Characterization of a Melanosomal Transport System in Murine Melanocytes Mediating Entry of the Melanogenic Substrate Tyrosine*

S. Brian Potter†, Jacqueline Muller‡, Isa Bernardini‡, Frank Tietze¶, Takeshi Kobayashi‡, Vincent J. Hearing‡, and William A. Gahl**

From the Laboratory of Cell Biology, NCI, National Institutes of Health, Bethesda, Maryland 20892, the Division of Viral Products, Center for Biologics Evaluation and Research, FDA, Rockville, Maryland 20852, the Section on Human Biochemical Genetics, Heritable Disorders Branch, NICHD, National Institutes of Health, Bethesda, Maryland 20892-1830, and the Laboratoary of Molecular and Cellular Biology, NIDDK, National Institutes of Health, Bethesda, Maryland 20892

In this study, we identify a transport system for tyrosine, the initial precursor of melanin synthesis, in the melanosomes of murine melanocytes. Melanosomes preloaded with tyrosine demonstrated countertransport of 10 μM [3H]tyrosine, indicating carrier-mediated transport. Melanosomal tyrosine transport was saturable, with an apparent Kₘ for tyrosine transport of 54 μM and a maximal velocity of 15 pmol of tyrosine/unit of hexosaminidase/min. Transport was temperature-dependent (Eₗₐₜₗ = 7.5 kcal/mol) and showed stereospecificity for the L-isomer of tyrosine. Aromatic, neutral hydrophobic compounds (such as tryptophan and phenylalanine), as well as the small, bulky neutral amino acids (such as leucine, isoleucine, and methionine) competed for tyrosine transport. Tyrosine transport was inhibited by the classical system L analogue, 2-aminobicyclo[2.2.1]heptane-2-carboxylic acid and by moniodotyrosine, but not by cystine, lysine, glutamic acid, or 2-(methylamino)isobutyric acid. Tyrosine transport showed no dependence on Na⁺ or K⁺, and did not require an acidic environment or the availability of free thiols. These results demonstrate the existence of a neutral amino acid carrier in murine melanocyte melanosomes which resembles the rat thyroid FRTL-5 lysosomal system. This transport system is critical to the function of the melanosome since tyrosine is the essential substrate required for the synthesis of the pigment melanin.

Pigmentation in mammals occurs within melanosomes and results from the deposition of melanin pigment in cells of the skin, hair, and eye (1, 2). Some important biological functions of melanin include protective coloration (3), shielding from ionizing radiation (4), trapping of toxic metabolites (4), sexual attraction within species (3), and proper neurological (ocular) development (5). Melanin synthesis involves a complex series of biochemical reactions that are restricted to melanocyte-specific, membrane-bound organelles termed melanosomes (1–4). The initial, rate-limiting step in melanogenesis is the hydroxylation of tyrosine to 3,4-dihydroxyphenylalanine (DOPA)¹ by the enzyme tyrosinase (monophenyl monoxygenase, EC 1.14.18.1). Subsequent metabolism of DOPA and its derivatives by various melanocyte-specific enzymes, including tyrosinase, tyrosinase-related protein-1 (TRP-1), and tyrosinase-related protein-2 (TRP-2) results in the synthesis of eumelanin, a brown-black pigment (3). The synthesis of phaeomelanins (yellow to red pigments) is poorly understood, but involves the production of cysteinyldopa conjugates (6), also following the production of DOPA from tyrosine. With complete pigmentation, mature melanosomes are extruded from dendritic processes of the melanocyte and taken up by neighboring keratinocytes (4).

Since melanin production requires tyrosine, some mechanism, such as transmembrane import, must supply this amino acid to the melanosome and may be a critical element limiting pigment synthesis. We have previously characterized the transport of various ligands across the membranes of lysosomes, which are intracellular vesicles closely resembling early stages of melanosomes. Carrier-mediated lysosomal membrane transport systems are known to exist for cystine (7, 8) and sialic acid (9), and a tyrosine/moniodotyrosine transporter effecting iodine salvage has been characterized in the lysosomes of rat FRTL-5 thyroid cells (10). In view of these findings, it has been proposed that a tyrosine transport system must exist within the melanosomal membrane (11) and, furthermore, it has been hypothesized that this carrier may be defective in the specific disorder known as oculocutaneous albinism type II (5). However, recent evidence from our laboratories, using cultured melanocytes from the murine model of oculocutaneous albinism type II, the pink-eyed dilution mouse (12), has revealed no significant decrease in plasma or melanosomal membrane tyrosine transport attendant to this mutation (13).

In this study, we have identified a neutral amino acid transporter in murine melanocytes that is responsible for melanosomal tyrosine transport. We have characterized the carrier’s ligand specificity, energy of activation, trans stimulation, pH optimum, ion effects, among other parameters. In view of the requirement for tyrosine within the melanosome to facilitate pigment production, this transporter may serve a critical function in regulating melanogenesis.

EXPERIMENTAL PROCEDURES

Materials—L-[3,5-3H]Tyrosine (48 Ci/mmol) was obtained from Amersham Corp. Nonradioactive amino acids and amino acid analogues were obtained from Sigma Chemical Co., the enzyme tyrosinase (monophenyl monoxygenase, EC 1.14.18.1), and various amino acids and amino acid analogues as indicated.

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** To whom correspondence should be addressed: Section on Human Biochemical Genetics, NICHD, Heritable Disorders Branch, Bldg. 10, Rm. 9S-241, 10 Center Dr., MSC1830, Bethesda, MD 20892-1830. Tel.: 301-496-9101; Fax: 301-402-0234.

† The abbreviations used are: DOPA, 3,4-dihydroxyphenylalanine; TRP-1, tyrosinase-related protein 1; TRP-2, tyrosinase-related protein 2; CCCP, carbonyl cyanide m-chlorophenylhydrazone; MES, 2-(N-morpholino)ethanesulfonic acid; BCH, 2-aminobicyclo[2.2.1]heptane-2-carboxylic acid.

1 The abbreviations used are: DOPA, 3,4-dihydroxyphenylalanine;
resultant melanosome pellets were resuspended in ice-cold 0.25M sucrose, 10 mM HEPES, pH 7.0, and centrifuged at 17,000 g for 1 h at 4°C. The pellet was layered onto top of a discontinuous gradient (24 ml) containing 4% sucrose at 4°C. Samples were subsequently dehydrated with graded alcohols and embedded in epoxy resin. Thin sections were stained with uranyl acetate and lead citrate and examined with a Zeiss EM 912 electron microscope.

Preparation of Sucrose-purified Melanosomes—Highly purified melanosome fractions were prepared as described previously (13). Briefly, a post-nuclear supernatant was layered onto a discontinuous gradient of sucrose 0–10% of the activity measured at 37°C. Tyrosine uptake was detectable under these conditions. To differentiate between true uptake and nonspecific binding, granular fraction transport experiments and measurement of tyrosine loading. Tyrosine loading was measured by resuspending the sample in phosphate-buffered saline, sonicating, and precipitating the protein with 1/10 volume of 40% sulfosalicylic acid. Pellets were recovered by centrifugation and assayed for protein content. The supernatants were filtered through 10K MWCO Centrifree cartridges (Amicon, Danvers, MA), and applied to an LKB 4151 Alpha Plus amino acid analyzer equipped with a 200 × 4.6-mm cation exchange column for physiological fluids, a stepped elution system of five lithium citrate buffers and post-column ninhydrin detection. Post-run integration was carried out with an LKB 2220 integrator in external calibration standard mode. When compared to a panel of 40 standard amino acids, the only free amino acid present in detectable amounts after incubation of melanosomes with tyrosine methyl ester was tyrosine. The concentration of tyrosine could be calculated by comparison of the integrated peak area to that of the known standard.

Tyrosine Countertransport—Reaction mixtures of 4.6 ml of 0.25 M sucrose, 10 mM HEPES buffer containing 10 μM [3H]tyrosine were warmed to 37°C. The reaction was initiated by adding 500 μl of tyrosine-loaded or nonloaded melanosomes derived from the granular fraction (3.7 mg of protein/500 μl) or the sucrose gradient fraction (3.0 mg of protein/500 μl) obtained as detailed above. After vortexing briefly, 300-μl aliquots from triplicate samples were diluted into 10 ml of ice-cold 0.25 M sucrose, 10 mM HEPES (zero time), and vacuum filtered and washed on GF/B glass fiber filters (Whatman, Maidstone, UK) under vacuum, and washed twice with 10 ml of sucroseHEPES buffer. Radioactivity trapped on the filters was measured in an LKB 1219 scintillation counter and converted to picomoles of tyrosine using the specific radioactivity of tyrosine, determined by counting an aliquot of the incubation mixture containing a known concentration of tyrosine. Radioactivity measured at zero time was subtracted from the 1-min values. Tyrosine uptake was calculated as picomoles of tyrosine taken up by melanosomes per unit of hexosaminidase activity.

RESULTS

Centrifugation of a postnuclear supernatant derived from normal murine melanocytes yielded a pigmented granular fraction highly enriched in melanosomes, as indicated by their pigmentation and elliptical shape (Fig. 1, A and B), as well as by the presence of tyrosinase activity (data not shown). The lysosomal enzyme hexosaminidase (B, 16) was also present in this fraction, an activity that has been previously shown to co-purify with melanosomes (17, 18). Fragmented mitochondrial membranes were the major contaminant, but no lysosomal structures were seen in this granular fraction, which was subsequently examined for tyrosine transport activity.

At pH 7.0 and 37°C, [3H]tyrosine uptake was linear for at least 1 min of incubation (data not shown), allowing determination of an initial velocity of tyrosine uptake. This rate increased linearly with tyrosine concentration and then leveled off, indicating saturation kinetics (Fig. 2). The apparent K_m for melanosomal tyrosine uptake at pH 7.0 and 37°C was 54 μM, with a V_max of 15 pmol of tyrosine/unit of hexosaminidase/min. An Arrhenius plot at pH 7.0 yielded an energy of activation of 7.5 kcal/mol and a Q_10 of 1.5 (Fig. 3), which is consistent with carrier-mediated translocation of tyrosine. The initial velocity...
of tyrosine uptake into melanosomes also varied with extramelanosomal pH (Fig. 4). Uptake was maximal at pH 6.5, 20% higher than at pH 7.0, with the extremes of the pH range (6.0–7.5) showing decreased transport.

The structural requirements for ligand recognition by the tyrosine transporter were assessed by measuring competition of various amino acids and analogues with 10 μM [3H]tyrosine for melanosomal uptake. The murine tyrosine transporter resembled the lysosomal system, first described in rat FRTL-5 thyroid cell lysosomes (10), in its pattern of ligand specificity (Table I). It showed stereospecificity for the L-stereoisomer of tyrosine, with minimal competition by D-tyrosine. D-Tryptophan also competed poorly compared to L-tryptophan. Both the murine melanosomal and rat lysosomal carriers also showed the greatest affinity for large, hydrophobic aromatic compounds (i.e. tyrosine, tryptophan, and phenylalanine), as well as bulky neutral amino acids such as leucine and isoleucine. In addition, competition for tyrosine uptake was apparent using the classic system L analogue, BCH (20). The melanosomal

FIG. 1. Ultrastructure of melanosomes purified from melan-a melanocytes. A and B, granular fraction preparations × 9,324 (A) and × 18,500 (B). C and D, sucrose gradient × 13,840 (C) and × 18,500 (D). Melanosomes are elliptical in shape, and appear as highly electron-dense organelles in different stages of maturation (pigmentation), indicative of melanin deposition. Bars represent 1 μm.

FIG. 2. Initial velocity of tyrosine uptake into melanosomes as a function of tyrosine concentration. Melanosomes (granular fractions) were added to mixtures of 0.25 M sucrose, 10 mM HEPES and different concentrations of [3H]tyrosine at 37 °C. Aliquots were removed (zero time), diluted into ice-cold 0.25 M sucrose, 10 mM HEPES and collected onto glass fiber filters. After 1 min at 37 °C, further aliquots were removed and treated similarly. Results plotted represent the difference between 1 min and 0 time points, converted to pmol of tyrosine (based upon the specific radioactivity of tyrosine) and are the means of triplicate determinations at each concentration performed on different days.

FIG. 3. Arrhenius plot for [3H]tyrosine uptake by melanosomes. Melanosomes (granular fractions) were added to 0.25 M sucrose, 10 mM HEPES medium containing 10 μM [3H]tyrosine at 37, 30, or 25 °C for 1 min. The initial velocity of [3H]tyrosine uptake per unit of hexosaminidase was plotted as a logarithmic function of reciprocal temperature in degrees Kelvin.

FIG. 4. pH curve for tyrosine uptake by melanosomes. Buffers were either 50 mM MES (pH 6.0–6.8) or 50 mM HEPES (pH 7.0–7.5). Uptake rate at each pH was determined relative to the rate at pH 7.0 (100%). Points represent means of 3–5 determinations. The mean rate of 10 μM [3H]tyrosine uptake at pH 7.0 was 19.5 ± 5 pmol/min/mg protein or 1.0 ± 0.2 pmol/min/hexosaminidase unit (n = 5).
carrier also had a high affinity for moniodotyrosine, which is transported by the thyroid cell lysosomal transporter (19). 3-(p-Hydroxyphenyl)-propionic acid, which lacks the amino group of tyrosine, did not compete with [3H]tyrosine for uptake, indicating that the ω-amino acid configuration is necessary for ligand recognition. Removal of the ω-carboxyl, as in tyramine, also resulted in lack of competition. Addition of a methylene group to the aliphatic chain (homophenylalanine) had an inhibitory effect on tyrosine uptake similar to that of phenylalanine, suggesting that the length of the aliphatic chain is not a strict requirement for recognition. Adding substituents on the aromatic ring which confer less hydrophobicity also reduced competition compared to phenylalanine, as demonstrated by DOPA. DOPA, a melanin precursor and also a substrate for tyrosinase, inhibited tyrosine uptake by approximately 80%. However, other potential competitors examined, including L-cystine, L-alanine, L-glutamate, and L-lysine, had little or no competitive nature toward this transporter.

Melanosomal tyrosine transport was not affected by the presence of 20 mM ammonium chloride, 5 mM N-ethylmaleimide, or by high concentrations of sodium or potassium (Table II). CCCP and monensin (in the presence or absence of KCl or NaCl, respectively) failed to affect transport, while treatment of melanosomes with the ionophore nigericin in the presence or absence of KCl resulted in a modest decrease in tyrosine transport.

### Table I

| Competitor | Concentration | Melan-a | FRTL-5 |
|------------|---------------|---------|--------|
| L-Tyrosine | 8-10 μM       | 0%      | 0%     |
| 20         | 8%            |         |        |
| 80         | 37            |         |        |
| 100        | 53            |         |        |
| 150        | 76            |         |        |
| 300        | 85            |         |        |
| 500        | 94            | 92%     |        |
| o-Tyrosine | 500 μM        | 18%     | 29%    |
| L-Tryptophan| 500 μM       | 97%     | 96%    |
| L-Phenylalanine| 10 μM     | 99%     | 99%    |
| L-Leucine | 500 μM        | 96%     | 97%    |
| L-ISOleucine| 500 μM       | 91%     | 92%    |
| BCh        | 500 μM        | 81%     |        |
| L-Monoiodotyrosine| 10 μM | 28%     |        |
| L-Methionine| 500 μM       | 30%     |        |
| L-Histidine| 500 μM        | 80%     |        |
| 3-(p-Hydroxyphenyl)-propionic acid | 500 μM | 11% | 11% |
| Tyramine   | 500 μM        | 0%      |        |
| L-Homophenylalanine| 500 μM | 89% |        |
| L-DOPA     | 500 μM        | 79%     |        |
| L-Cystine  | 500 μM        | 30%     |        |
| L-Alanine  | 500 μM        | 38%     |        |
| L-Glutamate| 500 μM        | 11%     |        |
| L-Lysine   | 500 μM        | 16%     | -1%    |
| ω-(Methylamino)-isobutyric acid (MeAIB)| 500 μM | 4% |        |

Results for the FRTL-5 thyroid cell lysosomes are taken from Bernar et al. (10) and converted to % competition.

### Table II

| Compound | [3H]Tyrosine uptake |
|----------|---------------------|
| None     | 100%                |
| NH4Cl (20 mM) | 106%           |
| N-Ethylmaleimide (5 mM) | 93%       |
| NaCl (100 mM) | 102%        |
| KCl (100 mM) | 93%         |
| CCCP (25 μM) | 101%        |
| CCCP/KCl (25 μM/100 mM) | 99%       |
| Monensin (20 μM) | 100%       |
| Monensin/NaCl (20 μM/100 mM) | 86%       |
| Nigericin (25 μM) | 63%        |
| Nigericin/KCl (25 μM/100 mM) | 68%       |

Uptake of 10 μM [3H]tyrosine was measured in the presence of the noted compounds and is expressed as a percentage of control uptake, which averaged 2.5 ± 2.2 pmol of [3H]tyrosine uptake/unit of hexosaminidase. Incubation mixtures were warmed to 37°C before addition of melanosomes. For NH4Cl studies, melanosomes were pre-incubated for 20 min at 37°C before adding [3H]tyrosine. Results are the means of triplicate determinations in two independent experiments.

In order to address the possible contribution of contaminating lysosomes to the measured tyrosine transport by melanosomes in the granular fraction, further purification on a discontinuous sucrose gradient was performed, yielding highly purified melanosomes at density ≥1.8 M (Fig. 1, C and D). Over 90% of the organelles in these preparations were estimated visually to be melanosomes and no lysosomal structures were seen in these fractions (i.e. with density ≥1.8 M). When comparable subcellular fractions derived from non-pigmenting melanocytes (e.g. pink-eyed dilution melanocytes (13), or albino melan-c (21) melanocytes) were similarly purified by sucrose density gradients, no hexosaminidase activity could be found in the ≥1.8 M fractions (data not shown). Since these non-pigmented melanocytes contained a normal lysosome content, the lack of hexosaminidase activity reflects a lack of authentic lysosomes sedimenting at sucrose densities ≥1.8 M.

Melanosomes were found at that density, however, and possessed an esterase activity necessary to convert amino acid methyl esters to the free amino acids (22); this allowed melanosomes to be loaded for more definitive countertransport experiments. In fact, free tyrosine concentrations of 12.2 nmol/mg protein and 2.5 nmol/mg protein could be achieved for granular fraction melanosomes and sucrose gradient purified melanosomes, respectively, after incubation in 1 mM tyrosine methyl ester for 25 min at 37°C. These levels represented 15–30-fold increases in tyrosine content compared to controls not exposed to tyrosine methyl ester, and allowed for a demonstration of trans stimulation, or countertransport, of tyrosine across the melanosomal membrane.

In countertransport, which serves as classical evidence for carrier-mediated rather than diffusional transport (23), tracer amounts of a radiolabeled substance will cross a membrane at an increased rate if there is a substantial concentration of the nonradioactive substance on the opposite side of the membrane. Once the radioactive tracer has moved across the membrane it is effectively trapped due to competition with saturating amounts of the nonradioactive compound within; it therefore cannot exit the vesicle (16). In this study, the radioactive tracer used outside of the melanosomes was 10 μM [3H]tyrosine, and the nonradioactive competitor was tyrosine placed inside the melanosomes by pre-exposure to 1 mM tyrosine methyl ester. The tyrosine-loaded melanosomes took up much more [3H]tyrosine than did non-loaded melanosomes in both the granular fraction (Fig. 5A) and in the sucrose gradient purified fraction (Fig. 5B). The countertransport activity of the...
highly purified melanosomes derived from the ≥1.8 M sucrose gradient fraction amounted to 10% of the total tyrosine transport activity of the postnuclear supernatant. When tyrosine transport activity was expressed as units, i.e. picomoles of tyrosine/min/unit of hexosaminidase, the values of the granular and sucrose fractions were essentially the same (1.4 and 0.8, respectively), verifying carrier-mediated tyrosine transport in a melanosomal fraction.

DISCUSSION

The primary function of melanosomes within melanocytes is to produce melanin, whose synthesis is initiated by the tyrosinase-catalyzed conversion of tyrosine to DOPA (1–4). This process creates a tyrosine “sink” by consuming the amino acid, and emphasizes the critical nature of tyrosine entry into melanosomes in order for pigmentation to occur. We now show that tyrosine entry into melanosomes is carrier-mediated, as demonstrated by its saturability (Fig. 2), its ligand competition (Table I), and its trans stimulation, or countertransport, both in melanosome-rich granular fractions and in sucrose-gradient purified melanosomes (Fig. 5, A and B). Furthermore, melanosomal tyrosine transport displayed a temperature dependence (Q_{10} = 1.5, Fig. 3) consistent with carrier-mediated uptake rather than other mechanisms such as diffusion or movement via a membrane channel. Recently, Pisoni et al. described a cysteamine transporter in human fibroblast lysosomes with a Q_{10} value similar to that of this melanosomal tyrosine transporter (24).

It is not surprising that a carrier for amino acid transport resides within the melanosomal membrane, in view of the many lysosomal transporters known to exist (20, 22) and the similarities in organellogenesis between melanosomes and lysosomes (17, 18). Melanosomes contain the lysosomal integral membrane protein LAMP-1 (25), as well as acid phosphatase (26) and various other “lysosomal” hydrolases such as β-N-hexosaminidase and cathepsins B and L (17). In this study, we have demonstrated the presence of an esterase activity within murine melanosomes which cleaves tyrosine methyl ester to yield free tyrosine. How the various lysosomal enzymes are directed to the melanosome remains to be determined; they may enter by the same mechanism used by the melanocyte-specific enzymes tyrosinase, TRP-1, and TRP-2. These proteins are targeted to “premelanosomes” via coated vesicles, which originate from the trans-Golgi network (27), and apparently lack mannose 6-phosphate receptor binding sites (28). Alternatively, the tyrosine carrier itself may be directed to the melanosomal membrane via mechanisms independent of small vesicle delivery. For example, the melanocyte-specific protein, Pmel 17/silver (29), has been shown to co-purify with immature and mature melanosomes but is not detected in small vesicle fractions and presumably is targeted to melanosomes via another processing route (30).

Using tyrosinase as a marker for melanosomes, only a 2-fold purification of melanosomes is achieved in the granular fraction and sucrose gradient fraction compared with the whole cell homogenate (data not shown). This may be due to the fact that tyrosinase, and even typical lysosomal enzymes such as hex-
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**osaminidase, sediment also in a light, coated vesicle fraction** which does not sediment with either the granular fraction or the denser sucrose gradient fraction (27). Additionally, as melanosomes become highly pigmented and travel to the periphery of the melanocyte (i.e. reach maturation), they may lose their tyrosinase activity due to dilution or inhibition of enzymatic activity. They may also lose their esterase activity, and this may be the reason for the 5-fold higher level of tyrosine loading in the less pure (and less mature) granular fraction compared with the sucrose gradient purified melanosomes. The greater manipulation involved in preparing the sucrose gradient purified melanosomes may also have influenced its tyrosinase and esterase activities.

The biochemical similarities between melanosomes and lysosomes (31) make it difficult to eliminate a small lysosomal contribution to tyrosine transport in our melanocyte granular fraction, since both melanosomes and lysosomes contain hepxosaminidase activity (8, 16–18) and other shared hydrodases (16, 17). Therefore, we set out to demonstrate that highly purified melanosomes (i.e. purified by high density sucrose gradient ultracentrifugation) exhibited tyrosine countertransport. In fact, this fraction contained a specific activity of tyrosine countertransport, expressed as picomole/min/unit of hexosaminidase, similar to that of the crude granular fraction. Yet these highly purified melanosomes lacked discernible lysosomes whether morphological (Fig. 1, C and D) or genetic/biochemical means were employed to identify them. Specifically, non-pigmented murine melanocytes (pink-eyed dilution and melan-c, both of which have mutations that affect pigmentation but not their lysosome content) exhibited no hexosaminidase activity (i.e. they had no melanosomes or lysosomes) in the sucrose fractions where pigmented melan-a melanosomes sedimented at ≥1.8 M (data not shown). Therefore, to the best of our ability to detect them, lysosomes are not present in the pigmented melanosomal fraction exhibiting tyrosine countertransport. Based on the morphological and biochemical evidence presented above, we conclude that the tyrosine transport we measured in these studies is largely melanosomal. It is critically important to demonstrate that melanosomes (as opposed to possibly contaminating lysosomes) effect tyrosine transport because pigmentation, which utilizes enzymes not associated with lysosomes, occurs only in melanosomes. This organelle has a unique requirement for the import of tyrosine in order for the process of melanogenesis, or pigment production, to proceed.

Melanosomal tyrosine transport resembled lysosomal tyrosine transport in many ways. It was not affected by alkalinization of the melanosome with ammonium chloride (32), by blocking of free thiolis with N-ethylmaleimide (7), or by high concentrations of sodium or potassium (Table I). Therefore, there was no evidence that the carrier operated through a structural change involving a protonated intermediate or that it required free thiolis for binding, translocation, or release of tyrosine; furthermore, the system did not appear cation-dependent. The ionophores CCCP and monensin, with or without potassium or sodium, respectively, had no effect on tyrosine transport. Nigericin alone, or in the presence of KCI, effected a modest decrease in tyrosine uptake, perhaps indicating a small dependence on membrane potential. Since high concentrations of potassium did not inhibit tyrosine transport, nigericin was presumably responsible for this decrease.

The melanosomal tyrosine carrier showed a preference for the large, aromatic compounds tyrosine, phenylalanine, and tryptophan, as well as smaller neutral amino acids such as leucine and isoleucine and to some extent, methionine and histidine. It also displayed a lack of dependence upon extra-melanosomal sodium, and was inhibited by the classical system L analogue, BCH, suggesting a similarity to rat thyroid lysosomal carrier (8, 16–18) and human fibroblast lysosomal carrier (9). The melanosomal tyrosine carrier did not recognize the classic plasma membrane system A analogue, MeAIB, or other amino acids which primarily utilize carriers other than lysosomal systems I and h (see Refs. 11, 20, and 22 for reviews). Including the cysteine carrier, system C for lysine, systems e and f for alanine, or system d for glutamate (Table I), Collarini et al. (33) described the existence of a membrane transporter in human fibroblast lysosomes, termed system t, that is more selective for aromatic amino acids than systems I and h, having a higher affinity for tyrosine, tryptophan, and phenylalanine. Other characteristics of system t include affinity for D-isomers of aromatic amino acids, and sensitivity to the sulfhydryl-reactive agent, N-ethylmaleimide. In contrast, the melanosomal tyrosine carrier characterized in this study displayed a strong preference for the L-stereoisomer of tyrosine, and was not inhibited by D-tyrosine and only modestly by D-tryptophan. Furthermore, melanosomal tyrosine transport was not sensitive to N-ethylmaleimide, suggesting that in melanocytes, the primary route for tyrosine entry into melanosomes is via a single carrier that resembles systems h and t. Genetic similarities between the rat and mouse may explain the lack of system t in these species, while human fibroblasts may have evolved more differentiated transport systems.

While most lysosomal carriers transport the small molecule products of lysosomal hydrolysis out of vesicles (20, 22), melanosomes appear to effect a net inward flux of tyrosine for melanogenesis. The melanosomal tyrosine carrier does resemble one lysosomal amino acid transport system in this respect; the cysteine transporter appears to favor a net inward flux, which Pisoni et al. (34) have proposed promotes proteolytic digestion within lysosomes. In melanosomes, the inward flux of cysteine could have a physiological basis since cysteine is required for the synthesis of the cysteinylidopa conjugates which eventually form the yellow to red pigment, phaeomelanin (6). It remains to be determined if the melanosomal membrane possesses a carrier capable of transporting cysteine.

The melanosomal tyrosine carrier strongly resembles the lysosomal system h first described in rat FRTL-5 thyroid cell lysosomes (10). The two systems share ligand specificities, even for moniodotyrosine (16) (Table I), and have similar apparent K_m values for tyrosine and similar energies of activation, 7.5 kcal/mol (Fig. 3) and 9.7 kcal/mol (10). But most intriguing is the role that each vesicular transport system plays in achieving the differentiated function of its specific cell type. While membrane transport is bidirectional and its flux is determined by ligand concentrations on the two sides of the membrane (20, 22), tyrosine and moniodotyrosine egress from lysosomes is required for salvage of iodine and tyrosine for subsequent reincorporation into thyroglobulin in thyroid cells (16); tyrosine entry into melanosomes is necessary for the synthesis of melinin in melanocytes. Both of these processes are fostered by a sink on one side of the membrane, and both processes highlight how physiological conditions and the functional imperative of the cell dictate the net direction of movement across membranes. In addition, both processes may be subject to hormonal regulation. In rat FRTL-5 cells, thyroid-stimulating hormone (or thyrotropin) markedly up-regulates the lysosomal tyrosine/moniodotyrosine carrier at the transcriptional level (35). In the mouse, the expression of various melanocyte-specific enzymes localized to the melanosome and involved in melanogenesis is regulated in response to a-melanocyte-stimulating hormone (2). Whether melanosomal tyrosine transport is also regulated by these or other factors remains an interesting
possibility to be determined.

Finally, since both melanosomes and lysosomes contain amino acid carriers and known lysosomal transport defects result in certain diseases (i.e. cystinosis and Salla disease), some tyrosinase-positive forms of human albinism may be due to impaired tyrosine uptake across the melanosomal membrane.

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J. Biol. Chem. 1996, 271:4002-4008.
doi: 10.1074/jbc.271.8.4002

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