The 4F2 Antigen Heavy Chain Induces Uptake of Neutral and Dibasic Amino Acids in Xenopus Oocytes*

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The 4F2 cell surface antigen is a disulfide-linked heterodimer induced during the process of cellular activation and expressed widely in mammalian tissues (Parmacek, M. S., Karpinski, B. A., Gottesdiener, K. M., Thompson, C. B., and Leiden, J. M. (1989) Nucleic Acids Res. 17, 1915–1931). The human heavy chain component, a type II membrane glycoprotein, has 29% identity to the amino acid transport-related protein encoded by the recently cloned rat D2 cDNA. We have demonstrated that Xenopus oocytes injected with in vitro transcribed cRNA from D2 take up cystine and dibasic and neutral amino acids (Wells, R. G., and Hediger, M. A. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 5596–5600). In the present study, we examine the role of the human 4F2 heavy chain in amino acid transport. In vitro transcribed 4F2 cRNA was injected into Xenopus oocytes which were assayed for the uptake of radiolabeled amino acids. Our results show that cRNA from 4F2 stimulates the uptake of dibasic and neutral amino acids into oocytes at levels up to 3-fold higher than for water-injected control oocytes. There is no demonstrable uptake of cystine. Uptake is saturable, with characteristics of high affinity transport, and inhibition data suggest that uptake occurs via a single transporter. Dibasic amino acids are taken up by both 4F2 and D2 cRNA-injected oocytes in a sodium-independent manner. In contrast, 4F2-induced but not D2-induced neutral amino acid uptake has a significant component of sodium dependence. Likewise, neutral amino acids in excess inhibit the 4F2-induced uptake of radiolabeled arginine but not leucine in a sodium-dependent manner. The 4F2-induced uptake we observe most likely represents the activity of a single transport system with some characteristics of systems y* and b0,+, and B0,+*. We suggest that 4F2 and D2 represent a new family of proteins which induce amino acid transport with distinct characteristics, possibly functioning as transport activators or regulators.

The 4F2 cell surface antigen is a 125-kDa disulfide-linked heterodimer composed of an 85-kDa glycosylated heavy chain and a 41-kDa non-glycosylated light chain (6, 7). It was originally identified by the production of a mouse monoclonal antibody (mAb4F2) against the human T-cell tumor line HSB-2 (6, 8). cDNA clones for the human and mouse heavy chain antigens have been isolated and are 75% identical at the amino acid level (1, 5, 9). The heavy chain antigens are type II membrane glycoproteins with cytoplasmic N termini, short transmembrane domains, and glycosylated extracellular C termini. Northern analysis with a mouse heavy chain cDNA probe has demonstrated that 4F2 heavy chain expression is widespread in mouse tissues (1).

The function of the 4F2 proteins remains unclear despite nearly a decade of research. 4F2 expression is induced during the process of cellular activation and remains at constant levels in exponentially growing cells (1); some antibodies against 4F2 inhibit the proliferation of tumor cells in vitro (10). There were early suggestions that the protein represented the Na+/Ca2+ exchanger or a regulator of the exchanger because binding of the monoclonal antibody inhibited Na+/ Ca2+ exchange in sarclemma vesicles (11), and mAb4F2 was known to increase intracellular calcium in parathyroid cells in culture (12, 13). The distribution of the molecule, however, is not consistent with this explanation of its function (1).

We and others (2–4) have recently used expression cloning to isolate kidney- and intestine-specific cDNAs from rat (D2) and rabbit (rBAT) kidney which encode type II membrane glycoproteins with similarity to the family of α-glucosidase and to the 4F2 heavy chain cDNA (29% identity between the human 4F2 heavy chain cDNA and the rat (2) and human D2 amino acid sequences). D2 or rBAT cRNA, when injected into Xenopus oocytes, stimulates the uptake of cystine and dibasic and neutral amino acids up to 400 times background (2, 3). The predicted structure of the D2 protein is not typical of a membrane transporter. We have suggested that D2 may instead act as an activator or a regulatory subunit of the actual transport molecule, although its specific function is not yet known.

The similarity between the D2 and 4F2 amino acid sequences prompted speculation that the two proteins might have similar functions. We report here the results of studies of amino acid uptake into Xenopus oocytes injected with human 4F2 heavy chain cRNA. We show that 4F2 induces the uptake of dibasic and neutral amino acids (but not cystine) and that it has different sodium requirements for the handling of the two groups of amino acids. The transport stimulated by 4F2 shares some characteristics with the mouse blastocyst dibasic and neutral amino acid transport system b0,+(14) and with the recently cloned dibasic amino acid transporter y* (15, 16). We suggest that 4F2 may function as an activator or a regulatory subunit of the actual transport molecule, possibly a sodium cotransporter. Understanding 4F2-induced transport may lead to insights into D2-induced transport.

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MATERIALS AND METHODS

Preparation of 4F2 cRNA—The cDNA encoding the 4F2 heavy chain cell surface antigen (5) was inserted into the EcoRI site of the expression vector pBluescriptII KS+ (Stratagene). In vitro transcription of 4F2 (using T7 RNA polymerase) and D2 (using T3 RNA polymerase) was performed according to a modification of the method of Krieg and Melton (17). cRNA from the y- transporter clone used for the experiments reported in Fig. 1 was prepared as described (18) and kindly provided by James Cunningham (Brigham and Women’s Hospital, Boston, MA).

Transport Measurements—Collagenase-treated and manually defolliculated Xenopus oocytes were injected with 50 nl of water or cRNA (generally 40–50 ng as previously described (18)). Injected oocytes were incubated in ND96 medium (96 mM NaCl, 2 mM KCl, 1.8 mM CaCl2, 1 mM MgCl2, 5 mM HEPES; pH 7.4) supplemented with pyruvate (2.5 mM) and gentamicin (50 µg/ml). Uptakes of 14C-amino-acids were measured 3 days after injection according to the methods of Hediger et al. (18). Oocytes were incubated in groups of 5–8 for 1 h in 0.75 ml of uptake solution containing 0.5–1.5 µCi of the radiolabeled substrate and 100 mM Na+ or, in the case of Na+-free incubations, 100 mM NMDG. Unlabeled amino acids were added to the incubation mixture where necessary to bring the total amino acid concentration to 10 µM (or to the concentrations specified for KM determinations). For each inhibition experiment, an additional unlabeled amino acid was added to a final concentration of 2 mM (0.5 mM for cystine, which is poorly soluble in water). Uptakes were stopped by washing each group of oocytes 4–5 times with ice cold Na+-free uptake solution, then placing each individual oocyte into 250 µl of 10% sodium dodecyl sulfate. In certain experiments, as noted, the pH of the uptake and wash solutions was altered from the standard of 7.4 by addition of HCl. Each data point represents the mean of uptake into 5–8 oocytes. Radiolabeled amino acids were obtained from Du Pont-New England Nuclear. All amino acids were in the L-form.

RESULTS

Oocytes injected with water (controls) or cRNA from the D2, y-, or 4F2 clones were assayed in the presence of 100 mM sodium for the ability to take up a variety of amino acids (Fig. 1). 4F2 cRNA-injected oocytes are consistently able to induce uptake of dibasic and neutral amino acids at levels up to 3 times greater than water-injected oocytes. 4F2-induced amino acid uptake is similar to D2-induced amino acid uptake, with the exception that there is no inductive cystine uptake. Oocytes injected with cRNA from the y- system clone (15) are able to take up lysine, arginine, and ornithine, but not cystine or neutral amino acids. None of the cRNA species are able to induce uptake of proline, an imino acid. This pattern of 4F2-induced uptake is similar to that seen in water-injected control oocytes (see Fig. 1, open bars). Uptake was lower for 4F2 than for D2 or y- despite injection of similar amounts of cRNA, but was consistent over several experiments.

Additional evidence for the significance of the 4F2-induced uptake we observe comes from kinetic analysis. Uptake of arginine, as shown on a Michaelis-Menten plot, is saturable (Fig. 2). The KM of 4F2-induced arginine uptake is 43 µM; this is similar to the KM arginine values for water-injected oocytes (48 µM; Fig. 2), D2-injected oocytes (74 µM), and y- injected oocytes (70 µM (15)) and is representative of high affinity transport.

Dibasic amino acid uptake into 4F2 cRNA-injected oocytes is sodium-independent, with nearly identical uptakes for arginine and lysine in the presence versus the absence of sodium (Fig. 3, left panel). In contrast, uptake of the neutral amino acids leucine and methionine is highly sodium-dependent (96 and 89% sodium dependence, respectively) and is barely above levels for control oocytes when assayed in the absence of sodium. The background uptake of neutral and dibasic amino acids is zero for the ability to take up a variety of amino acids.
acids into water-injected oocytes shows similar patterns of sodium dependence (data not shown).

Uptake of histidine by dibasic amino acid transport system y+ is pH-dependent, with increased uptake as the pH is lowered from neutral toward the pK (15). Histidine is mostly neutral at pH 7.4 (the pH of the standard uptake solution) and becomes increasingly dibasic at pH values closer to 6.0, the pK of the imidazole group. Because 4F2, like y+, stimulates uptake of dibasic amino acids, we evaluated the pH dependence of 4F2-induced histidine uptake. For 4F2-injected oocytes, histidine uptake at pH 6.0 is twice as high as uptake at pH 7.4 (Fig. 3, right panel).

Fig. 4 shows mutual inhibition of uptake of dibasic and neutral amino acids. In the experiment represented in the left panel, water (open columns) and 4F2 (solid columns) cRNA-injected oocytes were assayed for the uptake of 15 μM [14C]arginine in the presence and absence of an excess of different unlabeled amino acids. The right panel shows results from the same experiment performed with 15 μM [14C]leucine. Unlabeled leucine causes a significant inhibition of [14C]arginine uptake. In both water- and 4F2-injected oocytes. MeAIB, an amino acid analogue that is a specific substrate for the sodium-dependent neutral amino acid transport system A (19), has no effect on the uptake of arginine. Cystine, which is not taken up into water- or 4F2-injected oocytes but is a substrate of the D2-induced transport system, causes a surprising 50% decrease in arginine uptake; it has no effect on leucine uptake.

Fig. 5 demonstrates that neutral amino acids inhibit arginine but not leucine uptake in a sodium-dependent manner. The left panel shows that arginine uptake in 4F2 cRNA-injected oocytes is inhibited by an excess of unlabeled neutral amino acids significantly more in the presence than in the absence of 100 mM sodium. Homoserine, a specific sodium-dependent inhibitor of the y+ dibasic amino acid transport system, decreases arginine uptake by about 60% in the presence of sodium, but has no effect with 15 μM [14C]leucine. Unlabeled leucine addition to a [14C]leucine uptake solution decreases leucine uptake in both water- and 4F2-injected oocytes. MeAIB, an amino acid analogue that is a specific substrate for the sodium-dependent neutral amino acid transport system A (19), has no effect on the uptake of arginine. Cystine, which is not taken up into water- or 4F2-injected oocytes but is a substrate of the D2-induced transport system, causes a surprising 50% decrease in arginine uptake; it has no effect on leucine uptake.

The 4F2 Antigen Heavy Chain Induces Amino Acid Uptake

We present here evidence that the human 4F2 heavy chain antigen, part of a cell surface disulfide-linked heterodimer associated with cell activation and tumor cell growth, stimulates amino acid uptake up to 3-fold over controls when cRNA synthesized by in vitro transcription is injected into Xenopus oocytes. This uptake is reproducible, statistically significant and saturable. The spectrum of uptake is broad, encompassing both neutral and dibasic amino acids. 4F2-induced uptake is clearly different from uptake induced by cRNA from either the y+ system transporter or the D2 clone; the y+ clone induces uptake of dibasic amino acids only, as has been previously reported (15), and the D2 clone (and its rabbit counterpart) induces uptake of cystine as well as dibasic and neutral amino acids (2, 3) (see also Fig. 1). It seems unlikely that 4F2 induces system y+ uptake in animal tissues. Although the distributions of the 4F2 and y+ mRNAs as evaluated by Northern analysis are similar for most tissues (1, 15), the properties of the amino acid uptake induced by the two are quite different. 4F2-induced uptake is also distinct from uptake induced by the broad spectrum mouse blastocyst transporters Bε+ and Bε+, which have different patterns of sodium dependence (see below).

The sodium dependence of 4F2-induced uptake is perhaps its most surprising characteristic. Uptake of the dibasic amino acids arginine and lysine is sodium-independent; uptake of the neutral amino acids leucine and methionine is mostly Na+-dependent. Likewise, neutral amino acids inhibit the uptake of arginine significantly more in the presence of sodium than in its absence. This pattern of sodium dependence has not been noted before for an amino acid transport system. D2-induced dibasic and neutral amino acid uptake are clearly sodium-independent (2, 3), with the exception that D2-induced alanine uptake has about 40% sodium dependence (2). Neutral amino acid transport systems A and ASC are sodium-dependent. The broad spectrum mouse blastocyst transporters Bε+ and Bε+ induce the sodium-dependent and sodium-independent transport, respectively, of both dibasic and neu-
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cellular amino acids, but they appear to be distinct systems (14, 20–22). Sodium-dependent inhibition of arginine uptake by neutral amino acids has been reported for system y’, but is most pronounced for homoserine (19); our data for 4F2 show this effect for leucine and methionine as well as for homoserine. It has been proposed that homoserine and sodium are cotransported by system y’ (19). The sodium-dependent neutral amino acid transport we observe for 4F2 may represent a similar phenomenon of sodium cotransport but involving a broader spectrum of neutral amino acids.

4F2, like y’, induces the uptake of histidine in a pH-dependent manner. Since y’ carries only dibasic amino acids, it follows that histidine in its dibasic form would be a better substrate for the y’ system. The explanation for this phenomenon in 4F2-induced transport is less clear, since neutral and dibasic amino acids appear to be transported equally well (see Fig. 1).

The role of the D2 and 4F2 proteins in amino acid transport is not clear, although 4F2 probably induces uptake via a single transport system. The inhibition data in Figs. 4 and 5 demonstrate that dibasic and neutral amino acids are mutually inhibitory, supporting the idea that they are carried by the same transporter. An alternative possibility is that 4F2 activates more than one transport system in the oocyte and that our data represent the combined uptake. The uptake we measure has characteristics particularly of systems y+, Boa+, and Asc+. Activation of these systems together, however, is not consistent with our observation that leucine and methionine as well as homoserine are sodium-dependent inhibitors of arginine uptake, or with the observations that dibasic amino acid uptake has no component of sodium dependence, and neutral amino acid uptake little or no component of sodium independence. Our data are most consistent with the activation of a single broad spectrum transporter that has not been previously described. Van Winkle et al. (21) have suggested that transport systems b^+, y’, ASC (Na^+-dependent neutral amino acid uptake), and asc (Na^+-independent neutral amino acid uptake) are evolutionarily related and have similar substrate receptor sites. These speculations may be relevant to the 4F2-induced transporter.

Both 4F2 and D2 are predicted to be type II membrane glycoproteins, with a structure unlike most other cloned transporters (including the y’ transporter) which have multiple membrane-spanning regions. We have suggested that D2 may function as a transport activator or as the regulatory subunit of a transporter (2); the same is possible for 4F2. If we postulate that D2 and 4F2 function as regulators or regulatory subunits of transporters, it will be important to identify those transporters. D2-induced transport in oocytes has different characteristics than native oocyte transport (as seen in water-injected oocytes), so activation of a native oocyte transporter by D2 would require significant up-regulation of that transporter or a change in its substrate specificity (2). 4F2-induced uptake, in contrast, has similar characteristics to the uptake seen in water-injected oocytes (see Fig. 1, example). In all of our experiments with the exception of the pH dependence of histidine uptake, the effects of various manipulations on 4F2-induced uptake were qualitatively the same as the effects on uptake in water-injected oocytes. The 4F2 heavy chain is known to be disulfide-linked to a 41-kDa, highly hydrophobic light chain subunit (6), which has not to our knowledge been cloned. Its structure may provide an explanation for the amino acid uptake we report here. In our oocyte experiments, it is likely that the 4F2 heavy chain associates with a Xenopus light chain analogue. The human and mouse light chains are similar or identical (23); the Xenopus protein, however, may be significantly different and its ability to associate with the human heavy chain antigen may be compromised. This is a possible explanation for the relatively low level of 4F2-induced uptake we observe. D2 has not yet been evaluated for associated subunits, although there is a cysteine residue located at position 111 of the rat clone (4 residues C-terminal to the transmembrane region) which is conserved in the human and rabbit D2 amino acid sequences (2, 3). One of the two cysteine residues found in the human and mouse 4F2 heavy chain sequence is located in a similar position (residue 106, which is 5 residues C-terminal to the transmembrane region) (1, 5, 9). It will be interesting to learn whether the D2 protein is disulfide-linked at this conserved residue to a second subunit.

The D2 protein and the 4F2 heavy chain define a new family of proteins involved in amino acid transport. Additional experiments involving expression of D2 and 4F2 clones in transformed cell lines in addition to oocytes will better define the transport systems associated with this family. Characterization of this group of proteins may yield important insight into the sodium dependence and substrate specificity of amino acid transport.

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