, designated ATA2 for amino acid transporter A2, was cloned from rat skeletal muscle. It is distinct from the neuron-specific glutamine transporter (GlnT/ATA1). Rat ATA2 consists of 504 amino acids and bears significant homology to GlnT/ATA1 and system N (SN1). ATA2-specific mRNA is ubiquitously expressed in rat tissues. When expressed in mammalian cells, ATA2 mediates Na\(^{+}\)-dependent transport of \(\alpha\)-(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. When expressed in *Xenopus laevis* oocytes, transport of neutral amino acids via ATA2 is associated with inward currents. The substrate-induced current is Na\(^{+}\)-dependent and pH-sensitive. The amino acid transport system A is particularly known for its adaptive and hormonal regulation and therefore the successful cloning of the protein responsible for this transport activity represents a significant step towards understanding the function and expression of this transporter in various physiological and pathological states.
Amino acid transport system A was originally described in Ehrlich cells (1,2). It was named system A because of its preference for alanine as a substrate. Subsequently it became known that this transport system is expressed ubiquitously in mammalian tissues (3-5). The characteristics of system A include Na\textsuperscript{+}-dependence, preference for short-chain neutral amino acids such as alanine, serine, proline and glutamine as substrates, pH sensitivity and trans-inhibition. Bulky hydrophobic amino acids (e.g., leucine, tryptophan), anionic amino acids (glutamate and aspartate) and cationic amino acids (arginine and lysine) are excluded by the system. System A can be discriminated however from other known transport systems for neutral amino acids by its unique ability to transport N-methylated amino acids (2). In fact, system A is defined as the component of Na\textsuperscript{+}-dependent transport of any neutral amino acid that is inhibitable by the model substrate $\alpha$-(methylamino)isobutyric acid (MeAIB\textsuperscript{1}) (3-5). Alternatively, Na\textsuperscript{+}-dependent transport of MeAIB can be used directly as a measure of system A activity. Another important feature of system A is its regulatory properties (6-8). System A is up-regulated by a variety of hormones, growth factors and mitogens. It is also subject to adaptive regulation mediated by altered levels of intracellular amino acids. The expression of system A in the skeletal muscle and liver and its regulation by glucagon and insulin in these tissues are particularly important with respect to various physiological conditions such as starvation and pregnancy and pathological states such as diabetes.

In recent years, several amino acid transporters have been identified at the molecular level (9-11). However, the molecular identification of the protein responsible for system A activity has been elusive. Most recently, Varoqui et al (12) reported the cloning of a Na\textsuperscript{+}-dependent glutamine transporter (GlnT) from rat brain that is capable of transporting the system A-specific model substrate MeAIB. But, the expression of GlnT is restricted to the brain. GlnT-specific transcripts are not detectable in the skeletal muscle, liver and other tissues. This suggests that GlnT may represent a subtype of system A that is expressed specifically in the brain. The ubiquitously expressed system A
has not yet been cloned. System A activity has been shown to be induced in X. laevis oocytes following microinjection of mRNA from the liver (13,14), but identification of the mRNA responsible for the activity has not been accomplished.

Here we describe the cloning of an amino acid transporter from rat skeletal muscle that is capable of Na\textsuperscript{+}-dependent transport of MeAIB and expressed ubiquitously in rat tissues including skeletal muscle, liver and brain. The cloned transporter is distinct from GlnT. We have characterized the transport function of the cloned transporter in two different heterologous expression systems using mammalian cells and X. laevis oocytes. The transporter is Na\textsuperscript{+}-dependent, pH-sensitive and electrogeneric and prefers short-chain neural amino acids as substrates.

**EXPERIMENTAL PROCEDURES**

*Materials* —[^14C]MeAIB (specific radioactivity, 50 mCi/mmol) was purchased from American Radiolabeled Chemicals (St. Louis, MO). Human retinal pigment epithelial (HRPE) cells were originally provided by Dr. Del Monte (University of Michigan, Ann Arbor, MI) and have been in use in our laboratory for several years for heterologous expression of a variety of cloned transporters (15-17). Cell culture media, TRIzol reagent, oligo(dT)-cellulose, and Lipofectin were from Life Technologies (Gaithersburg, MD). 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).

*Probe Preparation* — A cDNA fragment of the recently cloned rat GlnT (12) was used as the probe in the screening of the rat skeletal muscle cDNA library. The probe was prepared by RT-PCR using poly(A)\textsuperscript{+} mRNA isolated from rat brain. The sense primer was 5'-TGGAGAAAAGGAAAATGCGAC-3' which corresponded to the nucleotide position 406-425 in rGlnT cDNA and the antisense primer was 5'-ACGAAGGAAAAGGATGAAAATGAG-3' which corresponded to the nucleotide position 1512-1533 in rGlnT cDNA. RT-PCR was done using the
GenAmp RNA PCR kit from Perkin-Elmer (Norwalk, CT). The conditions for reverse transcription were as follows: denaturation of the template mRNA for 10 min at 70°C; reverse transcription for 60 min at 42°C. The conditions for PCR amplification were as follows: denaturation for 45 sec at 95°C; annealing for 45 sec at 56°C and extension for 2 min at 72°C; 39 cycles; final extension for 7 min at 72°C. The resultant product (~1.1 kbp) was subcloned in pGEM-T vector and sequenced to establish its molecular identity.

**Construction of cDNA Library** — The SuperScript Plasmid system (Gibco-BRL) was used to establish a unidirectional cDNA library with poly(A)$^+$ mRNA isolated from rat skeletal muscle. 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 kbp were separated by size-fractionation and used for ligation at SalI/NotI site in pSPORT1 vector.

**cDNA Library Screening and DNA sequencing** — The rat skeletal muscle cDNA library was screened under low stringency conditions as described previously (15-17) using the rGlnT-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).

**Functional Expression in HRPE Cells** — This was done using the vaccinia virus expression system as described previously (15-17). 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.

*Functional Expression in X. Laevis Oocytes* — cRNA from the cloned cDNA was synthesized using the mMESSAGE mMACHINE™ kit (Ambion). The plasmid containing the cDNA insert was linearized with NotI, and the cDNA was transcribed *in vitro* using T7 RNA polymerase in the presence of an RNA cap analog. The procedures for oocyte isolation, cRNA injection and current measurements with the two-microelectrode voltage clamp technique have been described in several of our previous publications (18-21).

*Northern Blot* — A commercially available rat multiple tissue blot (Origene, Rockville, MD) 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.

**RESULTS**

*Structural Features of ATA2* — Screening of the rat skeletal muscle cDNA library with a rGlnT-specific cDNA probe resulted in the identification of a clone that was different from rGlnT but was able to induce Na$^+$-dependent transport of MeAIB in mammalian cells and in X. laevis oocytes (see below). To date, this is only the second cloned transporter that is capable of transporting the system A-specific substrate MeAIB. Therefore, we designate the newly cloned transporter ATA2 (amino acid transporter A2), GlnT being ATA1. Rat ATA2 cDNA is 4632 bp long (GenBank
accession no. AF249673) and codes for a protein of 504 amino acids (Fig. 1A). At the level of amino acid sequence, rat ATA2 bears significant homology to rat ATA1 (55% identity; 65% similarity).

The tissue distribution pattern of ATA2 mRNA in the rat was studied by Northern blot (Fig. 1B). All twelve tissues tested (brain, thymus, lung, heart, skeletal muscle, stomach, small intestine, liver, kidney, spleen, testis, and skin) were positive for ATA2 mRNA. In all tissues, two different transcripts were detected with sizes 4.7 kb and 2.5 kb. The larger transcript was more predominant than the smaller transcript.

*Functional Characteristics of rATA2 in HRPE Cells* — Heterologous expression of rATA2 in HRPE cells using the vaccinia virus expression technique led to the induction of MeAIB transport (Table I). Transport of MeAIB in the presence of NaCl was 10-fold higher in rATA2 cDNA-transfected cells than in vector-transfected cells. The cDNA-specific transport was obligatorily dependent on the presence of Na$^+$ since replacement of Na$^+$ 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 only a small effect on the transport, suggesting that Cl$^-$ ions are not involved in the transport process mediated by rATA2.

Competition experiments revealed that rATA2 was able to recognize several neutral amino acids as substrates (Table II). Alanine, methionine, proline, serine, asparagine, glutamine, histidine, glycine, and phenylalanine were very effective in competing with $[^{14}\text{C}]$MeAIB for the transport process mediated by rATA2. Tryptophan was comparatively less effective. Cationic amino acids (arginine and lysine) and anionic amino acids (aspartate and glutamate) were very weak in competing with rATA2-mediated transport of $[^{14}\text{C}]$MeAIB. The transport function of rATA2 was markedly pH-sensitive (Fig. 2A). 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. There was a 28-fold increase in cDNA-specific transport with a
pH change from 6.0 to 7.5 and an additional 1.5-fold increase with a pH change from 7.5 to 8.5. The cDNA-specific transport of MeAIB was saturable (Fig. 2B) 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 $0.23 \pm 0.02$ mM. Fig. 3 describes the Na$^+$-activation kinetics of rATA2. 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 $1.05 \pm 0.15$ was obtained for the Hill coefficient. The $K_{0.5}$ (i.e., the concentration of Na$^+$ necessary for half-maximal activation) was $22 \pm 4$ mM. These results show that the Na$^+$:MeAIB stoichiometry for rATA2 is 1:1.

**Functional Characteristics of rATA2 in X. Laevis Oocytes** — rATA2 was expressed heterologously in X. laevis oocytes to study the electrophysiological characteristics of the transporter. Perifusion of rATA2-expressing oocytes with 5 mM alanine induced marked inward currents in the presence of NaCl at pH 8.0 (Fig. 4A). The magnitude of the currents was unaffected when Cl$^-$ in the buffer was replaced with gluconate, showing that Cl$^-$ does not play any role in the transport process. In contrast, there were no alanine-induced currents when Na$^+$ in the buffer was replaced with NMDG, demonstrating the obligate requirement for Na$^+$ for the transport process. The magnitude of the alanine-induced currents was influenced by pH (Fig. 4B). The currents decreased substantially when the pH of the perifusion buffer was changed from 8.0 to 6.0. The currents were saturable with increasing concentrations of alanine (Fig. 4C). The data were found to fit very well to the Michaelis-Menten equation describing a single saturable system. The $K_{0.5}$ (i.e., concentration of alanine necessary for induction of half-maximal current) was $0.23 \pm 0.02$ mM at a testing membrane potential of −70 mV.

Substrate specificity of rATA2 was studied by assessing the ability of 17 different amino acids (5 mM) to induce currents in rATA2-expressing oocytes in the presence of NaCl at pH 8.0. Alanine induced the maximum current ($73 \pm 13$ nA in 4 different oocytes). The relative currents induced by other amino acids as percent of the alanine-induced current in the same oocyte are given in Table II.
The neutral amino acids glycine, serine, proline, methionine, histidine, asparagine, and glutamine were good substrates for rATA2 with abilities to induce currents in the range of 50-100% of the alanine-induced current. Threonine, leucine, phenylalanine, and tryptophan induced comparatively much less currents (10-20%). Cationic amino acids and anionic amino acids induced very little currents (<10%).

DISCUSSION

We have cloned the amino acid transport protein that is responsible for system A activity known to be expressed in most mammalian tissues, including the skeletal muscle, liver, and brain. This transporter, designated ATA2, is distinct from the recently cloned brain-specific glutamine transporter GlnT (12). Since GlnT was the first cloned amino acid transporter with ability to transport the system A-specific model substrate MeAIB, we identify GlnT as ATA1 in this paper. Functionally, ATA2 behaves similar to ATA1. Both of them are Na\(^+\)-dependent and pH-sensitive. Both of them are able to transport the system A-specific substrate MeAIB. In the present study, we have investigated the transport function of ATA2 in a much greater detail. The most important feature of ATA2 is its electrogenericity. The transport process mediated by ATA2 is associated with the transfer of net positive charge into the cells. The Na\(^+\): amino acid stoichiometry is 1:1. Since only neutral amino acids are recognized as substrates by ATA2, the cotransport of one Na\(^+\) ion with one amino acid molecule provides the molecular basis for the electrogenicity of the transport process. Such information is not available for ATA1. Another significant finding in the present study is the relatively smaller magnitude of currents induced by MeAIB in rATA2-expressing oocytes compared to most neutral amino acids. Alanine induced the maximum currents. MeAIB-induced currents were only 14% of the alanine-induced currents in spite of the fact that the affinities of the transporter for the two amino acids are comparable. This suggests that even though MeAIB is a highly specific model substrate for system A, it is not a good substrate for rATA2, a subtype of system A, in terms of the relative transport rate in comparison with
other naturally occurring neutral amino acid substrates. Whether or not the same is true for ATA1, another subtype of system A, remains to be seen.

The marked pH-dependence of ATA2 is very interesting. The transport activity of ATA2 increases markedly when the extracellular pH is higher than 7.5 and decreases markedly when the extracellular pH is lower than 7.5. ATA1 also exhibits this characteristic (12). Based on the amino acid sequence, ATA1 and ATA2 are related to the recently cloned amino acid transport system N (SN1), a Na\(^+\) and H\(^+\)-coupled glutamine transporter (22). SN1 is also pH-sensitive and the influence of extracellular pH on the transport activity of SN1 is similar to that described in the case of ATA1 and ATA2. But, the basis for the pH influence on SN1 is that this transporter mediates the influx of Na\(^+\) and amino acid (glutamine) into the cells coupled to the efflux of H\(^+\) (22). Thus, H\(^+\) is a transportable coupling ion for SN1 and an outwardly directed H\(^+\) gradient serves as an energy source for the transporter in addition to the inwardly directed Na\(^+\) gradient. Our recent studies have shown that SN1 is electrogenic with a Na\(^+\): amino acid stoichiometry of 2:1. (Y.J., Fei, M. Sugawara, T. Nakanishi, W. Huang, H. Wang, P.D. Prasad, F.H. Leibach, and V. Ganapathy, manuscript submitted for publication).

Taken collectively, the data suggest that SN1 mediates the influx of two Na\(^+\) and one amino acid coupled to the efflux of one H\(^+\). The Na\(^+\): amino acid: H\(^+\) stoichiometry of 2:1:1 corroborates with the electrogenic nature of SN1. In contrast to SN1, the Na\(^+\): amino acid stoichiometry for ATA2 is 1:1. Therefore, even though ATA2 is highly pH-sensitive, it is unlikely that H\(^+\) is a transportable coupling ion for this transporter. This is supported by studies done with system A in rat skeletal muscle (23). These studies have shown that the alterations in system A activity observed with different extracellular pH are actually induced by intracellular pH which changes with extracellular pH. The activity of this transporter is inhibited when the intracellular pH is lowered below physiological pH and is stimulated when the intracellular pH is raised above physiological pH. These findings argue against a role of H\(^+\) as an exchange ion coupled to the influx of Na\(^+\) and amino acid. Available evidence suggests that the
transporter may possess a pH-sensing domain on the cytoplasmic region, most likely a histidine residue, which is responsible for intracellular pH-induced changes in transport function (24).

We have cloned ATA2 from rat skeletal muscle. Northern blot analysis shows that ATA2 mRNA is expressed ubiquitously in rat tissues, in contrast to the brain-specific ATA1. Based on the tissue distribution pattern, it is most likely that ATA2 represents the amino acid transport system A known to be regulated by hormones such as insulin and glucagon in the skeletal muscle and liver. The present studies on the molecular identification of this transporter are very important for future investigations of the mechanisms of the hormonal control of the expression and activity of the transporter.

Acknowledgements — The authors thank Vickie Mitchell for expert secretarial assistance.
REFERENCES

1. Oxender, D.L., and Christensen, H.N. (1963) J. Biol. Chem. 238, 3686-3699

2. Christensen, H.N., Oxender, D.L., Liang, M., and Vatz, K.A. (1965) J. Biol. Chem. 240, 3609-3616

3. Christensen, H.N. (1985) J. Membr. Biol. 84, 97-103

4. Christensen, H.N. (1989) Meth. Enzymol. 173, 576-616

5. Christensen, H.N. (1990) Physiol. Rev. 70, 43-77

6. Shotwell, M.A., Kilberg, M.S., and Oxender, D.L. (1983) Biochim. Biophys. Acta 737, 267-284

7. Haussinger, D., Lang, F., and Kilberg, M.S. (1992) In: Mammalian Amino Acid Transport. Mechanisms and Control (eds., Kilberg, M.S., and Haussinger, D.), pp.113-130. Plenum Press, New York

8. Kilberg, M.S., Bracy, D.S., and Handlogten, M.E. (1986) Fed. Proc. 45, 2438-2440

9. Malandro, M.S., and Kilberg, M.S. (1996) Annu. Rev. Biochem. 65, 305-336

10. Palacin, M., Estevez, R., Bertran, J., and Zorzano, A. (1998) Physiol. Rev. 78, 969-1054

11. Ganapathy, V., and Leibach, F.H. (1999) In: Textbook of Gastroenterology (ed. Yamada, T.), pp. 456-467. Lippincott Williams & Wilkins: Philadelphia

12. Varoqui, H., Zhu, H., Yao, D., Ming, H., and Erickson, J.D. (2000) J. Biol. Chem. 275, 4049-4054

13. Palacin, M., Werner, A., Dittmer, J., Murer, H., and Biber, J. (1990) Biochem. J. 270, 189-195

14. Tarnuzzer, R.W., Campa, M.J., Qian, N.X., Englesberg, E., and Kilberg, M.S. (1990) J. Biol. Chem. 265, 13914-13917

15. Wu, X., Kekuda, R., Huang, W., Fei, Y.J., Leibach, F.H., Chen, J., Conway, S.J., and Ganapathy, V. (1998) J. Biol. Chem. 273, 32776-32786
16. Rajan, D.P., Kekuda, R., Huang, W., Wang, H., Devoe, L.D., Leibach, F.H., Prasad, P.D., and Ganapathy, V. (1999) J. Biol. Chem. 274, 29005-29010

17. Wu, X., Huang, W., Prasad, P.D., Seth, P., Rajan, D.P., Leibach, F.H., Chen, J., Conway, S.J., and Ganapathy, V. (1999) J. Pharmcol. Exp. Ther. 290, 1482-1492

18. Mackenzie, B., Loo, D.D.F., Fei, Y.J., Liu, W., Ganapathy, V., Leibach, F.H., and Wright, E.M. (1996) J. Biol. Chem. 271, 5430-5437

19. Ganapathy, M.E., Prasad, P.D., Mackenzie, B., Ganapathy, V., Leibach, F.H. (1997) Biochim. Biophys. Acta 1324, 296-308

20. Wang, H., Huang, W., Fei, Y.J., Xia, H., Yang-Feng, T.L., Leibach, F.H., Devoe, L.D., Ganapathy, V., and Prasad, P.D. (1999) J. Biol. Chem. 274, 14875-14883

21. Fei, Y.J., Romero, M.F., Krause, M., Liu, J.C., Huang, W., Ganapathy, V., and Leibach, F.H. (2000) J. Biol. Chem. 275, 9563-9571

22. Chaudhry, F.A., Reimer, R.J., Krizaj, D., Barber, D., Storm-Mathisen, J., Copenhagen, D.R., and Edwards, R.H. (1999) Cell 99, 769-780

23. Munoz, P., Guma, A., Camps, M., Furriols, M., Testar, X., Palacin, M., and Zorzano, A. (1992) J. Biol. Chem. 267, 10381-10388

24. Bertran, J., Roca, A., Pola, E., Testar, X., Zorzano, A., and Palacin, M. (1991) J. Biol. Chem. 266, 798-802
HRPE cells were transfected with either pSPORT alone or pSPORT-rATA2 cDNA. Transport of $[^{14}\text{C}]\text{MeAIB}$ (16 µM) was measured in these cells at 37°C for 15 min in the presence of NaCl, Na gluconate, NMDG chloride, LiCl or mannitol under isoosmotic conditions. The pH of the transport buffer was 8.0 in all cases. Values are means ± S.E. from three independent determinations. Values in parenthesis are percent of transport measured in the presence of NaCl.

| Ionic composition  | [14C]MeAIB transport |     |
|--------------------|----------------------|-----|
|                    | pSPORT               | pSPORT-rATA2 cDNA | cDNA-specific |
| NaCl               | 341 ± 4              | 3664 ± 37          | 3323 ± 37 (100) |
| Na gluconate       | 282 ± 15             | 2866 ± 62          | 2584 ± 62 (78) |
| NMDG chloride      | 105 ± 24             | 171 ± 19           | 66 ± 19 (2)    |
| LiCl               | 98 ± 7               | 299 ± 12           | 201 ± 12 (6)   |
| Mannitol           | 62 ± 2               | 185 ± 16           | 124 ± 16 (4)   |

Values are means ± S.E. from three independent determinations. Values in parenthesis are percent of transport measured in the presence of NaCl.
TABLE II

*Substrate specificity of rat ATA2*

HRPE cells were transfected with either pSPORT alone or pSPORT-rATA2 cDNA. Transport of $[^{14}\text{C}]\text{MeAIB}$ (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 $[^{14}\text{C}]\text{MeAIB}$ 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. from four determinations. rATA2 was also expressed in *X. laevis* oocytes by injecting rATA2 cRNA and the currents induced by various amino acids (5 mM) were measured using the two-microelectrode voltage clamp technique. The perifusion medium contained NaCl (pH 8.0). Values are means ± S.E. from three oocytes and represent normalized data (i.e., alanine-induced current in each oocyte was taken as 100% and the currents induced by other amino acids in the same oocyte were calculated as percent of the alanine-induced current). The value for alanine-induced current in three oocytes was 73 ± 13 nA (100%).

| Substrate | Control | rATA2 | rATA2 cRNA |
|-----------|---------|-------|------------|
| Alanine   | 73      | 73    | 100        |
| Aspartate | 24      | 24    | 100        |
| Glutamate | 45      | 45    | 100        |
| GABA      | 32      | 32    | 100        |
| Serine    | 21      | 21    | 100        |
| Cysteine  | 16      | 16    | 100        |
| Glycine   | 9       | 9     | 100        |

Values are means ± S.E. from three determinations.
| Unlabeled amino acid | cDNA-specific [14C]MeAIB Current in transport in HRPE Cells | Current in X. laevis oocytes |
|----------------------|----------------------------------------------------------|----------------------------|
|                      | pmol/10^6 cells/15 min %                                  |                            |
| Control              | 3503 ± 519 (100)                                          |                            |
| Alanine              | 353 ± 53 (10)                                             | 100 ± 16                   |
| MeAIB                | 213 ± 33 (6)                                              | 14 ± 1                     |
| Methionine           | 253 ± 36 (7)                                              | 83 ± 2                     |
| Proline              | 416 ± 56 (12)                                             | 80 ± 6                     |
| Serine               | 477 ± 107 (14)                                            | 97 ± 6                     |
| Asparagine           | 562 ± 112 (16)                                            | 85 ± 4                     |
| Glutamine            | 621 ± 115 (18)                                            | 50 ± 3                     |
| Histidine            | 690 ± 83 (20)                                             | 46 ± 3                     |
| Glycine              | 722 ± 124 (21)                                            | 67 ± 3                     |
| Threonine            | 1292 ± 181 (37)                                           | 22 ± 2                     |
| Phenylalanine        | 1451 ± 239 (41)                                           | 14 ± 3                     |
| Tryptophan           | 2582 ± 435 (74)                                           | 9 ± 3                      |
| Arginine             | 2923 ± 334 (83)                                           | 6 ± 2                      |
| Lysine               | 2932 ± 425 (84)                                           | 10 ± 3                     |
| Aspartic acid        | 2963 ± 375 (85)                                           | 8 ± 1                      |
| Glutamic acid        | 2666 ± 378 (76)                                           | 7 ± 1                      |
FIGURE LEGENDS

FIG. 1. Primary structure of rATA2 (A) and tissue expression pattern of ATA2 mRNA in rat (B). Putative transmembrane domains are boxed.

FIG. 2. (A) pH-Dependence of the transport function of rATA2. Transport of \(^{14}\text{C}\)MeAIB (16 µM) was measured in vector-transfected cells (○) and rATA2 cDNA-transfected cells (●) at 37º 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. from four determinations. (B) Saturation kinetics of MeAIB. Transport of MeAIB (0.05-2 mM) was measured in vector-transfected cells and rATA2 cDNA-transfected cells at 37º C for 15 min in the presence of NaCl (pH 8.0). Values (means ± S.E.) represent only the cDNA-specific transport. *Inset*, Eadie-Hofstee plot.

FIG. 3. Na\(^+\) - activation kinetics. Transport of \(^{14}\text{C}\)MeAIB (16 µM) was measured in vector-transfected cells and rATA2 cDNA-transfected cells at 37º C for 15 min. Concentration of Na\(^+\) in the transport buffer (pH 8.0) was varied over the range of 1-140 mM. Values (means ± S.E.) represent only the cDNA-specific transport. *Inset*, Hill plot.

FIG. 4. Functional characteristics of rATA2 expressed in X. laevis oocytes. (A) Ionic dependence of rATA2. Oocytes expressing rATA2 were perifused with 5 mM alanine in buffers (pH 8.0) containing NaCl, Na gluconate, or NMDG chloride. (B) pH-Dependence of rATA2. Oocytes expressing rATA2 were perifused with 5 mM alanine in NaCl-containing buffers of varying pH. (C) Saturation kinetics of rATA2. Oocytes expressing rATA2 were perifused with varying concentrations of alanine (0.05-2.5 mM) in NaCl-containing buffer (pH 8.0). *Inset*, Eadie-Hofstee plot. In all cases (A, B, and C), currents were monitored using the two-microelectrode voltage clamp technique.
A.

1  MKKTEMGRFN ISPDEDSSY SSNGDFNYSY PTKQAALKSH YVDVDQPENQN
51  FLLESNLGKK KYETDFHPGT TSFGMSVFNLSNAIVGSGILGLSYAMANTG
101  IALFIILLTFVSIFSLSVTL LLLKTNEGG SLLYEQLGHKA YGLAGKLAA
151  SGSITMQNIG AMSSYLFLVK YELPLVIKAL MNIETNGLW YLNGDYLVLILL
201  VSFVLILPLS LLRNGLYLGY TSGFSLCCMIFFLIVVIKKFQIPCPVEVA
251  LMANETVNGTFTOVALAALASONSTAADTCQPRYIFNSQTVAVPILTFS
301  FVCHPAVLPF YEELKSRSSRMNNVSKISFFAMFLMYLLAALFGYLTFFY
351  HVESELLHTYSAIVGTDILLLVVRLAVLVA VTLTPVVFIPRIRSVTHLL
401  CPTKEFSWFRHSVITVTLAFTNLLVFVP TIMIFGFYASIADAMLIF
451  LPSAFYIKLV KKEPMRSVQK IGAALCFLLSG VVVMIGSMGL IVLDWVHDAS
501  AGGH

B.
Cloning of an Amino Acid Transporter with Functional Characteristics and Tissue Expression Pattern Identical to That of System A
Mitsuru Sugawara, Takeo Nakanishi, You-Jun Fei, Wei Huang, Malliga E Ganapathy, Frederick H Leibach and Vadivel Ganapathy

*J. Biol. Chem.* *published online March 31, 2000*

Access the most updated version of this article at doi: [10.1074/jbc.C000205200](http://doi.org/10.1074/jbc.C000205200)

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts