Functional heterodimeric amino acid transporters lacking cysteine residues involved in disulfide bond

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Abstract The protein mediating system L amino acid transport, AmAT-L, is a disulfide-linked heterodimer of a permease-related light chain (AmAT-L-lc) and the type II glycoprotein 4F2hc/CD98. The *Schistosoma mansoni* protein SPRM1 also heterodimerizes with *h*4F2hc, inducing amino acid transport with different specificity. In this study, we show that the disulfide bond is formed by heavy chain C109 with a Cys residue located in the second putative extracellular loop of the multi-transmembrane domain light chain (C164 and C137 for *X*AmAT-L-lc and SPRM1, respectively). The non-covalent interaction of Cys-mutant subunits is not sufficient to allow coimmunoprecipitation, but cell surface expression of the light chains is maintained to a large extent. The non-covalently linked transporters display the same transport characteristics as disulfide bound heterodimers, but the maximal transport rates are reduced by 30–80%.

Key words: Amino acid transporter; Disulfide bond; Site directed mutagenesis; *Xenopus* oocyte

1. Introduction

The glycoprotein *h*4F2hc (CD98 in mouse) induces low levels of amino acid transport of L- and y^+L-type when expressed alone in *Xenopus laevis* oocytes and/or some other cells [1–4]. Because of its likely topology with only one single transmembrane domain (type II membrane glycoprotein), it was suggested that *h*4F2hc acts as an activator or modulator of amino acid transport [1,2], possibly by binding to endogenous 'light chains'. Indeed, from older studies, this surface antigen is known to migrate at the level of ~80 kDa on SDS-PAGE and to be covalently associated with a hydrophobic light chain which appears as a 40-kDa band on SDS-PAGE [5,6].

We recently described the permease-related human protein E16 (*h*AmAT-L-lc) as the first light chain of *h*4F2hc [7]. We showed that indeed *h*AmAT-L-lc and its *Xenopus laevis* homologue *X*AmAT-L-lc (ASUR4) induce high levels of L-type amino acid transport (Na^+-independent exchange of large neutral amino acids) when coexpressed with *h*4F2hc in *Xenopus* oocytes, but not when expressed alone. Similar transport properties were reported for the rat homologue TA1 [8]. We showed that *X*AmAT-L-lc and *h*4F2hc form a heterodimer (AmAT-L) that migrates as a band of approximately 120 kDa on SDS-PAGE in non-reducing conditions and that, in the presence of a reducing agent, additional bands of 80 kDa and 40 kDa appear, indicating that heavy and light chains are linked by a disulfide bond [7]. SPRM1, a *Schistosoma mansoni* membrane protein with 40% identity to *X*AmAT-L-lc was also shown to heterodimerize with *h*4F2hc. This led to its surface expression and to the induction of amino acid transport with a different specificity, i.e. Na^+-independent transport of cationic amino acids and partially Na^+-dependent transport of large neutral amino acids [7].

In the present study we show by site directed mutagenesis that C109 of the heavy chain *h*4F2hc and C164 and C137 of the light chains *X*AmAT-L-lc and SPRM1, respectively, form the disulfide bond linking the heterodimer subunits covalently together and that the absence of disulfide bond does not preclude, but only decreases transporter surface expression and/or function.

2. Materials and methods

2.1. Point mutations

For introduction of the point mutation C109S in the *h*4F2hc cDNA the primer 5′-AGCGCCGCGTTCTCGCGAGCTACC-3′ (sense) and the corresponding antisense primer containing the mutation were generated (mutated bases are underlined). These primers were used to amplify the plasmid containing the template cDNA of *h*4F2hc by PCR using Pfu DNA polymerase (Stratagene) according the manufacturers protocol. Following temperature cycling, template DNA was selectively digested with the DNA methylation-dependent restriction enzyme *Dpn*I. The same method was used to introduce the point mutations C164S in *X*AmAT-L-lc and C137S in SPRM1 using the primers 5′-TTCCCTACCTCCCCCGTGCCCGAT-3′ and 5′-CGTCTACATGGACAGTGTTACTCCTAC-3′ (sense).

2.2. Xenopus laevis oocytes

Oocytes were treated with collagenase A for 2–3 h at room temperature in Ca^{2+}-free buffer containing: 82.5 mM NaCl, 2 mM KCl, 1 mM $MgCl_2$, 10 mM HEPES/Tris, pH 7.4, to remove follicular cells and then kept at 16°C in ND96 buffer containing: 96 mM NaCl, 2 mM KCl, 1 mM $MgCl_2$, 1.8 mM $CaCl_2$, 5 mM HEPES/Tris, pH 7.4.

2.3. Amino acid uptake

Oocytes were injected with 5 ng of each cRNA dissolved in 33 nl water. Oocytes were then kept for 24 h at 16°C in ND96 buffer. Before experiments, oocytes were washed 6 times in uptake buffer containing: 100 mM choline-Cl, 2 mM KCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 10 mM HEPES/Tris, pH 7.4. Six oocytes per uptake condition were preincubated at 22°C for 2 min. Then, the buffer was exchanged for 100 μl uptake buffer supplemented with amino acid at the indicated concentration and tritiated amino acid as tracer. Uptakes were performed for 1 min because initial experiments showed linear uptake during this time. Oocytes were then washed 5 times with 3 ml buffer, distributed to individual vials and lysed in 2% SDS. Radioactivity was counted by liquid scintillation.

2.4. Labeling of oocytes and immunoprecipitation

After cRNA injection (as for uptake experiments), oocytes were

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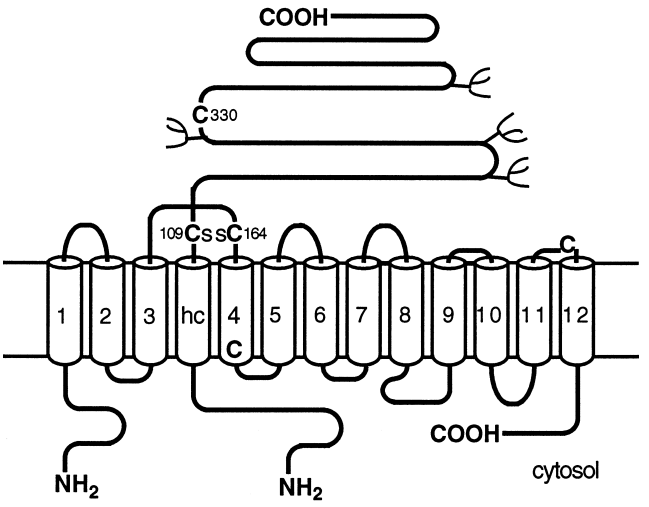

Fig. 1. Membrane topology model of a heterodimeric amino-acid transporter of the AmAT family. hc indicates the single transmembrane domain of the heavy chain *h*4F2hc. Putative transmembrane domains of the lipophilic light chain are numbered 1–12. The Cys residues 109 in *h*4F2hc and 164 in *X*AmAT-L-lc (corresponds to C137 in SPRM1) are linked by a disulfide bond. Two other Cys residues which are present in *X*AmAT-L-lc and SPRM1 as well as C330 of *h*4F2hc which is also present in CD98 are indicated. Potential *N*-glycosylation sites are indicated by forks.
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incubated for 48 h in ND96 buffer supplemented with 1 mCi/ml [^{35}S]methionine. Oocytes were then washed twice in ND96 buffer and lysed in 20 μl/oocyte oocyte lysis buffer containing: 120 mM NaCl, 50 mM Tris-HCl, pH 8.0, 0.5% NP40 supplemented with protease inhibitors. Lysates were vortexed for 20 s and incubated shortly on ice and centrifuged for 10 min at 12 000 rpm at 4°C. Incorporated radioactivity was determined by TCA-precipitation.

Antibodies for *h*4F2hc (monoclonal antibody [6] and SPRM1 (affinity purified rabbit anti-peptide antibody) were provided by L. Kühn and P. Skelly, respectively. Antibody for *X*AmAT-L-lc was raised against a peptide corresponding to amino acids 30–45 coupled to keyhole limpet hemocyanin (Eurogentec, Seraing, Belgium) and affinity purified. Aliquots of lysate containing equal amounts of counts were precleared twice for 30 min at 4°C with protein G plus/protein A agarose beads (Calbiochem). Antibodies were bound to protein G plus/protein A agarose for 2 h at room temperature in oocyte lysis buffer and added to the precleared lysate. After overnight incubation at 4°C beads were washed 6 times in buffer containing: 100 mM NaCl, 20 mM Tris-HCl, pH 8.0, 1 mM EDTA, 500 mM LiCl, 0.5%

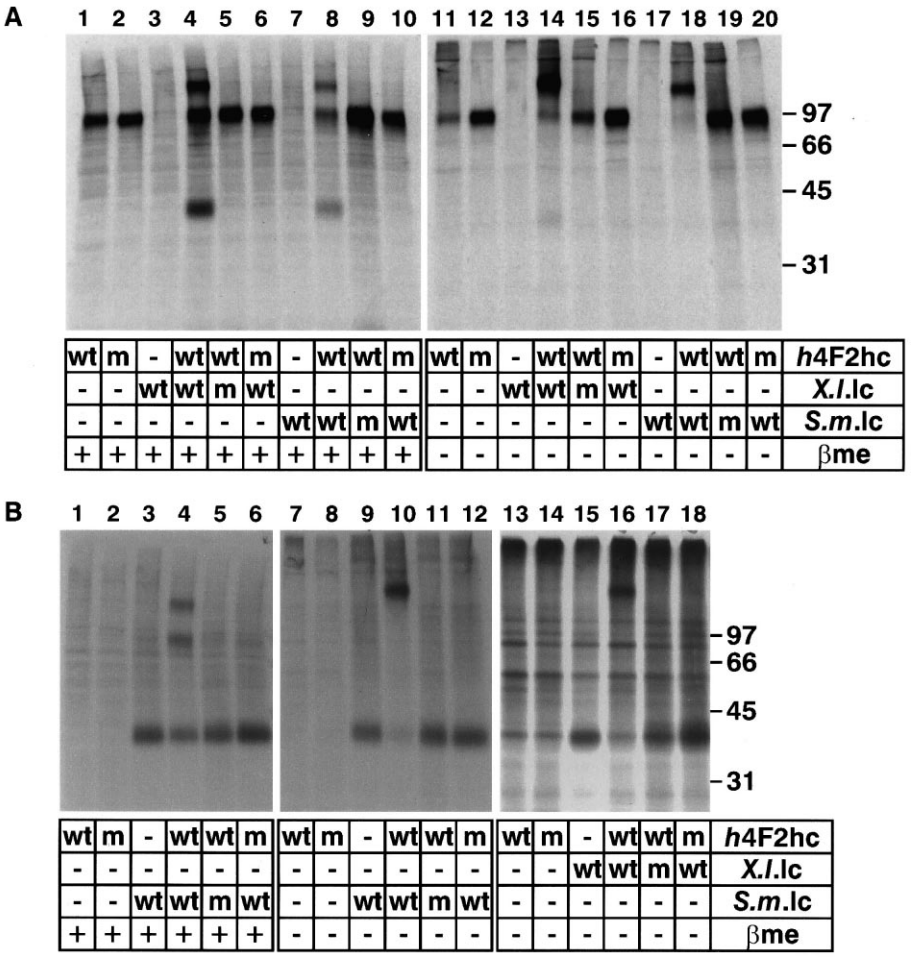

Fig. 2. Immunoprecipitation of wild type and Cys-mutant *h*4F2hc and light chains *X*AmAT-L-lc and SPRM1 expressed in oocytes with anti-*h*4F2hc (A), anti-SPRM1 (B1–10) and anti-*X*AmAT-L-lc antibody (B10–15), followed by SDS-PAGE and fluorography. A: *X*AmAT-L-lc or SPRM1 coprecipitated with *h*4F2hc appears as additional band of 40 kDa in reducing conditions and remains associated with *h*4F2hc in non-reducing conditions migrating as heterodimer of ~120 kDa. In ^{C109S}*h*4F2hc and ^{C164S}*X*AmAT-L-lc or ^{C137S}SPRM1 expressing oocytes the light chain is not coprecipitated. B: Wild-type *h*4F2hc is coprecipitated with wild-type *X*AmAT-L-lc and SPRM1 light chain. No coprecipitation of the heavy chain is seen with ^{C109S}*h*4F2hc and ^{C146S}*X*AmAT-L-lc or ^{C137S}SPRM1. wt: wild type; m: Cys to Ser mutants (see Section 2); *X.l.*lc: *X*AmAT-L-lc; *S.m.*lc.: SPRM1; βme: β-mercaptoethanol.

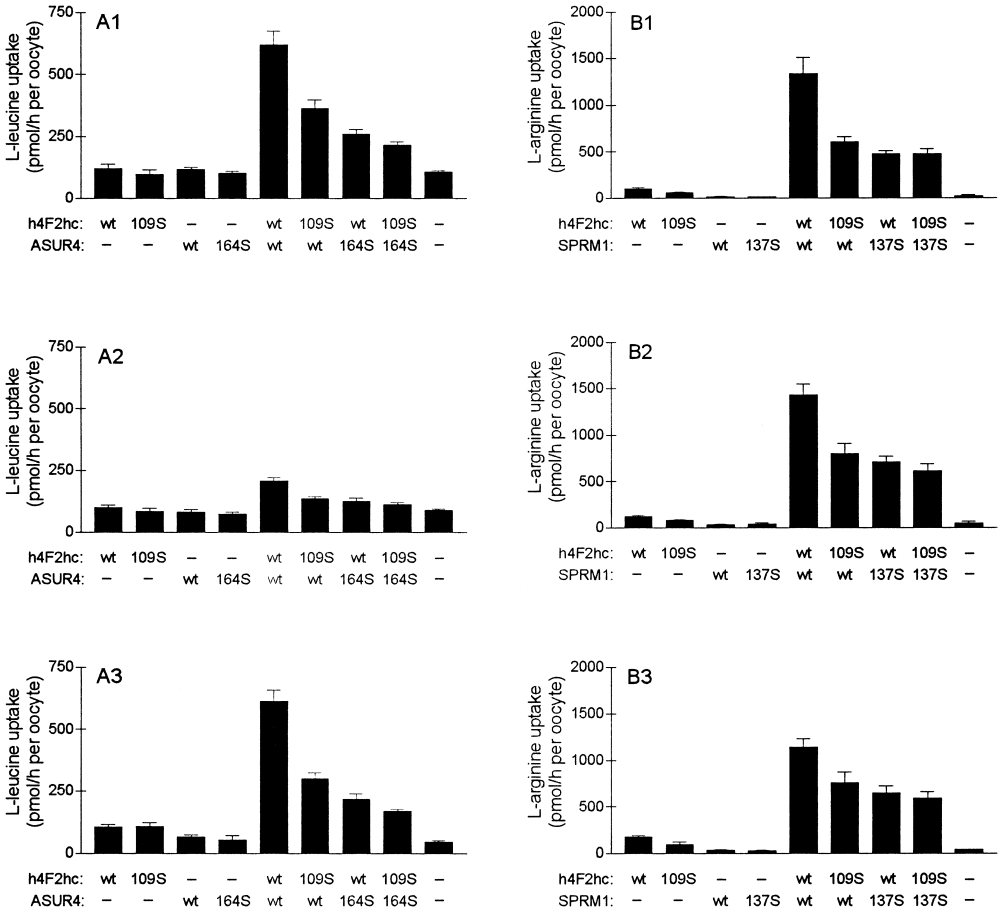

Fig. 3. Amino acid uptake in *Xenopus* oocytes expressing wild-type *h*4F2hc and *X*AmAT-L-lc or SPRM1 and Cys mutants (wt: wild type; 164S: ^{C164S}*X*AmAT-L-lc; 137S: ^{C137S}SPRM1). A: L-Leucine (1 mM) uptake was similar in non-injected oocytes and oocytes expressing *h*4F2hc, ^{C109S}*h*4F2hc, *X*AmAT-L-lc or ^{C164S}*X*AmAT-L-lc only. The increase in L-leucine uptake observed in oocytes coexpressing *h*4F2hc and *X*AmAT-L-lc was reduced in oocytes coexpressing ^{C109S}*h*4F2hc. Coexpression of ^{C109S}*h*4F2hc and ^{C164S}*X*AmAT-L-lc results in a further reduction of uptake. L-Leucine uptake was inhibited by an excess of BCH (10 mM) (A2), but not by MeAIB (10 mM) (A3). B: L-Arginine (1 mM) uptake by oocytes expressing ^{C109S}*h*4F2hc, SPRM1 or ^{C137S}SPRM1 only was slightly less than that by *h*4F2hc expressing oocytes. The increase in L-arginine transport observed in oocytes coexpressing *h*4F2hc and SPRM1 is diminished in oocytes coexpressing ^{C109S}*h*4F2hc and SPRM1 and further reduced in oocytes coexpressing ^{C109S}*h*4F2hc and ^{C137S}SPRM1. L-Arginine transport by the heterodimer of *h*4F2hc and SPRM1 is not inhibited by BCH (10 mM) (B2) and MeAIB (10 mM) (B3). Means of 18 oocytes ± S.E.M. pooled from 3 independent experiments are shown.

NP40 and 6 times in buffer containing: 100 mM NaCl, 20 mM Tris-HCl, pH 8.0, 1 mM EDTA, 0.5% NP40. Beads were resuspended in SDS-PAGE sample buffer and heated to 65°C for 15 min. β-Mercaptoethanol was added where indicated and SDS-PAGE analysis was performed. Gels were stained in Coomassie blue and fixed. After incubation in Amplify (Amersham), gels were dried and exposed to film.

2.5. Immunocytochemistry

Oocytes were fixed with 3% paraformaldehyde in PBS for 4 h at 4°C, 24 h after injection of the cRNAs. Anti-*h*4F2hc antibody (2 μg/ml) followed by a 1:1000 dilution of a Cy-3 conjugated goat anti-mouse Ig (Jackson Immuno Research Laboratories, West Grove, PA, USA) and anti-SPRM1 antibody diluted 1:4000 and followed by a 1:100 dilution of a horseradish peroxidase (HRP)-conjugated donkey-anti-rabbit Ig (Jackson) were used on cryosections (6 μm thick) of the fixed oocytes. The HRP-conjugated secondary antibody was revealed with FITC-tyramide conjugates (TSA-Direct, NEN, Boston, MA, USA) which were diluted 1:75 in the TSA amplification diluent. Sections were studied by epifluorescence and digitized images were acquired with a VISICAM CCD camera (Visitron, Puchheim, Germany) and processed by Image-Pro Plus v3.0 software (Media Cybernetics, Silver Spring, MD, USA) (same settings for all images with same antibody).

3. Results

For the different light chains belonging to the AmAT-lc family, hydropathy analysis as well as analysis with the TMpred program (ISREC) predict a 12 transmembrane domain topology with the N- and C-termini in the cytosol. According to this prediction, the only extracellular Cys residue which is conserved in AmAT-L-lc's, SPRM1 and the light chains which induce system y^+L-type amino acid transport (AmAT-y^+L-lc's) (Pfeiffer et al., manuscript submitted) is located in the second extracellular loop and corresponds to C164 in *X*AmAT-L-lc and C137 in SPRM1, respectively. This suggested the involvement of these Cys residues in the disulfide bond linking the light chain to the heavy chain and prompted us to test this possibility by mutating them to Ser and testing the resulting mutant light chains in *Xenopus* oocytes.

The heavy chain of the heterodimers, the type II glycoprotein *h*4F2hc, has a large extracellular domain containing only 2 Cys residues, one of which is close to the transmembrane

domain. This is also the only one to be conserved between *h*4F2hc and the related glycoprotein rBAT, a glycoprotein expressed at the apical surface of small intestine and kidney proximal tubule which induces $b^{0,+}$-like amino acid transport when it is expressed in *Xenopus* oocytes. This, as well as results obtained with *h*4F2hc-transferrin receptor chimeras (Rothenberger and Kühn, personal communication) and with *h*4F2hc expressed alone in *Xenopus* oocytes (Estevés et al., in press) prompted us to test by site directed mutation whether this Cys^{109} residue indeed is forming the disulfide bridge with the light chain. The model shown in Fig. 1 corresponds to the heterodimer formed by *h*4F2hc and the *Xenopus* light chain *X*AmAT-L-lc or the *Schistosoma mansoni* chain SPRM1 as proposed above and subsequently confirmed by the experimental work described below.

3.1. Coimmunoprecipitation of heavy and light chains depends on the Cys residues indicated in the model

To test the hypothesis that the Cys residues C164 in *X*AmAT-L-lc or C137 in SPRM1 and C109 in *h*4F2hc are forming disulfide bridges in heterodimers, we performed coimmunoprecipitations using wild type and the mutated chains described above expressed in *Xenopus* oocytes. Fig. 2A shows the SDS-PAGE analysis of immunopreciptiations made with the anti-*h*4F2hc antibody. The expected band of 80 kDa appeared in oocytes expressing *h*4F2hc only (lanes 1, 2, 11, 12), and, in contrast, no protein was precipitated when oocytes were injected with either of the light chains alone (lanes 3, 7, 13, 17) [7]. In non-reducing conditions, coinjection of *h*4F2hc/*X*AmAT-L-lc or *h*4F2hc/SPRM1 resulted, as previously shown, in the appearance of a band at ~120 kDa, which corresponds to a heterodimer (lanes 14, 18). As expected, the heterodimer could be separated by the addition of the reducing agent β-mercaptoethanol as shown by the additional band at 40 kDa corresponding to the coprecipitated light chain (lanes 4, 8). No coprecipitation was seen with ^{C109S}*h*4F2hc coexpressed with a wild-type light chain nor with wild-type *h*4F2hc coexpressed with a mutant light chain (lanes 5, 6, 9, 10, 15, 16, 19, 20). These results confirm that C109 of *h*4F2hc, and C164 and C137 in *X*AmAT-L-lc and SPRM1, respectively, form a disulfide bond in the heterodimer. The lack of coprecipitation of Cys-mutants is compatible with the possibility that the formation of the interaction between heavy and light chain or its stability during cell lysis and/or immunoprecipitation depends on the covalent bond.

Immunoprecipitations were also performed using anti-SPRM1 antibody (Fig. 2B) which confirmed the results obtained with anti-*h*4F2hc antibody. The expected band at a molecular weight of 40 kDa was seen with lysate of oocytes injected with SPRM1 alone (lanes 3, 9). In *h*4F2hc/SPRM1 coinjected oocytes, *h*4F2hc was coprecipitated and migrated as a 80-kDa band (lane 4) or a ~120-kDa band (heterodimer, lane 10) in reducing or non-reducing conditions, respectively. Again there was no coprecipitation of ^{C109S}*h*4F2hc coinjected with SPRM1 or wild-type *h*4F2hc coinjected with ^{C137S}SPRM1 (lanes 5, 6, 11, 12). Anti-*X*AmAT-L-lc antibody also precipitated *X*AmAT-L-lc (lane 15) and ^{C164S}*X*AmAT-L-lc and coprecipitation of *h*4F2hc was only seen when wild-type *X*AmAT-L-lc and *h*4F2hc where coexpressed (lane 16). The fact that three different antibodies directed against epitopes were situated on different sites (heavy chain extracellular domain, light chain N-terminal or C-terminal region) supports the idea that the absence of coprecipitation in our experimental conditions is due to the intrinsic instability of the subunit interaction and not a destabilizing effect of the antibody.

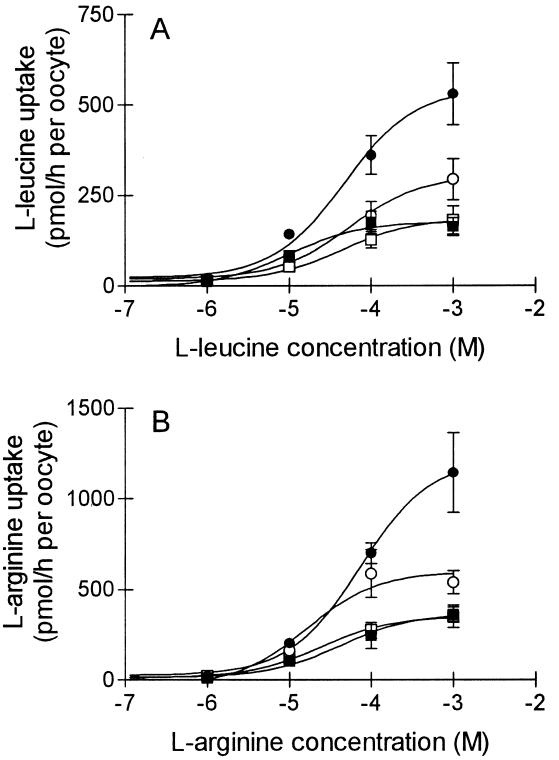

Fig. 4. Dose dependence of amino acid uptake in *Xenopus* oocytes coexpressing *h*4F2hc and *X*AmAT-L-lc or SPRM1 and Cys mutants. A: L-Leucine uptake by oocytes coexpressing *h*4F2hc/*X*AmAT-L-lc (●), ^{C109S}*h*4F2hc/*X*AmAT-L-lc (○), *h*4F2hc/^{C164S}*X*AmAT-L-lc (■) and ^{C109S}*h*4F2hc/^{C164S}*X*AmAT-L-lc (□). B: L-Arginine uptake by oocytes coexpressing *h*4F2hc/SPRM1 (●), ^{C109S}*h*4F2hc/SPRM1 (○), *h*4F2hc/^{C137S}SPRM1 (■) and ^{C109S}*h*4F2hc/^{C137S}SPRM1 (□). Uptakes were performed at 4 different amino acid concentrations, background uptake by oocytes expressing *h*4F2hc or ^{C109S}*h*4F2hc was subtracted and curves corresponding to Michaelis-Menten kinetics were fitted to the experimental data using non-linear regression analysis. Means of 12 oocytes ± S.E.M. pooled from 2 independent experiments are shown.

3.2. Amino acid transport is only reduced in the absence of covalent binding between subunits

To test the role of the disulfide bond in the function of AmAT, wild-type heavy and light chains and mutated heavy and/or light chains were expressed in *Xenopus* oocytes (Fig. 3A,B). L-Leucine uptakes in *X*AmAT-L-lc (Fig. 3A1) and L-arginine uptakes in SPRM1 (Fig. 3B1) expressing oocytes, respectively, were performed in the absence of Na^+. After 24 h expression, the background uptake of L-leucine and L-arginine by oocytes injected with ^{C109S}*h*4F2h, *h*4F2hc, ^{C164S}*X*AmAT-L-lc, ^{C137S}SPRM1 or the wild-type light chains alone was similar to that of non-injected oocytes. Coexpression of wild-type heavy and light chain induced a substantial increase in amino acid uptake, as expected [7]. However, when ^{C109S}*h*4F2hc was coexpressed with wild-type *X*AmAT-L-lc or SPRM1, the induced transport was reduced to ~30–70% of that obtained with wild-type *h*4F2hc. The transport was even

slightly lower (~20–50% of wild type), when a mutated light chain was coexpressed with wild-type *h*4F2hc. Transport rate of oocytes coinjected with mutated heavy and light chains was similar to that of oocytes coinjected with wild-type *h*4F2hc and mutated light chain.

Qualitatively, the transport characteristics of the non-covalently bound heterodimeric transporter were not discernible from those of wild-type transporter. For instance, L-leucine transport by *X*AmAT-L-lc/*h*4F2hc is blocked to the same extent in all combinations of mutants by the competitive inhibitor 2-amino-2-norbornane-carboxylic acid (BCH) (Fig. 3A2). As expected, methyl-aminoisobutyrate (MeAIB), the specific inhibitor of system A amino acid transport had no effect (Fig. 3A3). Amino acid transport mediated by the heterodimer of SPRM1 and *h*4F2hc has been shown to be different from that mediated by the heterodimer of *X*AmAT-L-lc and *h*4F2hc and differs from all described mammalian amino acid transport systems. As expected BCH (Fig. 3B2) and MeAIB (Fig. 3B3) had no inhibitory effect on SPRM1.

To investigate further the potential effect of the mutations on transport kinetics, uptakes of L-leucine in the case of *X*AmAT-L-lc (Fig. 4A) and L-arginine (Fig. 4B) in the case of SPRM1 were performed at 4 different amino acid concentrations in the absence of Na^+. Oocytes were injected with *h*4F2hc or ^{C109S}*h*4F2hc alone or the indicated combinations of heavy and light chains. Background uptake by oocytes injected with *h*4F2hc or ^{C109S}*h*4F2hc alone was subtracted from uptake by coinjected oocytes and sigmoidal curves were fitted to the experimental data such that amino acid concentrations for half-maximal activation (apparent K_m) could be derived. No major reproducible difference in apparent K_m was observed in oocytes coinjected with *h*4F2hc and any of the mutated light chains, ^{C109S}*h*4F2hc and wild-type light chains or ^{C109S}*h*4F2hc and mutated light chains (apparent K_m 10–54 μM for L-leucine uptakes by *h*4F2hc/*X*AmAT-L-lc combinations and 16–73 μM for L-arginine uptake by *h*4F2hc/SPRM1 combinations). However, V_{max} was reduced to a similar extent as seen in Fig. 3.

3.3. *Surface expression is not prevented by lack of disulfide bridge between subunits*

We have previously shown by immunofluorescence that *h*4F2hc expressed alone was localized at the surface of oocytes and that, in contrast, the AmAT transporter light chain was

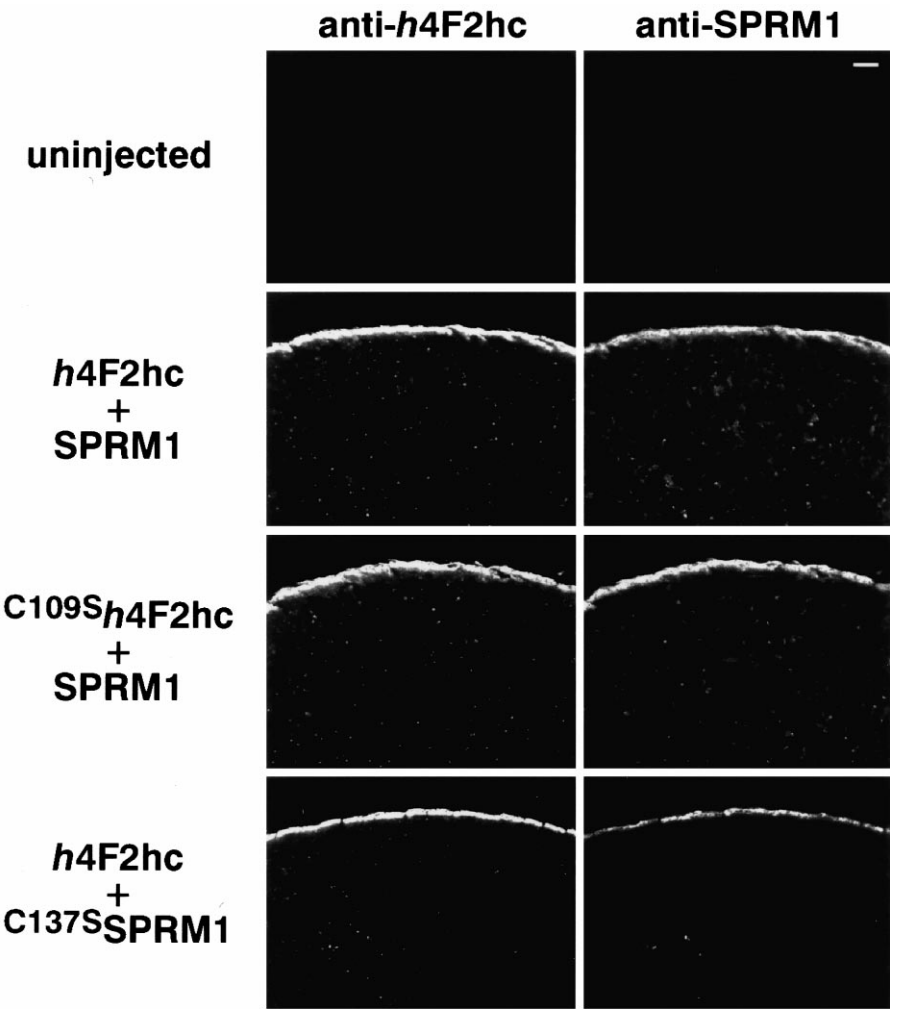

Fig. 5. Double immunofluorescence of *h*4F2hc and the light chain SPRM1 in oocyte sections. Injected cRNAs are indicated at the left side of each panel pair (same section) while the detecting antibody is indicated on the top of each panel. *h*4F2hc and SPRM1 colocalize at the cell surface, whether or not they are linked by a disulfide bond, in contrast to SPRM1 expressed alone which is not localized at the surface [7]. Bar: 20 μm.

localized intracellularly when expressed alone, and required coexpression of *h*4F2hc to localize at the surface [7]. To test whether *h*4F2hc is able to promote the surface expression of a non-covalently bound AmAT-light chain, double immunofluorescence experiments were performed using antibodies to *h*4F2hc and SPRM1. As shown in Fig. 5, coexpression of mutant chains (^{C137S}SPRM1 and ^{C109S}*h*4F2hc), which cannot form a disulfide bridge with their heterodimer partner, nevertheless leads to a substantial cell surface localization and no intracellular accumulation of the light chain. This result suggests that heterodimer interaction takes place also in the absence of disulfide bridge formation between the subunits and that this interaction is stable enough to allow cell surface expression of the light chain, in the context of the oocyte system.

4. Discussion

AmAT-L, composed of *h*4F2hc and *h*AmAT-L-lc or *X*AmAT-L-lc is the first heterodimeric transporter for organic substrates of vertebrates known as yet [7]. Characteristics of transport depend on the light chain associated with the 4F2 heavy chain. The function(s) of the heavy chain are not yet entirely elucidated. It has been shown in oocytes that the light chain was localized mainly intracellularly when injected alone, however, it was localized to a large extent at the plasma membrane when coexpressed with *h*4F2hc [7]. This led to the hypothesis that the heavy chain *h*4F2hc is important for maturation, trafficking and/or surface residence of the light chain. Interestingly, *h*4F2hc has an overall structure (glycosylated type II membrane protein) which resembles that of the β-subunit of Na,K-ATPase. This plasma membrane enzyme has a minimal functional unit composed of an α-β heterodimer which is, though, not covalently linked. Similarly to the AmAT amino acid transporters, it also performs a transmembrane exchange of substrates, i.e. three intracellular Na^+ for two extracellular K^+. For the Na,K-ATPase, it has been shown that the type II glycoprotein β-subunit is needed for stabilization, functional maturation and transport, from the endoplasmic reticulum to the plasma membrane, of the catalytic multi-transmembrane domain α-subunit [9]. We hypothesize that the 4F2 heavy chain type II glycoprotein fulfills a similar function towards its permease-related AmAT light chains.

Heavy and light chain are linked by a disulfide bridge which is shown in this study to be not essential for the surface expression and the function of the transporters. Nevertheless, the disulfide linked Cys-residues in the light chain are conserved in amphibia and mammals and even the flat worm *Schistosoma mansoni*. This indicates that covalent linkage of the heterodimer must be of importance. Our experiments show that the non-covalent interaction of heavy and light chain is not very strong, since coimmunoprecipitation was not observed after cell lysis and immunoprecipitation in non-denaturing conditions. In vivo, a missing disulfide bond might decrease the ability of the heavy chain to heterodimerize, and/or reduce the stability of interaction and thus decrease the amount of light chain brought to the membrane. A reduced stability of interaction might also decrease the time of residence of functional units at the cell surface.

Interestingly, the Cys residue in the heavy chain is also conserved in rBAT, another glycoprotein heavy chain which induces amino acid transport of type $b^{0,+}$-like characteristics [10,11]. Possibly (a) light chain(s) of the same AmAT-lc family heterodimerize with rBAT. Interestingly, expression experiments with rBAT truncated at its C terminus [12] have suggested that this other putative 'heavy chain' might modulate the transport characteristics of its light chain or, alternatively, that the mutation influenced the affinity for different putative endogenous light chains of the oocyte. As 4F2hc and rBAT are localized at the basolateral and apical membrane of epithelial cells, respectively [13,14], they might determine the basolateral or apical surface localization of their respective light chains.

In conclusion, we have identified the Cys residues forming the disulfide bond between heavy and light chain of heterodimeric amino acid transporter of the AmAT family. We have shown that transport characteristics and apparent K_m remain unchanged in the non-covalently bound transporter whereas maximal transport activity is reduced. These findings are compatible with the hypothesis that the heavy chain is important for maturation, trafficking and/or surface residence of the catalytic light chain.

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