

Isoform Selectivity of 3-¹²⁵I-Iodo- α -Methyl-L-Tyrosine Membrane Transport in Human L-Type Amino Acid Transporters

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3-¹²³I-iodo- α -methyl-L-tyrosine (¹²³I-IMT) has been developed for SPECT of amino acid transport imaging. We examined the isoform selectivity of ¹²⁵I-IMT transport of the 2 human L-type amino acid transporters, hLAT1 and hLAT2, with human 4F2hc-coexpressed *Xenopus laevis* oocytes. **Methods:** An uptake study of ¹²⁵I-IMT was performed using transporter-expressed *X. laevis* oocytes. Oocytes were injected with 17.6 ng of hLAT1 or hLAT2 complementary RNA (cRNA) and 7.4 ng of h4F2hc cRNA in a molar ratio of 1:1. Two days after injection, the uptake of ¹²⁵I-IMT was measured in the Na⁺-free uptake solution containing 18.5 kBq of noncarrier-added ¹²⁵I-IMT. After incubation for 30 min at room temperature, radioactivity of the oocytes was determined. **Results:** Of the 2 hLAT isoforms and h4F2hc-coexpressed *X. laevis* oocytes, ¹²⁵I-IMT uptake via hLAT1 was 5.95-fold higher than that via hLAT2 ($P < 0.005$). **Conclusion:** ¹²⁵I-IMT transport was hLAT1 selective. Investigations on the isoform selectivity of ¹²⁵I-IMT transport with other transporters are anticipated.

Key Words: membrane transport; 3-¹²⁵I-iodo- α -methyl-L-tyrosine; human L-type amino acid transporter family; human 4F2hc; *Xenopus laevis* oocyte

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The artificial amino acid 3-¹²³I-iodo- α -methyl-L-tyrosine (¹²³I-IMT), which is derived from tyrosine, was developed as a functional imaging agent for neutral amino acid transport in the brain and pancreas and has been used clinically for SPECT of tumors (1,2). In this pilot study, we first compared the transporter selectivity of ¹²⁵I-IMT and L-¹⁴C(U)-Tyr in isoforms of human L-type amino acid transporters (LATs) designated hLAT1 and hLAT2 (Fig. 1) (3,4). Human LAT1 and hLAT2 requires a heavy chain of human 4F2 cell-surface antigen (h4F2hc) for system L-like functional expression (3,4). Uptake studies of ¹²⁵I-IMT were

performed with hLAT1 or hLAT2 and h4F2hc-coexpressed *Xenopus laevis* oocytes. Among the amino acid transport systems described, system L is a Na⁺-independent transport system and a major route for providing cells with large neutral amino acids, including branched or aromatic amino acids (5). The hypothesis has been proposed that amino acid transporters in transformed cells are upregulated to support high-level protein synthesis for continuous growth and proliferation (5). In cultured human glioma cells, membrane transport of ¹²⁵I-IMT is dominated by amino acid transport system L (2).

MATERIALS AND METHODS

Labeled Compounds

Reagent grade chemicals (Aldrich Chemical Co., Milwaukee, WI) were used in this experiment. ¹²⁵I-NaI (8.1×10^{19} Bq/mol) was obtained from Amersham Pharmacia Biotech (Buckinghamshire, U.K.). Noncarrier-added ¹²⁵I-IMT was prepared by the conventional chloramine-T method as described (1). L-¹⁴C(U)-Tyr was obtained from American Radiolabeled Chemicals (St. Louis, MO).

Uptake Studies with *X. laevis* Oocytes

As described previously (3,4), in vitro transcription was performed to obtain complementary RNAs (cRNAs) for hLAT1 and hLAT2 using T3 RNA polymerase for hLAT1 and hLAT2 in pBluescript II SK- (Stratagene, La Jolla, CA) linearized with *Xho* I and T7 RNA polymerase for h4F2hc in plasmid pZL1 (Invitrogen, Carlsbad, CA) linearized with *Bam*HI. For *X. laevis* oocyte expression studies, 17.6 ng of hLAT1 or hLAT2 cRNA and 7.4 ng of h4F2hc cRNA (molar ratio, 1:1) were injected into *X. laevis* oocytes. The control group consisted of *X. laevis* oocytes injected with water instead of cRNA solution. Uptake of radiolabeled amino acids was measured 2 d after injection in an Na⁺-free uptake solution (100 mmol/L choline chloride, 2 mmol/L KCl, 1 mmol/L CaCl₂, 1 mmol/L MgCl₂, 10 mmol/L Hepes, 5 mmol/L Tris, pH 7.4; incubation for 30 min at room temperature) containing 18.5 kBq/mL ¹²⁵I-IMT or L-¹⁴C(U)-Tyr.

RESULTS

Labeling efficiency was >80% and, after purification, radiochemical purities of ¹²⁵I-IMT were >95%. Specific radioactivity was > 8.1×10^{19} Bq/mol.

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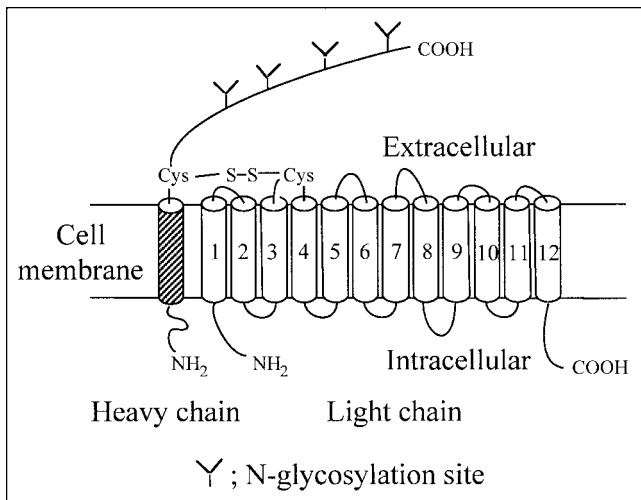


FIGURE 1. Expected structure of functional unit of L-type amino acid transporter. Human 4F2 heavy chain (CD98; h4F2hc), type II membrane glycoprotein, has molecular weight of approximately 80 kDa. This protein facilitates transport of ^{125}I -IMT by forming heterodimer by disulfide bond with light chain. Light chain has molecular weight of approximately 40 kDa. Human LAT1 and hLAT2 (light chains) have putative 12 transmembrane domains. Complementary DNAs of these transporters have been cloned.

^{125}I -IMT uptake of *X. laevis* oocytes via hLAT1-h4F2hc heterodimer was 5.95-fold higher than uptake of hLAT2-h4F2hc heterodimer ($P < 0.005$), whereas L- ^{14}C (U)-Tyr uptake was not significantly different between hLAT1-h4F2hc- and hLAT2-h4F2hc-coexpressing *X. laevis* oocytes (Fig. 2).

DISCUSSION

LAT1 and LAT2, belonging to the mammalian LAT family, selectively transport neutral amino acids by obligatory exchange mechanisms (3,4). These transporters possess

broad substrate selectivity for various neutral amino acids and have different roles. Table 1 shows the distribution of transporter expression (3,4). LAT1 exhibits higher affinity ($K_m = 20\text{--}40 \mu\text{mol/L}$) but lower capacity toward Leu, Ile, Phe, Met, Tyr, His, Trp, and Val. D-Isomers of Leu, Phe, and Met are also accepted as substrates (3). The heterodimeric complex of LAT2 and 4F2hc is involved in transcellular transport of neutral amino acids through epithelia and blood-tissue barriers (4). Compared with LAT1, which prefers larger neutral amino acids with branched or aromatic side chains, LAT2 exhibits lower affinity ($K_m = 30\text{--}300 \mu\text{mol/L}$) but higher capacity and remarkably broad substrate selectivity, including smaller neutral amino acids Gly, Ala, Ser, and Thr (4).

Although expression of LAT2 has not been detected in tumor cells (4), high expression of LAT1 has been confirmed in numerous types of tumor cells (3). Table 1 also lists tumor cell types that express LAT1 (3).

In this pilot study, membrane transport of ^{125}I -IMT and the parent L- ^{14}C (U)-Tyr differed in terms of isoform selectivity. Of the 2 human LAT family isoforms, ^{125}I -IMT transport preferred hLAT1 to hLAT2. The expression of amino acid transport proteins differs between cell types and is sometimes dependent on the differentiation state of cells (5). Development of isoform-selective artificial amino acids is expected to facilitate research into tumors, cerebral function, and other organs and to provide clues for research into amino acid transport. Further studies with transport of ^{125}I -IMT and other compounds via LATs are now in progress. Several studies have used cell lines to investigate ^{125}I -IMT transport and some have reported that systems L, A, T, and B $^{0,+}$ (or B 0) play a role (2,6). Na $^+$ -independent carrier systems such as b $^{0,+}$ and y $^+$ do not play a role in ^{125}I -IMT uptake (6). However, although system y $^+$ L cannot yet be excluded, evidence of its involvement is inconclusive (6). Investigations into iso-

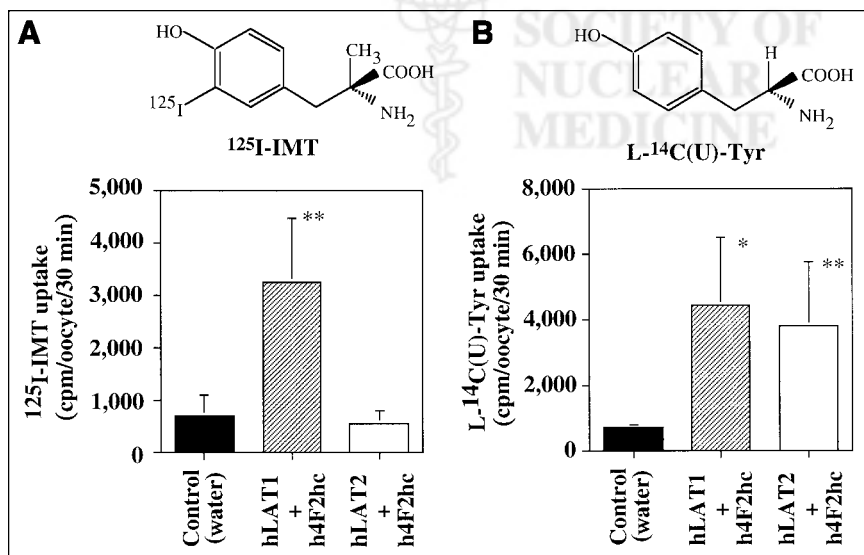


FIGURE 2. Comparison of isoform selectivity of ^{125}I -IMT transport (A) and L- ^{14}C (U)-Tyr (B) in hLAT1 or hLAT2 and h4F2hc-coexpressing *X. laevis* oocytes (water-injected oocytes acted as control). Uptake of radio-labeled amino acids was measured in Na $^+$ -free uptake solution containing 18.5 kBq ^{125}I -IMT or L- ^{14}C (U)-Tyr. ^{125}I -IMT transport showed hLAT1-h4F2hc selectivity. * $P < 0.05$; ** $P < 0.005$.

TABLE 1
Expression of LAT1 and LAT2 Confirmed in Mammalian Organs and Tumor Cell Types

Distribution	LAT1	LAT2
Organs	Heart, brain*, placenta*, lung, liver, skeletal muscle, kidney, pancreas*, spleen, thymus, prostate, testis*, ovary, small intestine, colon, peripheral leukocytes*, bone marrow*, fetal liver*	Brain, placenta*, skeletal muscle, kidney*, testis*, small intestine*
Tumor cell types	Teratocarcinoma*, bladder carcinoma*, lung small cell carcinoma*, uterine cervical carcinoma*, leukemia*, lymphoma*	

*High expression.

form selectivity of ¹²⁵I-IMT transport with other transport systems will no doubt be undertaken.

CONCLUSION

Of the 2 heterodimeric complexes of hLAT1–4F2hc and hLAT2–4F2hc, ¹²⁵I-IMT transport was hLAT1–4F2hc selective, whereas the parent L-¹⁴C(U)-Tyr did not demonstrate isoform selectivity.

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