Characterization of Lysosomal Monoiodotyrosine Transport in Rat Thyroid Cells

EVIDENCE FOR TRANSPORT BY SYSTEM h

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Lysosomal transport of monoiodotyrosine was characterized in countertransport experiments using rat FRTL-5 thyroid cell lysosomes. Monoiodotyrosine carrier activity was temperature-dependent (Eₚ = 11.65 kcal/mol) and had a pH optimum of 7.5. Carrier activity was minimally inhibited by KCl and NaCl, but unaffected by the presence of other ions or ATP. Monoiodotyrosine transport was unaffected by the presence of carbonyl cyanide m-chlorophenylhydrazone, nigericin, or ammonium chloride, indicating that a proton or K⁺ gradient is not necessary for monoiodotyrosine transport across the lysosomal membrane. Monoiodotyrosine countertransport showed a 6-fold increase in lysosomes from FRTL-5 cells grown in medium containing thyrotropin by comparison to cells grown without this hormone. Thyrotropin responsiveness raised the possibility that monoiodotyrosine was transported by system h, the only known lysosomal carrier whose activity is enhanced by thyrotropin. Consistent with this, monoiodotyrosine-loaded lysosomes exhibited countertransport of [²⁺²S¹¹]thyroglobulin, [²⁺²H¹]phenylalanine, and [²⁺²H¹]leucine, three system h ligands, but not [²⁺²H¹]cysteine, a nonsystem h ligand. Unlabeled tyrosine, phenylalanine, and leucine, but not cystine or proline, inhibited [¹⁵¹⁰⁰]monoiodotyrosine countertransport, and leucine inhibition of [²⁺²H¹]tyrosine countertransport and [¹⁵¹⁰⁰]monoiodotyrosine countertransport yielded virtually identical Kᵢ values, 3.5 and 3.2 μM, respectively. Competition studies with monoiodotyrosine analogues showed that system h recognizes a broad range of ligands with an α-amino acid configuration at one end and a hydrophobic region at the other. Ring-substituted halogens, regardless of mass or ring position, but not amino, nitro, hydroxy, or methoxy groups, enhanced carrier recognition of system h analogues. It appears that a single system effects the transport of iodinated (e.g. monoiodotyrosine) and noniodinated (e.g. tyrosine) thyroglobulin catabolites into the lysosomal carrier for MIT, discovered in rat FRTL-5 thyroid cells (3).

Selective proteolysis of thyroglobulin within thyroid cell lysosomes yields the thyroid hormones thyroxine and triiodothyronine, which are delivered to the blood stream by an unknown mechanism. The proteolysis of thyroglobulin in the lysosomes also releases significant amounts of iodine, 11 atoms iodine/m01 thyroglobulin (1), in the form of monoiodotyrosine (MIT)¹ and diiodothyronine (DIT). The mono- and diiodothyronine residues formed after lysosomal degradation of the thyroglobulin are transported to the cytoplasm and deiodinated, and the iodine is reincorporated into newly synthesized thyroglobulin (2). The transport of iodotyrosines into the cytosol has recently been shown to occur by means of a lysosomal carrier for MIT, discovered in rat FRTL-5 thyroid cells (3).

In the present report, we characterize the lysosomal transport of MIT in FRTL-5 cells, demonstrate that it is stimulated by thyrotropin, and provide evidence that MIT is transported by system h, a lysosomal membrane carrier of neutral, hydrophobic amino acids (4, 5). We also detail the system’s structural requirements for ligand binding, in particular, the influence of a ring halogen upon ligand recognition and transport.

EXPERIMENTAL PROCEDURES

Materials—L-[¹²⁵]MIT (2200 Ci/mmol) was obtained from Du Pont-New England Nuclear. L-[²⁺²H¹]tyrosine (50 Ci/mmol), L-[²⁺²H¹]phenylalanine (53 Ci/mmol), L-[²⁺²H¹]leucine (136 Ci/mmol), and L-[²⁺²H¹]cysteine (688 mCi/mmol) were obtained from the Amersham Corp. Nonradioactive amino acids, amino acid methyl esters, and amino acid analogues were obtained from Sigma, Aldrich, or Chemical Dynamics Corp. (South Plainfield, NJ). Nonradioactive amino acid methyl esters were obtained from Sigma. Ions (NaCl, KCl, MgCl₂, NH₄Cl), NaATP, MgATP, and ionophores (carbonyl cyanide m-chlorophenylhydrazone, valinomycin, nigericin) were obtained from Sigma and Calbiochem.

Cells—Rat FRTL-5 cells (ATCC No. CRL 6306) are a continuous line of functional epithelial cells from normal Fischer rat thyroids which take up iodine, produce and iodinate thyroglobulin, and exhibit most characteristics of normal thyroid cells in vivo (6–11). They are capable of prolonged periods of culture in thyrotropin depleted medium and regain the functional characteristics of thyroid cells when reintroduced into thyrotropin containing medium. The cells were grown, unless otherwise stated, in Coon’s modified Ham’s F-12 medium supplemented with 5% fetal calf serum, 1 mM nonessential amino acids (Microbiological Associates, Bethesda, MD), and a six-hormone mixture, including thyroid-stimulating hormone (10⁻¹⁰ M), insulin (10 μg/ml), hydrocortisone (0.36 ng/ml), transferrin (5 μg /ml).

¹ The abbreviations used are: MIT, monoiodotyrosine; DIT, diiodothyronine; EGTA, ethyleneglycolbis(oxyethylenenitrito)tetrasurface acid; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; CT, countertransport; TSH, thyrotropin.
glycyl-L-histidyl-L-lysine acetate (10 ng/ml), and somatostatin (9 ng/ml) (6, 11). Cell growth conditions were 37°C in 5% CO₂, 95% air atmosphere; cells were passaged every 7-10 days with a maximal passage number of 28.

**Lysosomal Preparations**—Lysosomal preparations were obtained as described previously (3). Summarized briefly, FRTL-5 cells were scraped from 75-cm² dishes after incubation in Hanks' balanced salt solution containing 4 mM EGTA for 30 min at 37°C. After the cells were briefly sonicated (8 s) in 0.25 m sucrose, 10 mM Hepes, pH 7.0, a lysosome-rich granular fraction was produced by a 10-min, 17,000 x g centrifugation of the post-nuclear supernatant. This granular fraction was layered onto 22% Percoll in 0.25 m sucrose, 10 mM Hepes and centrifuged at 40,000 x g for 40 min in a Ti-50 vertical rotor. The concentrated-gradient, containing “heavy lysosomes,” were briefly sonicated (8 s) in 0.25 M sucrose, 10 mM Hepes x g centrifugation of the post-nuclear supernatant. This granular fraction was layered onto 22% Percoll in 0.25 M sucrose, 10 mM Hepes and centrifuged at 40,000 x g for 40 min in a Ti-50 vertical rotor. The concentrated-gradient, containing “heavy lysosomes,” were washed twice by centrifugation in 300 and 50 ml of sucrose/Hepes for 10 min at 17,000 x g to remove the Percoll. The final pellet was then centrifuged 3-4 fold in lysosomes, as gauged by β-hexosaminidase activity, and contained 7-20% of the total cell's hexosaminidase activity.

Small amounts of mitochondria and plasma membranes have been shown to contaminate this “heavy” lysosomal fraction (3). Contaminating organelles or membranes could not affect the outcome of any experiments since all transport results were based on the differential uptake of MIT into loaded and unloaded lysosomes (see “MIT Loading” and “MIT Countertransport” below). Since only lysosomes contain the hydrolases necessary to achieve MIT loading when exposed to MIT methyl ester, any countercurrent transport observed would be attributable to the lysosome.

**MIT Loading**—Lysosomes were loaded with MIT by incubating the lysosomal suspension in a tube into which MIT methyl ester in methanol had been added and the methanol evaporated; the amount of MIT methyl ester added was calculated to give a final concentration of 2 mM MIT methyl ester. After incubation at 37°C for 15 min, the lysosomes were pelleted in a 9,000 x g centrifugation, 4 min, and washed by resuspending the pellet in 3 ml of sucrose/Hepes and centrifugation at 17,000 x g, 10 min. Under these conditions, MIT methyl ester is effectively taken up by the lysosomes and hydrolyzed to MIT as previously shown (3). These FRTL-5 lysosomes (3; lysosomal loading averaged 2.2 ± 0.9 nmoI of MIT/unit of hexosaminidase, well above the level of MIT carrier saturation (3). The final, MIT-loaded pellet was resuspended in 0.8-1.0 ml of sucrose/Hepes and tested for countercurrent transport.

**MIT Countertransport—Countertransport (CT) of MIT was characterized in experiments assessing the effects of pH, temperature, MIT concentration, and the presence of ions (NaCl, MgCl₂, and NH₄Cl), MgATP, NaATP, and ionophores (carbonyl cyanide m-chlorophenylhydrazine, valinomycin, and nigericin). Ionophores were dissolved in acetone, placed into an empty incubation tube and the acetone was evaporated before the incubation mixture was added; an equal volume of acetone was evaporated from the control tube. The amount of CT was defined as the difference between the uptake of [¹²⁵I]MIT into lysosomes loaded with nonradioactive MIT and the uptake into nonloaded lysosomes. Increased uptake into loaded lysosomes results from trapping of MIT intralysosomally because it must compete with the high intralysosomal concentration of MIT for exit. Membrane binding could not account for CT since no difference in binding between loaded and nonloaded lysosomes should occur.

The experimental procedure was initiated by adding 200 μl of loaded or nonloaded lysosomes to the incubation mixture containing 50 or 100 nM [¹²⁵I]MIT (0.7-1.5 μCi/ml) in 6.2 ml of sucrose/Hepes. After washing quickly, a 1-ml aliquot, in duplicate or triplicate, was removed immediately and diluted into 10 ml of ice-cold phosphate-buffered saline (zero-time). This was poured over a Whatman GF-B filter on a suction apparatus. The filter was washed twice with 10 ml of ice-cold phosphate-buffered saline. The remaining incubation mixture was placed at 37°C, or the experimental temperature to be studied, in a water bath and a second aliquot, in duplicate or triplicate, was removed 5 min later and treated identically to the zero time sample. Filters were counted in a Stare γ counter. The difference in radioactivity between the timed samples was calculated; corrections were made for the specific radioactivity of [¹²⁵I]MIT and the amount of lysosomes in the incubation mixture, as gauged by hexosaminidase activity. The first experiment was expressed to fructoseos of MIT/unit of hexosaminidase/min. The difference between the uptake into loaded and unloaded lysosomes constituted countertransport. The amount of MIT CT using 0.05 μM extralysosomal [¹²⁵I]MIT has been previously shown to increase linearly with time up to 20 min (3). Even with 10 μM extralysosomal [¹²⁵I]MIT, CT increased linearly through at least 5 min (data not shown).

The effect of TSH in the growth media was tested by placing FRTL-5 cells, previously grown in normal media (6H), in TSH-depleted media (5H) for a minimum of 5 days. Cells were then harvested in two populations (5H and 6H) on the same day, and their lysosomes were independently prepared and analyzed for MIT CT as described above.

Competition experiments were performed by adding a competing ligand at the appropriate concentration to the incubation mixture prior to adding the lysosomes. Competing ligands were present in the incubation mixture of both loaded and unloaded lysosomes. With each group of competition experiments, a control without any competing ligand was performed. MIT CT in the presence of each competing ligand was then compared to the control, and expressed as a percent of control MIT CT.

Countertransport of [³H]lysine, [³H]leucine, and [³H]phenylalanine, and [³H]cystine in MIT-loaded or nonloaded lysosomes was performed in the same manner as described above for MIT CT. MIT-loaded or nonloaded lysosomes were incubated for 5 min in 2 μM concentration of the radioactive transport ligand (e.g. [³H]lysine), before being poured over filters, washed, and counted. [³H]lysine, [³H]leucine, [³H]phenylalanine, and [³H]cystine CT represents the difference in ligand uptake between the MIT-loaded and the non-loaded lysosomes.

Leucine inhibition of lysosomal CT was performed by measuring the CT of [³H]lysine (1, 2.5, 10, 50 μM) in tyrosine-loaded and unloaded lysosomes in the presence and absence of 6 μM leucine. Leucine inhibition of MIT CT was performed by measuring the CT of [¹²⁵I]MIT (0.2, 0.8, 2.5, and 10 μM) in MIT-loaded and unloaded lysosomes in the presence and absence of 10 μM leucine. The apparent Kᵅ values were derived from the Michaelis-Menten equation:

\[
SLOPE_{	ext{control}} = SLOPE_{(1 + \text{LEUCINE})/Kᵅ}\]

**Assay of Hexosaminidase Activity**—Each incubation mixture was corrected for lysosomal content by assay of hexosaminidase activity, a uniquely lysosomal enzyme, as described previously (12).

**RESULTS**

In CT experiments performed at 0 to 45°C, the velocity of MIT CT was shown to increase with increasing temperature, with a Q₁₀ of 1.95 and energy of activation of 11.7 kcal/mol. Velocity of MIT CT was maximal at pH 7.5 and fell off sharply above pH 7.5 (Fig. 1). All countertransport experiments were performed at pH 7.0 to allow comparison of results with previously published studies performed at pH 7.0. There was, however, little difference in MIT CT between pH 7.5 and 8.0.

The presence in the incubation mixture of NaCl (100 mM) or KCl (100 mM) inhibited MIT CT, 21 and 17%, respectively. NH₄Cl (20 mM) had no effect on MIT CT, and MgCl₂ (2 mM) slightly stimulated MIT CT (121% of control). Neither NaATP (2 mM) nor MgATP (2 mM) with or without MgCl₂ (2 mM) had any effect on MIT CT; MgCl₂ (2 mM) and MgATP

![Fig. 1. pH profile of MIT countertransport in FRTL-5 cell lysosomes. Percoll-purified lysosomes were either loaded with MIT, or not loaded, and incubated for 5 min in 50 nM [¹²⁵I]MIT at pH 6.0-8.0. After incubation, aliquots were immediately poured over filters, washed, and the radioactivity was counted. The difference between uptake of [¹²⁵I]MIT into MIT-loaded and nonloaded lysosomes constituted the amount of MIT countertransport, as described under “Experimental Procedures.”](http://www.jbc.org)
(2 mM) in the presence of KCl (100 mM) inhibited MIT CT to approximately the same degree (20%) as KCl (100 mM) alone. Ionophores such as valinomycin (1 μM), nigericin (25 μM), and carbonyl cyanide m-chlorophenylhydrazone (25 μM) also had no effect on MIT CT, although the presence of KCl (100 mM) with valinomycin (1 μM) inhibited MIT CT (32%) approximately as much as KCl (100 mM) alone. The mean uninhibited velocity of MIT CT was 4.6 ± 1.7 (SD; n = 7) fmol/unit of hexosaminidase/min.

MIT CT was studied as a function of extralysosomal MIT concentration. Linear regression analysis of a Lineweaver-Burk plot revealed an apparent Kᵢ for MIT of 1.8 μM and a Vₘₐₓ of 8.5 pmol/unit of hexosaminidase/min (R = 0.99) (Fig. 2A). The Kᵢ of tyrosine CT in tyrosine-loaded lysosomes was 20 μM and the Vₘₐₓ was 3.5 pmol/unit of hexosaminidase/min (Fig. 2B).

Countertransport of MIT across lysosomal membranes was enhanced 4–9-fold by growth of FRTL-5 cells in thyrotropin-containing medium, compared with MIT CT in lysosomes from thyrotropin-depleted medium (Fig. 3). In a representative experiment, lysosomes from cells grown in the presence of thyrotropin demonstrated a mean velocity of 53 fmol of [125I]MIT CT/unit of hexosaminidase/min (n = 2), compared with 14 ± 2 (SD; n = 3) fmol of [125I]MIT CT/unit of hexosaminidase/min in lysosomes from cells grown in thyrotropin-depleted medium.

Transport by system h, the carrier which transports tyrosine, has been shown to be stimulated by the presence of thyrotropin in the growth medium (3, 4). To determine if lysosomal MIT transport might occur via system h, we compared the degree of competition of various ligands against CT of both MIT and tyrosine (4). The Kᵢ for tyrosine (~20 μM; see Fig. 2B) is 12-fold greater than that for MIT (~1.8 μM) (3), and the standard concentrations of transport ligands were 0.1 μM for [125I]MIT and 8 μM for [3H]tyrosine. Therefore, the competing ligand concentrations used were 50 μM against MIT CT and 500 μM against tyrosine CT. The pattern of competition of the amino acids relative to each other was the same for MIT CT as for tyrosine CT (Table I). L-Tyrosine, L-leucine, and L-phenylalanine all competed strongly against both MIT and tyrosine CT, and D-tyrosine competed weakly against both. Valine and methionine each competed to an intermediate degree against both MIT and tyrosine countertransport.

Another approach to determining the identity of the MIT carrier and system h carriers, MIT-loaded and nonloaded lysosomes were examined for countertransport of amino acids carried by system h. [3H]Tyrosine, [3H]phenylalanine, and [3H]leucine exhibited significant countertransport (248, 152, and 192 fmol/unit of hexosaminidase/min, respectively), although less than that for [125I]MIT (333 fmol/unit of hexosaminidase/min) (Fig. 4). The greater amount of [125I]MIT CT is attributable to the much lower Kᵢ and higher Vₘₐₓ for MIT than for tyrosine (see below) [3H]Cystine, which is not a system h ligand, showed negligible countertransport (35 fmol/unit of hexosaminidase/min) to MIT-loaded lysosomes and served as a control for any nonspecific effect of loading. These data indicate that system h ligands (tyrosine, phenylalanine, and leucine) are transported by the MIT carrier. Consistent with these data, tyrosine-loaded lysosomes display MIT CT at a rate of 110 fmol/unit of hexosaminidase/min.

Another approach to determining the identity of the MIT carrier and system h involves the measurement of comparative inhibition of [3H]tyrosine and [125I]MIT countertransport by a system h ligand (leucine). The apparent Kᵢ of leucine against [125I]MIT countertransport (using MIT-loaded lysosomes) and against [3H]tyrosine countertransport (using tyrosine-loaded lysosomes) was derived from the Lineweaver-Burk plots of uninhibited and inhibited transport for each ligand (Fig. 2A and B). The Kᵢ for leucine against MIT CT

Fig. 2. Determination of the Kᵢ value of leucine against [125I]MIT countertransport (A) and against [3H]tyrosine countertransport (B) in FRTL-5 cell lysosomes. A, Percoll-purified lysosomes were loaded with MIT and incubated in 0.2, 0.8, 2, 5, and 10 μM [125I]MIT in the presence (○) or absence (●) of 10 μM leucine as a competitive inhibitor. Countertransport was calculated as the difference between the uptake over 5 min of [125I]MIT CT into loaded and unloaded lysosomes. Countertransport data were plotted on a double-reciprocal plot and the slopes were used to derive the Kᵢ value of leucine against [125I]MIT CT into MIT-loaded lysosomes (Kᵢ = 3.2 μM) as described under “Experimental Procedures.” B, Percoll-purified lysosomes were loaded with tyrosine and incubated in 1, 2, 5, 10, 50 μM [3H]tyrosine in the presence (○) or absence (●) of 5 μM leucine. Countertransport was calculated as the difference between the uptake over 5 min of [3H]tyrosine CT into loaded and unloaded lysosomes. These data were plotted on a double-reciprocal plot and the slopes allowed the calculation of the Kᵢ of leucine against [3H]tyrosine CT into tyrosine-loaded lysosomes (Kᵢ = 3.5 μM).

Fig. 3. Effect of TSH in cell growth medium on MIT countertransport in FRTL-5 cell lysosomes. FRTL-5 rat thyroid cell lysosomes were grown in medium containing TSH for 7–10 days at which point one-half the cells were placed in TSH-depleted medium (6H) for 5 days before harvesting, whereas the other half was maintained in medium plus TSH (6H). Countertransport of 0.1 μM [125I]MIT, determined in Percoll-purified lysosome preparations from cells grown in the presence and absence of TSH, was calculated as described under “Experimental Procedures.” Results are presented as a mean percent increase of MIT CT from two separate experiments, each performed in duplicate. Data were corrected for the amount of lysosomes in each lysosomal preparation to ensure that the TSH effect was not due to an increased number of lysosomes.
Cystine transport was performed twice. The Kᵣ for cystine is 12 times that for MIT. Results of 3-(p-Hydroxyphenyl)-propionic acid, which resembles tyrosine, did not compete against [³²P]MIT, suggesting that the position of the halogen substituent was not critical for recognition by the carrier.

As noted earlier, the iodine substituent decreased the Kᵣ against tyrosine CT by 12-fold but only minimally affected the Kᵣ for cystine. The effect of the iodine substituent illustrated by 3,4-Dihydroxyphenylalanine, 3-(p-Chlorophenylalanine, and L-3-Amino-3-phenylpropionic acid was similar competition. Phenylalanine derivatives with substituents on the aromatic ring which confer less hydrophobicity relative to phenylalanine competed less than phenylalanine, the most obvious example was 3,4-Dihydroxyphenylalanine.

**DISCUSSION**

A primary function of lysosomes is to degrade macromolecules to allow salvage of the breakdown products. Transport
of these small molecules out of the lysosome makes them available for reutilization via cytosolic pathways. In the past 7 years, lysosomal membrane transport systems have been elucidated for amino acids, carbohydrates, nucleosides and N-acetylated oxosamines in several cell types (14, 15).

Different lysosomal membrane transport systems for amino acids display different specificities for various ligands. The cystine carrier has been demonstrated in leukocyte (16), fibroblast (17), and lymphoblast (18) lysosomes, and its impairment in cystinosis results in intralysosomal accumulation of cystine in most tissues of the body (19). System c is a carrier for cationic amino acids elucidated in human fibroblast lysosomes (18). Tyrosine and neutral, hydrophobic amino acids are carried by system h in rat thyroid cell lysosomes (4) and transport by this carrier is stimulated by thyrotropin (5). Lysosomal transport systems for small neutral amino acids in human fibroblasts demonstrate some overlap of ligand specificities (15). Proline, for example, is transported by a low affinity system (p) as well as a high affinity system (f).

MIT transport in rat FRTL-5 cell lysosomes displays saturation kinetics and countertransport (9), providing strong evidence that transport was carrier-mediated. We now show that the temperature characteristics of MIT CT are also consistent with carrier-mediated transport, which requires a relatively high energy of activation, and typically have a Q10 of approximately 2.0. In contrast, noncarrier-mediated transport systems, such as membrane channels, have a Q10 closer to 1.0 (20). We also demonstrated that MIT transport is cation-independent and does not require ATP or a transmembrane gradient of K+ or protons.

MIT has a high affinity for its carrier as evidenced by an apparent K<sub>m</sub> of 1.8 μM, approximately 12-fold less than the K<sub>m</sub> of tyrosine for system h by our determination in Percoll-purified lysosomes. Tyrosine competes with MIT for uptake into loaded lysosomes (3) and lysosomal MIT transport is stimulated by thyrotropin, a characteristic which has thus far been attributed only to the system h carrier (5). These findings raised the possibility that MIT was transported by the neutral, hydrophobic amino acid carrier, system h.

If the two carriers were the same, they should have identical ligand specificities as demonstrated by competition studies. The relative amount of competition of various amino acids against MIT CT was the same as found by Bernar et al. (3) against tyrosine CT, suggesting essentially identical ligand specificity. This was further supported by the finding of virtually identical values of K<sub>m</sub> for leucine against both [123I]MIT and [3H]tyrosine CT. Since the K<sub>m</sub> reflects the affinity of a carrier for a competing ligand, it will be the same against all ligands transported by that carrier. The essentially identical values of leucine's K<sub>m</sub> against MIT and tyrosine CT suggest that all three ligands are transported by system h. Similarly, increased uptake of [3H]tyrosine, [3H]phenylalanine, and [3H]leucine into MIT-loaded lysosomes (i.e., tyrosine, phenylalanine, and leucine CT) supports the identity of the MIT and system h carriers.

The system h carrier recognizes a wide range of compounds, provided they contain the α-amino configuration. Chemical moieties which decrease the hydrophobicity of the molecule are unfavorable for recognition. The carrier also recognizes a variety of different structures, such as leucine, phenylalanine, and cyclohexylalanine, to a similar degree. This finding is highlighted by the fact that cyclohexylalanine's ring, which is capable of the "boat" and "chair" conformation, is much larger and capable of greater steric hindrance than the planar ring of phenylalanine.

Halogenated phenylalanine derivatives compete with MIT countertransport to a greater degree than phenylalanine. MIT also competes more strongly than tyrosine for MIT CT, and has a higher affinity than tyrosine for system h as judged by a lower K<sub>m</sub> value of MIT CT. The position of the halogen substitution or halogen size has no significant effect on MIT CT. Previous studies of the competitive effect of DIT, triiodothyronine, and thyroxine on MIT CT showed no significant difference between MIT and DIT in their ability to compete against MIT CT, but triiodothyronine and thyroxine competed much better than MIT or DIT against MIT CT (3). Whether this shows an additive effect of halogens on carrier affinity, or is related to the considerable difference in structures is unclear. The favorable influence of halogens, but not other substitutions, in all ring positions suggests that the carrier possesses a binding site which is specific for the halogen-ring complex.

The characteristics of MIT transport suggest that lysosomal system h is essential for the salvage of iodine in the thyroid cell cytoplasm. Rousset et al. (1) showed that of approximately 42 iodine atoms/molecule of thyroglobulin present in the colloid, 11 iodine atoms/molecule of thyroglobulin remain in mature lysosomes after selective hydrolytic release of triiodothyronine and thyroxine, of which approximately one-third was MIT and two-thirds was DIT. While lysosomal transport of DIT remains to be demonstrated, we have shown that DIT competes strongly against MIT uptake (3). Preliminary experiments in our laboratory suggest that [125I]DIT countertransport can be shown in MIT-loaded lysosomes using low temperatures (20 °C) and short incubation times (30-60 s), perhaps because transport in unloaded lysosomes occurs so quickly that this background uptake must be reduced to demonstrate the effect of loading. System h seems to be responsible for transporting MIT, and possibly DIT, across the lysosomal membrane for iodine salvage.

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