Regulation of Lysosomal Sulfate Transport by Thyroid Hormone*

Hsu-Fang Chou, Merry Passage, and Adam J. Jonas
From the Division of Medical Genetics, E4, Department of Pediatrics, Harbor-UCLA Medical Center, Torrance, California 90502

Sulfate transport was examined in rat liver lysosomes that were isolated from thyroid hormone-treated, thyroidec- tomed, and control animals. Sulfate uptake was significantly decreased in lysosomes from animals that had received intraperitoneal T3 (3,5,3′-triiodothyronine) at a dose of 20 μg/100 g body weight. The effect of T3 was maximal by 24 h post-injection and resulted in marked decreases in both Vmax (control: 155 ± 33 pmol/unit of β-hexosaminidase/30 s versus T3 treated: 24 ± 7 pmol/unit of β-hexosaminidase/30 s) and Km (control: 213 ± 34 μM versus T3 treated: 92 ± 6 μM). Thyroidectomy was associated with a significant increase in Vmax (control: 250 pmol/unit of β-hexosaminidase/30 s versus thyroidectomized: 564 pmol/unit of β-hexosaminidase/30 s), while Km was not significantly affected. The effect of thyroid hormone on lysosomal sulfate transport appeared to be relatively specific. In contrast to its effect on sulfate transport, T3 treatment had no effect on the uptake of either glucose or N-acetylglucosamine by rat liver lysosomes. Lysosomal pH, acidification in response to Mg/ATP, and the specific activities of α-L-iduronidase, β-hexosaminidase, β-N-glucosidase, and acid phosphatase were unaffected by T3 administration. Incubation of T3 with lysosomes from control animals had little or no effect on sulfate transport. Treatment of isolated lysosomes with either protein kinase A or alkaline phosphatase resulted in modest stimulation of transport. Thus, T3 does not appear to regulate transport by either direct interaction with the lysosomal transporter or protein kinase A-mediated phosphorylation. The exact mechanism for the inhibitory effect of T3 on lysosomal sulfate transport remains to be determined.

Macromolecules are digested in lysosomes, and the resulting degradation products are either released to the cytoplasm for reuse or are secreted from the cell. The exodus of these small molecular weight materials from lysosomes is mediated by an array of specific transport systems. Thus far, more than 20 carrier-mediated transport systems for sugars, amino acids, peptides, ions, vitamins, and nucleosides have been described in the lysosomes of rat liver as well as human white blood cells, cultured fibroblasts, and cultured Epstein-Barr virus transformed lymphocytes (1, 2). These systems are components of a complex series of steps involving the recycling of nutrients. A limited number of studies have suggested that at least some of the lysosomal transport systems are subject to regulation. However, in contrast to the large body of information that has been gathered regarding the hormonal regulation of plasma membrane transport systems, regulation of lysosomal transport systems has been largely unstudied.

Earlier studies have suggested that lysosomal membrane structure and permeability are influenced by a variety of hormones (3). Steroid hormones, like progesterone (4), estradiol-17β, testosterone (5), and hydrocortisone (6), have been shown to change the liability and permeability of the rat liver lysosomal membrane both in vitro and in vivo. These effects appear to be primarily due to the direct effects of steroid compounds on the physical state of the lipid bilayer. Recently, Jadot et al. (7) suggested a direct and more specific effect of steroids on lysosomal glucose transport. Inhibition of membrane permeability to glucose was observed when rat liver lysosomes were incubated with small amounts (0.1 μM) of diethylstilbestrol, an estrogen analog. The exact mechanism for this effect is uncertain. Significant morphological alteration of liver lysosomes has been observed when glucagon is administered to rats. Lysosomal swelling occurs following in vivo treatment with glucagon with a 400–800% increase in size as determined by electron microscopy and consequent increased fragility and osmotic sensitivity (8–10). The mechanism responsible for this in vivo effect was thought to be due to increased autophagic vacuole formation. Phosphorylation of several lysosomal membrane proteins has also been observed following glucagon administration, presumably through the action of protein kinase A (11) or cAMP independent kinase on lysosomal membranes (3). It is not clear that phosphorylation is directly related to the phenomenon of swelling associated with glucagon administration.

In one unique system, thyrotropin has been shown to have a direct stimulatory effect on lysosomal transport (12). Addition of 0.1 μM thyrotropin to the culture medium of rat thyroid epithelial cells results in up to a 7-fold increase in the rate of tyrosine countertransport in lysosomes subsequently isolated from these cells. Transport of other substrates of the tyrosine transporter such as phenylalanine and iuecine is also enhanced by thyrotropin. The system functions as a hormonally sensitive salvage pathway for iodine through transport of moniodothyrosine. The effect of thyrotropin appears to require RNA synthesis, protein synthesis, and cyclic AMP. These findings are consistent regulation of steps involved in thyroid hormone synthesis and release.

We have previously described a carrier-mediated system for the transport of sulfate in rat liver lysosomes (13). This system, which is sensitive to pH and membrane potential, allows sulfate, a major degradation product of glycosaminoglycans and sulfolipids, to exit the lysosome. The kinetics of lysosomal sulfate transport have been characterized through in vitro studies using isolated lysosomal membranes. Although such studies are extremely useful in terms of system identification, they can only provide limited insights regarding in vivo function and regulation of transport. Thyroid hormone is known to play a role in glycosaminoglycan metabolism by decreasing glycosaminoglycan synthesis (14). We postulated that as a consequence, decreased cellular requirements for free sulfate might have an effect on lysosomal recycling of sulfate. We report our observa-

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† To whom correspondence should be addressed.
Lysosomal Sulfate Transport and \( T_3 \)

Sulfate and glucose uptake were measured in lysosomes isolated from \( T_3 \)-treated and control rats. Lysosomes were incubated in isotonic sucrose buffered with 20 \( \mu \)M MES, pH 5.5 for 30 s for sulfate uptake or 15 s for glucose uptake as described under "Materials and Methods." Results were expressed as percent uptake of their paired controls. Values are mean ± S.D. Actual uptake rates for controls were 57 ± 23 pmol of sulfate/unit of \( \beta \)-hexosaminidase/30 s, mean ± S.D., \( n = 12 \) and 558 ± 155 pmol of glucose/unit of \( \beta \)-hexosaminidase/15 s, mean ± S.D., \( n = 9 \).

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<th>( T_3 ) treatment</th>
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TABLE 1

Effects of thyroid hormone on sulfate and glucose transport by lysosomes

The abbreviations used are: \( T_3 \), 3,5,3'-triiodothyronine; MES, 4-nor-
phellinethanesulfonic acid; FITC, fluorescein isothiocyanate.

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**Materials and Methods**

All chemicals used in this investigation were obtained from Sigma unless otherwise indicated. \( ^{35} \)SNa\(_2\)SO\(_4\) (1130 \( \mu \)Ci/ml), \( ^{25} \)U-D-glucose (250 \( \mu \)Ci/mmol), and \( \beta \)-acetyl-\( \beta \)-glucosaminide (10 \( \mu \)Ci/mmol) were purchased from ICN Biochemicals, Inc., Scinventure II from Fisher, Dulbecco’s phosphate-buffered saline without calcium from Irvine Scientific, and cy-L-iduronidase (17) were measured fluorometrically (16) using 4-methylumbelliferyl-N-\( \beta \)-acetyl-\( \beta \)-glucosaminide, 4-methylumbelli-
fril-\( \beta \)-glucopyranoside, and 4-methylumbelliferyl-\( \alpha \)-d-glucoside as substrates, respectively. Alkaline phosphatase activity was determined spectrophotometrically as described previously using \( p \)-nitrophenyl phosphate as substrate (18). Protein content was measured colorimetrically using Coomasie Brilliant Blue (19). Plasma concentrations of \( T_3 \) were determined by radioimmunoassay (20).

**Lysosomal pH**—Lysosomal pH was determined by measurement of the fluorescence of fluorescein isothiocyanate-dextran (FITC-dextran) loaded lysosomes as described previously (21, 22). Rats received intra-
peritoneal injections of FITC-dextran (\( M_r = 70,000 \)) at a concentration of 20 mg/150 g body weight 16 h prior to isolation of lysosomes. Lysosomal pH was determined at room temperature using approximately 50 \( \mu \)g of lysosomal protein suspended in 2 ml of 0.25 M sucrose, 5 mM MgCl\(_2\), and 20 mM Heps, pH 7.0. Fluorescence was recorded in a spectrophotometer using an excitation wavelength of 495 nm and an emission wavelength of 550 nm. After establishment of baseline fluorescence, 40 \( \mu \)l of 100 mM ATP was added and the fluorescence was followed. Base-line and post-ATP measurements were adjusted for lysosomal breakage by subtracting the fluorescence value after the sample was filtered through a 0.22-\( \mu \)m filter from the fluorescence of the unfiltered sample. In order to determine total fluorescence, lysosomes were lysed by the addition of 20 \( \mu \)l of 10% Triton X-100 to the incubation mixture and fluorescence was again recorded. Relative fluorescence was then converted into pH using a standard curve of the fluorescence of 1 \( \mu \)g/ml FITC-dextran in Heps/sucrose buffer at varying pH values.

**Phosphorylation Studies**—Lysosomes isolated from controls or rats treated with \( T_3 \) for 2 days were incubated for 10 min at 37°C in 0.25 M sucrose, 2 mM Heps, pH 7.0, with 1 mM ATP, 1 mM MgCl\(_2\), 10 \( \mu \)M CaCl\(_2\), and 10 \( \mu \)M dibutyryl-cAMP. Protein kinase A was used as an enzyme for these experiments protein kinase A was incubated for 5 min with 40 \( \mu \)g of protein kinase inhibitor at 25°C before addition of lysosomes. For these phosphorylation studies, lysosomes were incubated in 0.25 M sucrose, 2 mM Heps, pH 7.0, with 1 mM MgCl\(_2\), and 10 \( \mu \)M CaCl\(_2\), and 0.138 units of alkaline phosphatase. In some experiments, alkaline phosphatase was inhibited by 20 \( \mu \)g of Tetramisole (alkaline phosphatase inhibitor) before incubation with lysosomes. After incubation, lysosomal sulfate transport was determined as described above.

**RESULTS**

In order to determine whether thyroid hormone has an effect on lysosomal sulfate transport, liver lysosomes were isolated from rats which had received daily intraperitoneal injections of \( T_3 \). Plasma \( T_3 \) concentrations were determined in three represen-
tative animals 24 h following the final intraperitoneal dose. Values were 845 ng/dl after a single dose, 1268 ng/dl after two doses, and 955 ng/dl after four doses as compared to 110 ng/dl in the untreated control rats. Lysosomal sulfate transport was determined as described above.

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phellinethanesulfonic acid; FITC, fluorescein isothiocyanate.
The effect of hypothyroidism on lysosomal sulfate transport was studied using thyroidectomized rats. Plasma T₃ concentration was decreased by approximately 60% over in thyroidectomized animals compared to control at the time of sacrifice with a mean value of 43 ± 7 ng/dl, n = 4, mean ± S.D. Liver lysosomes were isolated from these animals 7–13 days after removal of the thyroid gland so as to allow for decline in thyroid hormone concentration. Lysosomal sulfate transport was determined using the standard assay mixture and was increased in the thyroidectomized animals by 232 ± 64%, mean ± S.D., n = 5 compared to sham-operated controls. Kinetic analysis revealed a Kₘ of 299 μM and a Vₘₐₓ of 564 pmol/unit of β-hexosaminidase/30 s in the thyroidectomized animals versus a Kₘ of 289 μM and Vₘₐₓ of 250 pmol/unit of β-hexosaminidase/30 s in the sham-operated controls. Thus, the hypothyroid state was associated with an increase in Vₘₐₓ but no significant change in Kₘ. The Vₘₐₓ in the hypothyroid state was approximately 23-fold greater than that observed 24 h after induction of a hyperthyroid state by administration of T₃. It should be noted that the sham-operated controls exhibited moderately increased Kₘ and Vₘₐₓ when compared to control animals used in other experiments. This may reflect the increased age of the sham-operated controls. Hanes plot analysis of lysosomal sulfate uptake from controls, animals treated with T₃ for 2 days, thyroidectomized and sham-operated controls are shown in Figs. 1 and 2.
brane fragility, lysosomal protein import, or protein turnover in order to rule out a general effect of thyroid hormone on membrane transport has no apparent effects on lysosomal acidification. In vesicles from T$_3$-treated animals was 43.5 pmol/mg protein versus 0.1 for the controls (mean ± S.D., n = 3). Thus, thyroid hormone treatment was observed in membrane vesicles derived from T$_3$-treated animals compared to transport in membrane vesicles from controls. The initial rate of transport in membrane vesicles from T$_3$-treated animals was 43.5 pmol/mg protein/30 s, n = 2 versus 148 pmol/mg of protein/30 s, n = 2 in controls. Thus, administration of T$_3$ seems to have a primary effect on membrane transport rather than a secondary effect on the intralysosomal milieu. Since lysosomal sulfate transport is influenced by pH, direct measurement of lysosomal pH in FITC-dextran loaded liver lysosomes was performed using rats that had received supplemental T$_3$ 24 h earlier. The pH of lysosomes isolated from the T$_3$-treated animals was 5.3 ± 0.1 versus 5.4 ± 0.1 for the controls (mean ± S.D., n = 3). Thus, thyroid hormone treatment has no apparent effects on lysosomal acidification. In order to rule out a general effect of thyroid hormone on membrane fragility, lysosomal protein import, or protein turnover during the study interval, the specific activities of a number of soluble and membrane-bound lysosomal hydrolases were determined using lysosomes that had been isolated from T$_3$-treated and hypothyroid animals. These values were not significantly different from control values, allowing us to express transport in terms of hexosaminidase activity and again pointing to the specificity of the effect of T$_3$ (Table II). T$_3$ is recognized to influence membrane transport systems through a variety of mechanisms including direct effects, post-translational modification, and transcriptional regulation. Lysosomes which had been isolated from control animals were incubated with various amounts of T$_3$. Sulfate transport was measured at pH 5.0 over 1-min intervals. Values are mean ± S.D. (n = 3, from one single experiment). Differences between controls and T$_3$-treated lysosomes was compared by Student's t test.

**DISCUSSION**

We have demonstrated that sulfate transport in rat liver lysosomes is subject to regulation by thyroid hormone. The effect appears to be relatively specific in that other lysosomal membrane processes such as acidification and glucose transport are unaffected by T$_3$ administration. The fact that the effect of T$_3$ was demonstrable in resealed membrane vesicles with controlled internal environments also suggests it is not mediated through changes in lysosomal volume, pH, membrane potential, or substrate concentration. The specificity of
the effect argues against a generalized effect on membrane composition and fluidity as has been observed under some circumstances (24–27). The specific activities of a variety of lysosomal hydrolases, both soluble and membrane-bound, were unchanged following administration of T₃ in vivo making it unlikely that the changes seen in sulfate transport are due to generalized changes in lysosomal protein content.

While it appears that thyroid hormone has a relatively specific effect on lysosomal sulfate transport, this conclusion must be entertained with some caution. Since only two transport systems were examined in our study, it still remains possible that other lysosomal membrane transport systems are also subject to regulation by thyroid hormone. In addition, changes in the activity of a transporter with a very long half-life and a slow rate of synthesis might have gone undetected due to the limited length of the study period. Nevertheless, the effects of thyroid hormone on lysosomal sulfate transport are striking.

Since thyroid hormone has widespread effects on the membrane transport of amino acids (28, 29), glucose (23, 30), and ions (31–35), it is not surprising that it has an effect on a lysosomal transport process. Interestingly, studies of plasma membrane glucose transport have shown that thyroid hormone may have multiple effects on a single transport system. For instance, limited exposure of chick embryo cardiac myocytes to physiological doses of T₃ may stimulate glucose uptake via a mechanism that does not involve protein synthesis but is related to changes in cytoplasmic calcium concentration. However, prolonged exposure to T₃ results in a decrease in transport that is maximal by 24 h and which involves RNA and protein synthesis (36–38). Early stimulation followed by inhibition that is maximal by 24 h is similar to what we have observed with lysosomal transport following administration of T₃. It is possible that we are observing two processes with regard to lysosomal sulfate transport, an early activation phase such as might be seen with post-translational modification of the carrier followed by progressive inhibition. Transcriptional or translational control could conceivably account for the inhibitory phase. The impressive decline in both Vₘₐₓ and Kₘₐₓ after T₃ treatment suggests mixed effects with a decrease in carrier numbers and alteration of the carrier.

Most of our studies were performed using pharmacologic doses of T₃ (20 µg/100 g body weight) with subsequent 10-fold increases in serum T₃ concentration. It has been reported that 24 h after the last of four consecutive doses of 4 µg of T₃/100 g body weight/day, T₃ concentrations in rats are increased by approximately 50% (39). We found that administration of a single, smaller dose of 2 µg of T₃/100 g body weight had inhibitory effects on transport similar, albeit somewhat smaller, than those seen with pharmacologic doses. This argues that T₃ has a physiologic regulatory effect on lysosomal sulfate transport.

The mechanism(s) of action of thyroid hormone on lysosomal sulfate transport remains uncertain. Lysosomal sulfate transport is unchanged when purified lysosomes are incubated with T₃ in vitro suggesting that T₃ does not directly interact with the transporter. Obviously, this does not rule out in vivo activation of other systems by T₃ such as kinases which may modify the transport protein. Phosphorylation has been observed in the case of the GLUT1 and GLUT4 glucose transporters (40). Treatment of lysosomes with protein kinase A results in a modest stimulation of transport but it is not clear that this is a direct result of phosphorylation of the transporter. Issues of substrate specificity and indirect effects via phosphorylation of other proteins may account for the contradictory data obtained with alkaline phosphatase. Our results suggest that, while protein kinase A-mediated phosphorylation may affect sulfate transport, this does not appear to be the primary mechanism by which T₃ inhibits lysosomal sulfate transport. It remains possible, although unproven, that phosphorylation accounts for the short-term increase in transport seen after T₃ administration.

Since phosphorylation does not account for the inhibitory effect of T₃, another mechanism of action must be entertained. T₃ is known to have an effect on the expression of many genes (41–44) and such a mechanism may account for the regulation of lysosomal sulfate transport. It is possible that direct regulation of expression might account for the specificity of the T₃ effect. It is also possible that T₃ regulates expression of another gene that ultimately has a direct effect on the transporter. Definitive studies of the mechanism of thyroid action will be greatly facilitated by isolation of the protein and/or gene for the lysosomal sulfate transport system.

In addition to its effects on lysosomal sulfate transport, physiological doses of T₃ markedly inhibit the synthesis and accumulation of glycosaminoglycans, a major source for the production of lysosomal inorganic sulfate (14). Lysosomal sulfate transport may prove to be but one component of a coordinated series of steps involved in the synthesis and degradation of glycosaminoglycans. It is intriguing to speculate that T₃ serves a specific regulatory role for these events, perhaps at the level of transcription.

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REFERENCES