Differential Influence of the 4F2 Heavy Chain and the Protein Related to b0,+ Amino Acid Transport on Substrate Affinity of the Heteromeric b0,+ Amino Acid Transporter

Received for publication, January 13, 2000

D. Prasanna Rajan‡, Wei Huang‡, Ramesh Kekuda‡, Ronald L. George§, Jian Wang‡, Simon J. Conway¶, Lawrence D. Devoe‡, Frederick H. Leibach‡, Putter D. Prasad‡; and Vadivel Ganapathy‡

From the Departments of 2Biochemistry and Molecular Biology, 6Physiology and Endocrinology, and 7Obstetrics and Gynecology and the 1Institute of Molecular Medicine and Genetics, Medical College of Georgia, Augusta, Georgia 30912

We provide evidence here that b0,+ amino acid transporter (b0,+ AT) interacts with 4F2 heavy chain (4F2hc) as well as with the protein related to b0,+ amino acid transporter (rBAT) to constitute functionally competent b0,+–like amino acid transport systems. This evidence has been obtained by co-expression of b0,+ AT and 4F2hc or b0,+ AT and rBAT in human retinal pigment epithelial cells and in COS-1 cells. The ability to interact with 4F2hc and rBAT is demonstrable with mouse b0,+ AT as well as with human b0,+ AT. Even though both the 4F2hc b0,+ AT complex and the rBAT b0,+ AT complex exhibit substrate specificity that is characteristic of system b0,+ , these two complexes differ significantly in substrate affinity. The 4F2hc b0,+ AT complex has higher substrate affinity than the rBAT b0,+ AT complex. In situ hybridization studies demonstrate that the regional distribution pattern of mRNA in the kidney is identical for b0,+ AT and 4F2hc. The pattern of rBAT mRNA expression is different from that of b0,+ AT mRNA and 4F2hc mRNA, but there are regions in the kidney where b0,+ AT mRNA expression overlaps with rBAT mRNA expression as well as with 4F2hc mRNA expression.

The heavy chain of the 4F2 cell surface antigen (4F2hc)1 and the protein related to the b0,+ amino acid transporter (rBAT) are type II membrane glycoproteins with membrane topology atypical to most of the known transporters (1). However, these two proteins have been recently shown to be obligatory for the function of several amino acid transport systems. 4F2hc interacts with different proteins to constitute distinct amino acid transport activities. The 4F2hc-associated amino acid transport proteins thus far identified are LAT1 and LAT2, which are responsible for system L (2–6), y+LAT1 and y+LAT2, which are responsible for system y+L (7, 8), xCT, which is responsible for system xCT (9), and b0,+ AT (also called 4F2–1c6), which is responsible for system b0,+ (10). The interaction between 4F2hc and b0,+ AT to constitute the amino acid transport system b0,+ , reported from our laboratory recently (10), was an unexpected finding because available evidence suggests that only rBAT is associated with system b0,+ (1). The b0,+ amino acid transport system is Na+–independent and accepts zwitterionic amino acids as well as cationic amino acids as substrates (1, 11, 12). We observed that the cloned b0,+ AT interacts with 4F2hc to induce the Na+–independent transport of the zwitterionic amino acid alanine and that rBAT is unable to substitute for 4F2hc under similar conditions (10). The 4F2hc b0,+ AT complex-induced alanine transport is inhibitable by zwitterionic amino acids as well as cationic amino acids, confirming the identity of the induced transport activity as system b0,+ . Here we report an interesting finding that both 4F2hc and rBAT can interact independently with b0,+ AT to induce the Na+–independent transport of the cationic amino acid arginine. Of even greater significance of the present study are the findings that although the amino acid transport activities induced by the two heteromeric complexes 4F2hc b0,+ AT and rBAT b0,+ AT have similar substrate specificity and represent system b0,+ , there are marked differences in substrate affinities between the two complexes. The current prevailing notion is that 4F2hc and rBAT function to chaperone the associated proteins (LAT1, LAT2, y+LAT1, y+LAT2, xCT, and b0,+ AT) by covalent interaction to bring the heteromeric complex to the plasma membrane, and available the evidence strongly supports such a function (3, 5, 8, 13). Our present studies suggest that 4F2hc and rBAT also influence the catalytic function of the heteromeric complexes by modulating the substrate affinities. The studies reported here thus unravel a novel function of the heavy chains 4F2hc and rBAT in the transport activity of the heteromeric b0,+ amino acid transporter.

EXPERIMENTAL PROCEDURES

Materials—The human retinal pigment epithelial (HRPE) cell line 165, used in expression studies, has been described earlier from our laboratory (14). COS-1 cells were obtained from the American Type Culture Collection (Manassas, VA). The human rBAT cDNA and rat 4F2hc cDNA were provided by Matthias A. Hediger (Harvard University, Boston, MA). The human 4F2hc cDNA was isolated from a JAR human placental trophoblast cell line cDNA library (15) using the rat 4F2hc cDNA as a probe. The human b0,+ AT cDNA and the murine b0,+ AT cDNA were isolated from a Caco-2 cell (human intestinal cell line) cDNA library (16) and a mouse kidney cDNA library (17), respectively, using the rabbit b0,+ AT cDNA as a probe.

Functional Expression of the cDNAs in HRPE Cells—The vaccinia virus expression system was used to functionally characterize the cDNAs (4, 10, 15–17). The cDNAs of the mouse and human b0,+ ATs, human 4F2hc, and human rBAT were all cloned into pSPORT such that the sense transcription was under the control of the T7 promoter. The cDNAs were transfected into HRPE or COS-1 cells grown in 24-well tissue culture plates using Lipofectin, and the functional expression of the cDNAs was analyzed 12 h later by measuring the uptake of [3H]alanine. One microgram of the plasmid carrying the specific cDNA (b0,+ AT, 4F2hc, or rBAT) was used/well. Sister wells transfected iden-
FIG. 1. Transport of different amino acids (1 μM) in HRPE cells transfected with vector alone, human 4F2hc cDNA plus mouse b\(^{0,+}\)AT cDNA, or human rBAT cDNA plus mouse b\(^{0,+}\)AT cDNA. Uptake was measured for 15 min at 37 °C. For each amino acid, uptake in vector-transfected cells was taken as 100%. m, mouse.

Table I

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>4F2hc ( \cdot ) b(^{0,+})AT pmol/10^6 cells/15 min</th>
<th>4F2hc ( \cdot ) b(^{0,+})AT  ( [\text{H}])Glutamine uptake</th>
<th>rBAT ( \cdot ) b(^{0,+})AT pmol/10^6 cells/15 min</th>
<th>rBAT ( \cdot ) b(^{0,+})AT  ( [\text{H}])Arginine uptake</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>54.6 ± 2.1</td>
<td>3%</td>
<td>74.3 ± 8.1</td>
<td>100%</td>
</tr>
<tr>
<td>Alanine</td>
<td>1.6 ± 0.6</td>
<td>3%</td>
<td>6.2 ± 0.6</td>
<td>8%</td>
</tr>
<tr>
<td>Glutamine</td>
<td>2.1 ± 0.2</td>
<td>3%</td>
<td>11.2 ± 5.2</td>
<td>15%</td>
</tr>
<tr>
<td>Leucine</td>
<td>0.7 ± 0.2</td>
<td>1%</td>
<td>11.8 ± 2.6</td>
<td>16%</td>
</tr>
<tr>
<td>Arginine</td>
<td>0.5 ± 0.3</td>
<td>1%</td>
<td>2.0 ± 0.1</td>
<td>3%</td>
</tr>
<tr>
<td>Lysine</td>
<td>0.7 ± 0.5</td>
<td>1%</td>
<td>0.4 ± 0.1</td>
<td>1%</td>
</tr>
<tr>
<td>Glutamate</td>
<td>37.0 ± 2.0</td>
<td>68%</td>
<td>77.7 ± 11.7</td>
<td>105%</td>
</tr>
<tr>
<td>Methylaminoisobutyric acid</td>
<td>50.6 ± 7.6</td>
<td>93%</td>
<td>74.9 ± 7.8</td>
<td>101%</td>
</tr>
</tbody>
</table>

RESULTS AND DISCUSSION

Structural Features of Murine \( b^{0,+} \)AT—The murine \( b^{0,+} \)AT cDNA is 1795 base pairs long with an open reading frame coding for a protein of 487 amino acids (GenBank\textsuperscript{TM} accession no. AF192310). The predicted amino acid sequence is highly homologous to rabbit \( b^{0,+} \)AT with 85% identity and 93% similarity.

Transport Function of \( 4F2hc b^{0,+} \)AT and rBAT\( b^{0,+} \)AT Complexes—Our previous studies have shown that rabbit \( b^{0,+} \)AT interacts with human \( 4F2hc \) to induce Na\(^+\)-independent transport of alanine and that human or rat rBAT cannot substitute...
for human 4F2hc in inducing alanine transport (10). In the present study, we co-expressed mouse b0,-AT with either human 4F2hc or human rBAT and assessed the ability of these two heteromeric complexes to induce the transport of six different amino acids (Fig. 1). The concentration of the amino acids was 1 μM. The 4F2hc-b0,-AT complex was able to mediate the transport of the neutral amino acids alanine, glutamine, serine, threonine, and leucine as well as the transport of the cationic amino acid arginine. These results are similar to those reported previously with rabbit b0,-AT (10). In contrast, the rBAT-b0,-AT complex was unable to induce the transport of the neutral amino acids. Interestingly, the complex was able to mediate the transport of arginine.

We then compared the substrate specificity of the transport processes associated with the 4F2hc-b0,-AT and rBAT-b0,-AT complexes. This was done by assessing the ability of unlabeled amino acids (2 mM) to inhibit the transport of [3H]glutamine (1 μM) mediated by the 4F2hc-b0,-AT complex and the transport of [3H]arginine (1 μM) mediated by the rBAT-b0,-AT complex under identical conditions (Table I). The 4F2hc-b0,-AT complex-mediated [3H]glutamine transport was completely inhibited by the neutral amino acids alanine, glutamine, and leucine as well as by the cationic amino acid arginine. The anionic amino acid glutamate and the methylated amino acid methyaminosobutyric acid had no or minimal effect on this transport. Interestingly, the inhibitory pattern was identical in the case of rBAT-b0,-AT complex-mediated [3H]arginine transport. This transport also was almost completely abolished by neutral amino acids and cationic amino acids but not affected by glutamate and methyaminosobutyric acid. These data show that the 4F2hc-b0,-AT complex as well as the rBAT-b0,-AT complex induce amino acid transport activities with substrate specificity similar to that of system b0,-AT.

HRPE cells possess considerable transport activity for several amino acids mediated by transport systems that are expressed constitutively in these cells. These activities were detectable in cells transfected with vector alone. We did not attempt to characterize in detail these endogenous transport activities. However, when the transport of amino acids in vector-transfected cells was analyzed, there was evidence for the expression of a b0,-AT-like transport activity in these cells. The evidence included the inhibition of the Na+-independent glutamine transport by various neutral amino acids as well as by cationic amino acids and also the inhibition of the Na+-independent arginine transport by various neutral amino acids as well as by cationic amino acids. There was also evidence for the expression of a transport system belonging to the cationic amino acid transport family. This was suggested by the findings that the Na+-independent transport of radiolabeled arginine was completely inhibited by unlabeled arginine, whereas neutral amino acids showed only a partial inhibitory effect.

Whereas the interaction of the 4F2hc-b0,-AT complex with cationic and neutral amino acids was evident from actual transport measurements as well as from competition experiments, it was not the case with the rBAT-b0,-AT complex. The interaction of the rBAT-b0,-AT complex with cationic and neutral amino acids was demonstrable with competition experiments, whereas when actual transport measurements were made, only the transport of cationic amino acids was detectable. To determine if this difference is related to differences in substrate affinities between the two complexes, we compared the dose-response relationship for the inhibition of 4F2hc-b0,-AT complex-mediated [3H]glutamine (1 μM) transport and rBAT-b0,-AT complex-mediated [3H]arginine (1 μM) transport by three neutral amino acids (alanine, glutamine, and leucine), two cationic amino acids (arginine and lysine), and two heteromeric complexes to induce the transport of six different amino acids (Fig. 1). The concentration of the amino acids was 1 μM. The 4F2hc-b0,-AT complex was able to mediate the transport of the neutral amino acids alanine, glutamine, serine, threonine, and leucine as well as the transport of the cationic amino acid arginine. These results are similar to those reported previously with rabbit b0,-AT (10). In contrast, the rBAT-b0,-AT complex was unable to induce the transport of the neutral amino acids. Interestingly, the complex was able to mediate the transport of arginine.

![Table I](http://www.jbc.org/)

**Table I.** Comparison of Ki values for the inhibition of transport mediated by 4F2hc·b0,-AT and rBAT·b0,-AT complexes

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>4F2hc·b0,-AT</th>
<th>rBAT·b0,-AT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arginine</td>
<td>12.2 ± 1.8</td>
<td>115.8 ± 17.9 (9.5)</td>
</tr>
<tr>
<td>Alanine</td>
<td>56.5 ± 9.2</td>
<td>367.9 ± 43.8 (6.5)</td>
</tr>
<tr>
<td>Glutamine</td>
<td>83.2 ± 8.0</td>
<td>413.8 ± 27.8 (5.0)</td>
</tr>
<tr>
<td>Leucine</td>
<td>26.5 ± 6.0</td>
<td>73.6 ± 8.6 (2.8)</td>
</tr>
<tr>
<td>Cystine</td>
<td>155.2 ± 27.4</td>
<td>414.1 ± 75.9 (2.7)</td>
</tr>
<tr>
<td>Lysine</td>
<td>37.1 ± 11.6</td>
<td>72.2 ± 17.2 (2.0)</td>
</tr>
</tbody>
</table>

Interaction of b0,-AT with 4F2hc and rBAT

![Figure 2](http://www.jbc.org/)

**Figure 2.** Dose-response relationship for the inhibition of 4F2hc·b0,-AT complex-mediated [3H]glutamine (1 μM) uptake (A and C) and rBAT-b0,-AT complex-mediated [3H]arginine (1 μM) uptake (B and D). Only the uptake values specific to the heteromeric complexes were used in the analysis, m, mouse.
and cystine (Fig. 2). The IC\textsubscript{50} values (i.e. concentration of the amino acid causing 50\% inhibition) calculated from these studies are given in Table II. These IC\textsubscript{50} values are almost equal to \(K_i\) values (i.e. inhibition constant) under the experimental conditions because the concentration of radiolabeled glutamine and arginine used in these competition experiments is 1\(\mu\)M, a value severalfold lower than the known \(K_t\) values of system \(b_0,1\) for glutamine and arginine (11). A comparison of these values indicates that the affinities of the rBAT\(z\)\(b_0,1\)AT complex for neutral amino acids are three to seven times lower than the corresponding affinities of the 4F2hc\(z\)\(b_0,1\)AT complex. A similar difference is seen in the case of arginine (10-fold difference), lysine (2-fold difference), and cystine (3-fold difference). In accordance with these findings, the rBAT\(z\)\(b_0,1\)AT complex-mediated transport of the neutral amino acids alanine and glutamine became detectable when the concentration of the amino acids during uptake measurements was 50\(\mu\)M instead of 1\(\mu\)M (data not shown).

Because the transport function of the 4F2hc\(b_0,1\)AT complex and the rBAT\(b_0,1\)AT complex was detectable with arginine as the substrate, we determined directly the affinity of the two complexes for this amino acid (Fig. 3). The transport of arginine mediated by the 4F2hc\(b_0,1\)AT complex was saturable over the arginine concentration range of 2.5–60 \(\mu\)M. The Michaelis-Menten constant (\(K_m\)) for the transport activity was 12.4 \(\pm\) 1.1 \(\mu\)M. This value is comparable to the \(K_m\) value calculated for this amino acid (12.2 \(\pm\) 1.8 \(\mu\)M) to inhibit glutamine transport mediated by the complex. The transport of arginine mediated by the rBAT\(b_0,1\)AT complex was also saturable but at a much higher concentration range of arginine (10–400 \(\mu\)M). The \(K_m\) value for the transport activity was 88.3 \(\pm\) 8.7 \(\mu\)M. This value is comparable to the \(K_m\) value for this amino acid (unlabeled form) to inhibit the transport of radiolabeled arginine mediated by the complex (115.8 \(\pm\) 17.9 \(\mu\)M).

These results are very interesting as they provide evidence for the first time that 4F2hc and rBAT actually influence the transport of different amino acids (1 \(\mu\)M) in COS-1 cells transfected with vector alone, human 4F2hc cDNA plus mouse \(b_0,1\)AT cDNA, or human rBAT cDNA plus mouse \(b_0,1\)AT cDNA. For each amino acid, uptake in vector-transfected cells was taken as 100\%.

\(m\), mouse; \(h\), human.

**Fig. 3.** Saturation kinetics of arginine transport mediated by the 4F2hc\(b_0,1\)AT complex (A) and the rBAT\(b_0,1\)AT complex (B). Only the uptake values specific to the heteromeric complexes were used in the analysis.

**Fig. 4.** A, transport of different amino acids (1 \(\mu\)M) in COS-1 cells transfected with vector alone, human 4F2hc cDNA plus mouse \(b_0,1\)AT cDNA, or human rBAT cDNA plus mouse \(b_0,1\)AT cDNA. B, transport of different amino acids (1 \(\mu\)M) in HRPE cells transfected with vector alone, human 4F2hc cDNA plus human \(b_0,1\)AT cDNA, or human rBAT cDNA plus human \(b_0,1\)AT cDNA. For each amino acid, uptake in vector-transfected cells was taken as 100\%.

**Fig. 5.** In situ hybridization in mouse kidney for localization of mRNA transports specific for rBAT, 4F2hc, and \(b_0,1\)AT. The boxed regions in the top row were used for higher magnification and are shown in the bottom row.
functional characteristics of the heteromeric complexes in which 4F2hc or rBAT is associated with b0\(^{+}\)-AT as a common component. This is a novel finding because it is presently believed that 4F2hc and rBAT are only responsible for proper trafficking of the heteromeric amino acid transport complexes to the plasma membrane. These complexes consist of either 4F2hc or rBAT as the heavy chain and one of a multitude of transport proteins as the light chain. Neither the heavy chain nor the light chain is competent of amino acid transport when expressed independently. However, when co-expressed, the heavy chain and the light chain constitute a functional transport complex. The currently held notion is that the light chain is the actual transporter and that the heavy chain is obligatory to chaperone the light chain to the plasma membrane. Present studies show that the heavy chains have additional functions in the transport complexes. These subunits modulate the transport function of the complexes by influencing the substrate affinity. We were able to detect this novel function of 4F2hc and rBAT because of the fortuitous observation that b0\(^{+}\)-AT can interact with either of these heavy chains. Apparently, this is not the case with LAT1, LAT2, y\(^{-}\)LAT1, y\(^{-}\)LAT2, and xCT that are assumed to interact with only 4F2hc (2–9). The possible influence of 4F2hc in these transport systems could not be investigated because the light chains do not have detectable transport activity when expressed alone. In contrast, b0\(^{+}\)-AT is able to associate with either 4F2hc or rBAT to constitute a transport-competent heteromeric complex. This made it possible to detect the influence of the heavy chains on the substrate affinity of the transport system by comparing the characteristics of the two heteromeric complexes containing either 4F2hc or rBAT as the heavy chain.

While this manuscript was in preparation, there were two reports on the cloning and functional characterization of human b0\(^{+}\)-AT (22) and rat b0\(^{+}\)-AT (21). These reports showed that human b0\(^{+}\)-AT as well as rat b0\(^{+}\)-AT were able to associate only with rBAT. 4F2hc was unable to substitute for rBAT. These results contrast with the data from our previous (10) and current studies. The functional expression of human b0\(^{+}\)-AT and rat b0\(^{+}\)-AT, reported by other investigators, was carried out in COS-1 or COS-7 cells (21, 22). In contrast, our studies employed HRPE cells. To determine whether the observed differences could be explained on the basis of the different cell lines used, we investigated the transport function of the 4F2hc-b0\(^{+}\)-AT complex by co-expressing 4F2hc and mouse b0\(^{+}\)-AT in COS-1 cells (Fig. 4A). We found that the complex was able to induce the transport of alanine, glutamine, and arginine even in COS-1 cells. An alternative possibility is that the observed differences could be because of species-dependent variations in the ability of b0\(^{+}\)-AT to interact with 4F2hc and/or rBAT. Although mouse b0\(^{+}\)-AT is able to associate with 4F2hc as well as rBAT, it is possible that human and rat b0\(^{+}\)-ATs may interact only with rBAT. To test this possibility, we isolated the b0\(^{+}\)-AT cDNA from a Caco-2 cell cDNA library and investigated its transport function when co-expressed with either 4F2hc or rBAT in HRPE cells (Fig. 4B). These studies show that human b0\(^{+}\)-AT also is able to interact with 4F2hc as well as rBAT to constitute a functionally competent heteromeric transport complex. Therefore, we do not have an explanation at present for the data from other groups of investigators showing that b0\(^{+}\)-AT interacts with rBAT but not with 4F2hc.

Because b0\(^{+}\)-AT associates with 4F2hc as well as with rBAT, we investigated the regional distribution of mRNA for these three proteins in the mouse kidney by in situ hybridization (Fig. 5). b0\(^{+}\)-AT-specific mRNA was detectable in the cortex and also in the outer layer of the medulla. The hybridization signal was, however, higher in the cortex than in the outer layer of the medulla. An exactly identical pattern of expression was seen in the case of 4F2hc mRNA. In both cases, the signal was abundant in proximal convoluted tubules, but proximal straight tubules were also clearly positive for the signal. In contrast, the distribution pattern of rBAT mRNA was clearly different. The hybridization signal was more prominent in the outer layer of the medulla than in the cortex. The proximal straight tubules showed a very strong hybridization signal, but proximal convoluted tubules were also clearly positive for the signal. Even though the distribution pattern of b0\(^{+}\)-AT mRNA was similar to that of 4F2hc mRNA but was different from that of rBAT mRNA, there were regions in the kidney where the expression of all three mRNAs overlapped with one another. It is therefore very likely that the 4F2hc/b0\(^{+}\)-AT complex as well as the rBAT/b0\(^{+}\)-AT complex mediate the b0\(^{+}\)-like amino acid transport activity in the kidney. Interestingly, there were also regions in the kidney where only the expression of rBAT mRNA was detectable with no evidence for the expression of b0\(^{+}\)-AT mRNA and 4F2hc mRNA. The different distribution pattern of rBAT mRNA from that of b0\(^{+}\)-AT mRNA suggests that there may be other proteins, hitherto unidentified, that interact with rBAT. We speculated in our previous paper that b0\(^{+}\)-AT is a candidate gene for cystinuria (10). A recent study by the International Cystinuria Consortium (22) has identified mutations in the b0\(^{+}\)-AT gene in several patients with this disease.

Acknowledgment—We thank Ida O. Walker for excellent secretarial assistance.

REFERENCES
Differential Influence of the 4F2 Heavy Chain and the Protein Related to b$_0,+$ Amino Acid Transport on Substrate Affinity of the Heteromeric b$_0,+$ Amino Acid Transporter

D. Prasanna Rajan, Wei Huang, Ramesh Kekuda, Ronald L. George, Jian Wang, Simon J. Conway, Lawrence D. Devoe, Frederick H. Leibach, Puttur D. Prasad and Vadivel Ganapathy

doi: 10.1074/jbc.275.19.14331

Access the most updated version of this article at http://www.jbc.org/content/275/19/14331

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

This article cites 21 references, 16 of which can be accessed free at
http://www.jbc.org/content/275/19/14331.full.html#ref-list-1