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Inactivation of Tyrosine Aminotransferase in Neutral Homogenates and Rat Liver Slices

By F. AURICCHIO, L. MOLLICA and A. LIGUORI
Cattedra di Istituzioni di Patologia Generale, Istituto di Patologia Generale, Università di Napoli, S. Andrea delle Dame 2, 80138 *Napoli, Italy*

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Inactivation of tyrosine aminotransferase induced *in vivo* by triamcinolone was studied in a homogenate incubated at neutral pH values. The integrity and the presence of subcellular particles together with a compartment of acidic pH are necessary for inactivation of tyrosine aminotransferase. It is suggested that tyrosine aminotransferase is inactivated inside lysosomes. The system responsible for inactivation of tyrosine aminotransferase was partially purified and identified with lysosomal cathepsins B and B_1. Inactivation of tyrosine aminotransferase in liver slices is controlled by the amino acid concentration and strongly stimulated by cysteine. 3,3′,5-Tri-iodo-L-thyronine reversibly and strongly decreases the rate of inactivation of tyrosine aminotransferase. The effect is not due to an increased rate of tyrosine aminotransferase synthesis.

In spite of the increasing interest in protein degradation in bacteria (Nath & Koch, 1970; Goldberg, 1971) and in mammalian cells (Schimke, 1969; Auricchio *et al.*, 1969; Hershko & Tomkins, 1971; Hershko *et al.*, 1971) very little is known of the mechanisms responsible for the degradation of intracellular protein *in vivo* and their regulation.

A possible degradative system is the lysosome. However, lysosomes are thought to be involved more in cell involution and gross intracellular autophagy than in degradation of specific proteins under normal cellular conditions (Schimke, 1969).

Tyrosine aminotransferase (EC 2.6.1.5) is an enzyme inducible by glucocorticoids in rat liver (Lin & Knox, 1957) and in hepatoma cells (Thompson *et al.*, 1966). The rate of degradation of this enzyme is fast (Lin & Knox, 1958; Kenney, 1962; Auricchio *et al.*, 1969) and controlled by such factors as concentration of nutrients (Auricchio *et al.*, 1969), intracellular ATP concentrations (Hershko & Tomkins, 1971), and hormones (Hershko *et al.*, 1971).

We reported that tyrosine aminotransferase is inactivated in acidic rat liver homogenates (Auricchio & Liguori, 1971). This inactivation is due to a lysosomal thermolabile thiol-dependent system working only at acid pH values.

In the present paper we report that tyrosine aminotransferase is inactivated also in homogenates at neutral pH values, and that this inactivation seems to be due to the lysosomal system previously described (Auricchio & Liguori, 1971). It requires the integrity of subcellular particles, probably because tyrosine aminotransferase is inactivated within the lysosome. Inactivation of tyrosine aminotransferase is a degradative process caused by cathepsins. Both lysosomal cathepsins B (EC 3.4.4.–) and B_1 inactivate tyrosine aminotransferase. The inactivating system has been partially purified and characterized.

Comparative studies on the inactivation of tyrosine aminotransferase were done with rat liver slices. The lysosomal inactivating enzymes for tyrosine aminotransferase appear to be responsible for tyrosine aminotransferase inactivation in slices, and this finding suggests that lysosomes can degrade proteins *in vivo*. The regulation of the inactivation of tyrosine aminotransferase has been investigated. Compounds able to increase or decrease the rate of inactivation of tyrosine aminotransferase in both liver homogenates and slices have been found. Our results confirm that hormones can control protein concentrations by mechanisms other than protein synthesis (Hershko *et al.*, 1971).

A preliminary account of part of this work has been presented (Auricchio *et al.*, 1971).

Materials

Triamcinolone (9α-fluoro-16α-hydroxyprednisolone diacetate), a tyrosine aminotransferase inducer (Hayashi *et al.*, 1967), was generously given by Lederle Laboratories, Pearl River, N.Y., U.S.A. [^{3}H]Leucine was purchased from New England Nuclear Corp., Chicago, Ill., U.S.A. Cycloheximide was from Sigma Chemical Co., St. Louis, Mo., U.S.A. 3,3′,5-Tri-iodo-L-thyronine and dithiothreitol were from Calbiochem, Los Angeles, Calif., U.S.A. 'Stable' tyrosine aminotransferase was a partially purified tyrosine aminotransferase preparation free from the inactivating system; the first steps of a

published procedure were done, including the heat treatment (Valeriote *et al.*, 1969).

In all experiments, except for the purification of the tyrosine aminotransferase-inactivating system, male Wistar rats were injected intraperitoneally with 45mg of triamcinolone in 4ml of 0.15M-NaCl and killed after 10h.

Methods

Experiments with rat liver homogenate

Rat livers were homogenized in 10vol. of Krebs–Ringer phosphate buffer (Cohen, 1957), pH7.4, either with 10 strokes in a Potter–Elvehjem homogenizer fitted with a very loose Teflon pestle ('gentle' homogenization) or with 40 strokes in a Potter–Elvehjem homogenizer fitted with a tight pestle followed by 5min of homogenization in an MSE homogenizer ('drastic' homogenization). To measure inactivation of tyrosine aminotransferase the homogenate was incubated with gentle shaking at 37°C. Samples of 0.2ml were withdrawn at intervals and assayed for tyrosine aminotransferase activity and protein.

Purification of tyrosine aminotransferase-inactivating system

Ten rats starved for 12h were killed by a blow on the head. The livers were removed and 'gently' homogenized in 0.25M-sucrose. The homogenate was filtered through two layers of cheesecloth and then centrifuged at 750*g* for 10min. The supernatant was centrifuged again either at 13000*g* for 10min or at 28000*g* for 20min. The pellet was suspended in citrate–phosphate buffer, pH5, obtained by mixing 51.5vol. of 0.2M-Na_2HPO_4 and 48.5vol. of 0.1M-citric acid, containing 1mM-dithiothreitol, and was then frozen and allowed to thaw. The suspension was dialysed overnight against the same buffer and then clarified by centrifugation at 105000*g* for 30min. The supernatant was concentrated by dialysis under reduced pressure to a volume of about 3ml. This sample was applied to a Sephadex G-100 column (0.5cm diam. × 65cm) equilibrated with citrate–phosphate buffer and calibrated for molecular-weight determination as previously described (Auricchio & Bruni, 1966). The eluate was collected in 2ml fractions and assayed for cathepsin and for tyrosine aminotransferase-inactivating activity. All the operations were performed in the cold.

Experiments with rat liver slices

Three to six slices (50mg each) were prepared by hand and incubated by shaking in 5ml of Krebs–Ringer phosphate buffer (Cohen, 1957) at 37°C. At intervals the slices were homogenized in 20vol. of 0.05M-potassium phosphate buffer, pH7.6, containing 1mM-EDTA and 1mM-dithiothreitol with a hand-operated glass homogenizer. The homogenate was assayed for tyrosine aminotransferase activity and protein content.

The amino acid mixture added to Krebs–Ringer phosphate in some experiments is the same as used by Eagle for the minimum essential medium (Eagle, 1959).

Enzyme assay

Tyrosine aminotransferase activity was assayed as previously reported (Valeriote *et al.*, 1969).

Catheptic activity was measured by using 20mM-benzoyl-L-arginineamide as substrate in 0.1M-sodium acetate buffer, pH5.5 (Shibko & Tappel, 1965). The hydrolysis was measured colorimetrically with ninhydrin.

Tyrosine aminotransferase-inactivating activity during the purification procedure and in the presence of 0.1mM-3,3',5-tri-iodo-L-thyronine, amino acid mixture or 20mM-NaF was measured by incubating 14 units of 'stable' tyrosine aminotransferase and 20–100 units of tyrosine aminotransferase-inactivating activity for 1–6h in citrate–phosphate buffer, pH5. The unit of tyrosine aminotransferase activity is defined as the amount of enzyme catalysing the formation of 1 pmol of *p*-hydroxyphenylpyruvic acid/min at 37°C. The unit of tyrosine aminotransferase-inactivating activity is defined as the amount of enzyme that catalyses the inactivation of 40milliunits of 'stable' tyrosine aminotransferase/h. In our conditions the tyrosine aminotransferase-inactivating activity is proportional both to protein concentration and to incubation time.

The protein content was measured with the biuret reagent (Zamenhoff, 1957).

Protein synthesis was measured in rat liver slices to which [^{3}H]leucine (5μCi/ml) was added. The incubation medium was Krebs–Ringer phosphate (Cohen, 1957). At intervals two slices were removed, washed twice with Krebs–Ringer phosphate medium (pH7.4) and then homogenized in 50vol. of the same medium. A part of the homogenate (0.7ml) was used for protein assay and the rest (4.3ml) was precipitated with 5% (w/v) trichloroacetic acid. After 30min at 4°C, the sample was centrifuged at 2000rev./min for 15min. The pellet was washed twice with cold trichloroacetic acid and finally solubilized with 1ml of 1M-NaOH. A portion (0.15ml) was added to 10ml of Bray's solution (Bray, 1960) and radioactivity was measured in a liquid-scintillation spectrometer.

ATP was assayed in samples prepared from slices that had been frozen in a bath of acetone–solid CO_2, and homogenized in 0.6M-$HClO_4$ at 0°C. The homogenate was centrifuged and ATP measured in the supernatant (Adam, 1962).

Results

Inactivation of tyrosine aminotransferase in neutral homogenates

The homogenization conditions are of crucial importance for the process of inactivation of tyrosine aminotransferase. After 'gentle' homogenization of rat liver in Krebs–Ringer medium, the half-life for inactivation of tyrosine aminotransferase induced by triamcinolone is 8h (Table 1). By contrast, 'drastic' homogenization of the tissue or addition of Triton X-100 stops inactivation of tyrosine aminotransferase (Table 1). The importance of pH in the inactivation process is shown by the fact that addition of Triton X-100 to an acidified homogenate does not stop inactivation of tyrosine aminotransferase (Table 1).

To localize the inactivating system the inactivation of the transaminase was studied in the supernatants of homogenates centrifuged at different speeds. No inactivation of enzyme occurs in a neutral homogenate when particles sedimenting after 10min at 13000*g* are removed (Table 1). Removal of particles sedimenting after 10min at 750*g* does not affect the rate of inactivation of tyrosine aminotransferase.

The rate of enzymic inactivation is strongly increased by 10mM-cysteine (Table 1), a compound that also stimulates the activity of lysosomal cathepsins (see below).

We have compared the effect of 3,3′,5-tri-iodo-L-thyronine on enzyme inactivation in slices (see under 'Inactivation of tyrosine aminotransferase in liver slices' below) with the effect of this hormone in a neutral homogenate. In the latter system 3,3′,5-tri-iodo-L-thyronine strongly inhibits the transaminase inactivation (Fig. 5*b*).

Purification of tyrosine aminotransferase-inactivating system

By means of the simple procedure described above, a part (23%) of the system inactivating tyrosine aminotransferase was purified 170-fold (Table 2). The elution profile of this activity from a Sephadex column shows two peaks (Fig. 1) with elution volumes corresponding to molecular weights of 25000 and 50000. Similar elution patterns were obtained when the eluate was assayed for both cathepsin and tyrosine aminotransferase-inactivating activities. Both activities were completely inactivated by treatment at 67°C for 5min. Tyrosine aminotransferase-inactivating activity was also stimulated by 10mM-cysteine.

Tyrosine aminotransferase can be inactivated by acid phosphatase (Peterkofsky, 1971); we have therefore tested the effect of an inhibitor of phosphatases, NaF (20mM), on inactivation of tyrosine aminotransferase in both acidic and neutral homogenates and on 'stable' tyrosine aminotransferase (enzyme separated from the inactivating activity) by our most purified preparation of tyrosine aminotransferase-inactivating system (for conditions, see the assay for tyrosine aminotransferase-inactivating activity in the Methods section). No effect of NaF on inactivation of tyrosine aminotransferase was observed.

Table 1. *Inactivation under a variety of conditions of tyrosine aminotransferase induced in vivo by triamcinolone in rat liver homogenate*

The homogenate was incubated with shaking for 6h in Krebs–Ringer phosphate buffer (pH7.4) or in citrate–phosphate buffer (pH5.0) at 37°C. Conditions of 'gentle' or 'drastic' homogenization are described under 'Experiments with rat liver homogenate' in the Methods section.

Experimental conditions	Inactivation of tyrosine aminotransferase (half-time)
pH7.4 'Gentle' homogenization	8
pH7.4 'Drastic' homogenization	No inactivation
pH7.4 'Gentle' homogenization and incubation in the presence of 0.1% Triton X-100	No inactivation
pH5.0 'Gentle' homogenization and incubation in the presence or in the absence of 0.1% Triton X-100	10
pH7.4 13000*g* supernatant from a liver 'gently' homogenized	No inactivation
pH7.4 750*g* supernatant from a liver 'gently' homogenized	8
pH7.4 'Gentle' homogenization and incubation in the presence of 10mM-cysteine	2
pH7.4 'Gentle' homogenization and incubation in the presence of 10mM-cysteine and 0.1% Triton X-100	No inactivation

Table 2. *Purification of tyrosine aminotransferase inactivating activity*

For experimental details see the text.

Purification step	Vol. (ml)	Enzyme activity (units)	Protein (mg/ml)	Sp. activity	Recovery (%)
Homogenate	300	11180	37	1	100
105000*g* supernatant	7	2830	7	60	25
Sephadex G-100	22	2600	7	170	23

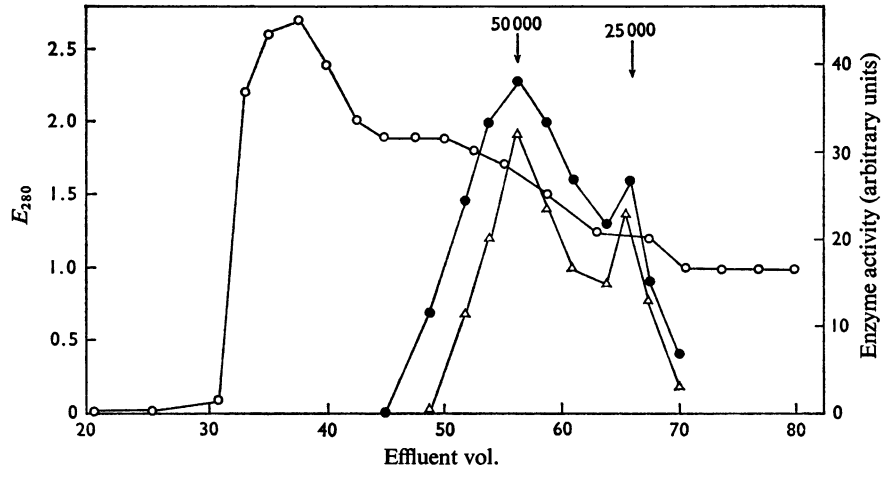

Fig. 1. *Elution profile of rat liver tyrosine aminotransferase-inactivating activities on a Sephadex G-100 column*

The column (0.5cm diam. × 65cm) was equilibrated with citrate–phosphate buffer containing 1mM-dithiothreitol and calibrated for molecular weights. The eluate was examined for E_{280} (○) and for catheptic (●) as well as for tyrosine aminotransferase-inactivating activity (△). The arrows indicate the molecular weights corresponding to the elution volumes of the enzymic activities.

Inactivation of tyrosine aminotransferase in liver slices

In a different set of experiments we have studied the process of inactivation of tyrosine aminotransferase in liver slices from rats treated with triamcinolone. In this system tyrosine aminotransferase is inactivated, with a half-life of 1–5h. Protein synthesis in liver slices linearly increases during the initial 4h of incubation (Fig. 2*a*). Addition of 1mM-cycloheximide to the incubation medium decreases protein synthesis to about 10% (Fig. 2*a*) but does not affect inactivation of tyrosine aminotransferase (Fig. 2*b*).

Addition of a mixture of amino acids and glucose to the incubation medium decreases the rate of inactivation of tyrosine aminotransferase (Fig. 3). Glucose (5–100mM) alone has no effect on the inactivation of tyrosine aminotransferase, whereas the amino acids have a definite small effect in the absence of glucose (Fig. 3). It is likely that glucose acts by increasing amino acid transport from the medium into the slices. The effect of the amino acid mixture is not due to an increased synthesis of the transaminase, because it is still observed in the presence of 1mM-cycloheximide (results not shown). The amino acid mixture has no effect on the rate of inactivation of 'stable' tyrosine aminotransferase by the purified inactivating system at acid pH values (for experimental conditions see the assay for tyrosine aminotransferase-inactivating activity in the Methods section).

Cysteine strongly increases the rate of inactivation of tyrosine aminotransferase in liver slices also (Fig. 4). No stimulation is observed when cysteine is

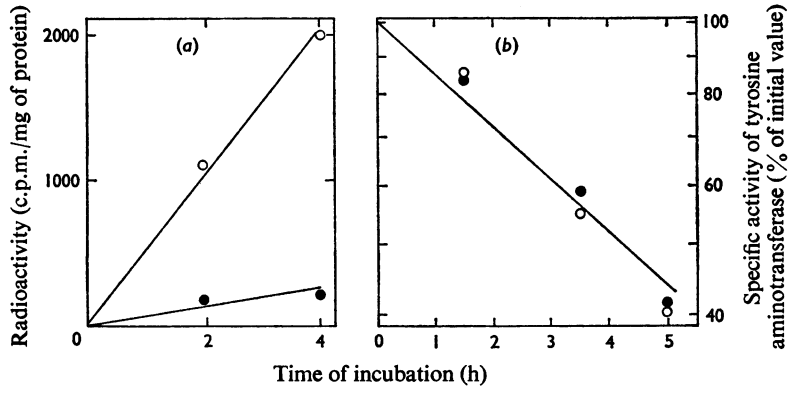

Fig. 2. *Rate of incorporation of [^3H]leucine (5μCi/ml) into proteins (a) and rate of inactivation of tyrosine aminotransferase (b) in rat liver slices*

The tissue was incubated with shaking in Krebs–Ringer phosphate medium, pH 7.4, at 37°C in the absence (○) and in the presence (●) of 1 mM-cycloheximide.

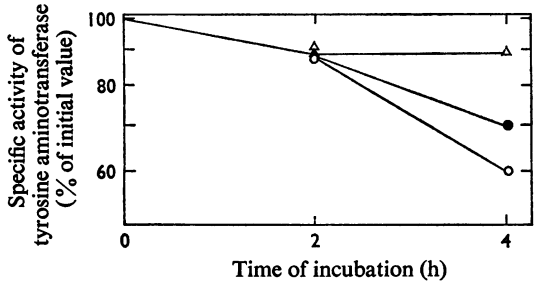

Fig. 3. *Protection by amino acids and glucose against inactivation of tyrosine aminotransferase*

Rat liver slices were incubated with shaking in Krebs–Ringer phosphate buffer, pH 7.4, at 37°C; the rate of inactivation of tyrosine aminotransferase is shown in the absence (○) and in the presence of amino acids (●) and amino acids plus 5.5 mM-glucose (△). The amino acids are the same as used by Eagle (1959).

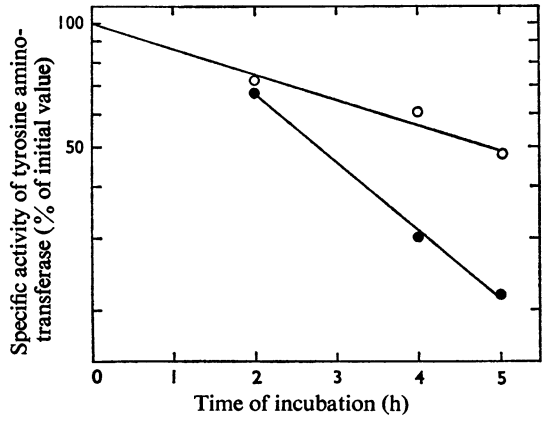

Fig. 4. *Effect of cysteine on the rate of inactivation of tyrosine aminotransferase in rat liver slices*

The slices were incubated with shaking in Krebs–Ringer phosphate buffer, pH 7.4, at 37°C, in the absence or in the presence of 16 mM-glucose (○) and in the presence of 16 mM-glucose and 10 mM-cysteine (●).

added in the absence of glucose. This finding suggests that glucose is necessary for penetration of the amino acid into cells.

3,3′,5-Tri-iodo-L-thyronine at 0.1 mM drastically decreases the rate of inactivation of tyrosine aminotransferase in liver slices (Fig. 5*a*). This inhibition is less at lower concentrations (0.01 mM) of the hormone (not shown). Addition of 1 mM-cycloheximide does not modify the effect of 3,3′,5-tri-iodo-L-thyronine on the inactivation of tyrosine aminotransferase; therefore this effect is not due to an increase in tyrosine aminotransferase synthesis. It has been reported that under some experimental conditions degradation of tyrosine aminotransferase depends on the intracellular concentration of ATP (Hershko & Tomkins, 1971). This is not so in our case. In fact 3,3′,5-tri-iodo-L-thyronine under our conditions does not modify ATP concentrations in liver slices (not shown). Further, 0.1 mM-3,3′,5-tri-iodo-L-thyronine also decreases the rate of inactivation in a neutral homogenate (Fig. 5*b*) but has no effect on the inactivation

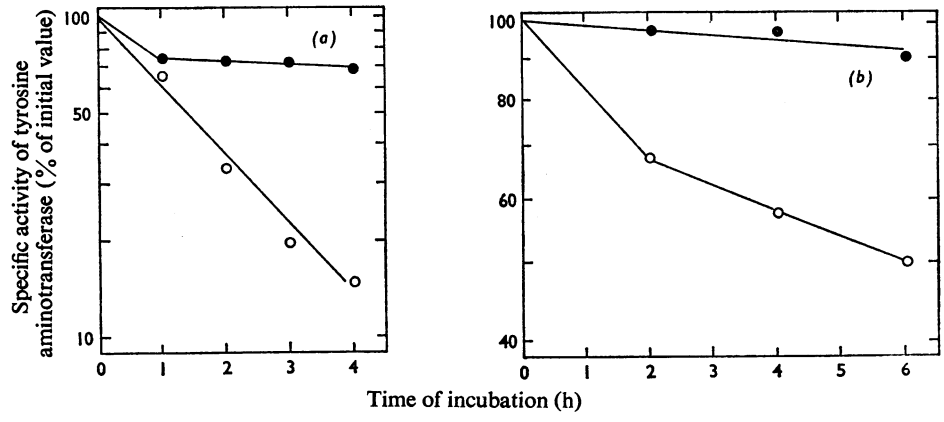

Fig. 5. *Effect of* 3,3′,5-*tri-iodo*-L-*thyronine on the rate of inactivation of tyrosine aminotransferase in rat liver slices* (*a*) *and in neutral homogenate* (*b*)

The preparations were incubated with shaking in Krebs–Ringer phosphate buffer, pH 7.4, at 37°C, in the absence (○) and in the presence (●) of 0.1 mM-3,3′,5-tri-iodo-L-thyronine.

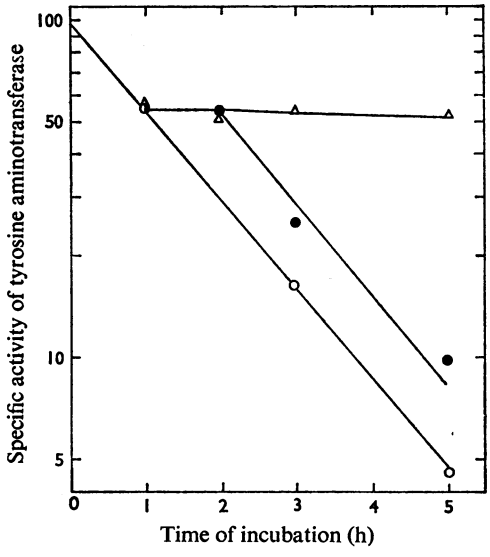

Fig. 6. *Reversible effect of* 0.1 *mM*-3,3′,5-*tri-iodo*-L-*thyronine on inactivation of tyrosine aminotransferase in rat liver slices*

Slices were incubated with shaking at 37°C in Krebs–Ringer phosphate medium not containing 3,3′,5-tri-iodo-L-thyronine (○), containing 3,3′,5-tri-iodo-L-thyronine during the first hour of incubation (●) and containing 3,3′,5-tri-iodo-L-thyronine during all the incubation time (△).

of 'stable' tyrosine aminotransferase by the purified inactivating system at acid pH (for experimental conditions see the assay for tyrosine aminotransferase-inactivating activity in the Methods section).

Further, the effect of 3,3′,5-tri-iodo-L-thyronine on inactivation of tyrosine aminotransferase is completely reversible (Fig. 6).

Discussion

Inactivation of tyrosine aminotransferase in liver homogenate

The isolation and characterization of the system responsible for degradation of tyrosine aminotransferase has been hindered by the fact that homogenization of rat liver at neutral pH values stops degradation of this enzyme as well as of tryptophan pyrrolase and in general of liver proteins (Schimke *et al.*, 1965). Neither addition of cysteine nor of lysed lysosomal preparation induced inactivation of tryptophan pyrrolase in neutral homogenate. Because of these studies liver slices were considered to be the simplest system capable of inactivating these enzymes and it was supposed that metabolically active tissue is required for protein degradation. Later we reported that tyrosine aminotransferase is inactivated in rat liver acidic homogenates by a lysosomal system (Auricchio & Liguori, 1971). However, if such inactivation is due to a system related to the degradation *in vivo* then it should also occur at the neutral pH values of the physiological intracellular pH environment.

We now find that tyrosine aminotransferase is inactivated in 'gently' homogenized liver incubated at neutral pH values (Table 1). In contrast the enzyme is stable at this pH after 'drastic' homogenization or addition of Triton X-100 (Table 1), presumably because the subcellular structure is completely destroyed. Specifically, the detergent action in neutral homogenate seems to be due to destruction of a subcellular compartment of acid pH, since in homogenates at acidic pH values inactivation of tyrosine aminotransferase proceeds in the presence as well as in the absence of Triton X-100 (Table 1).

The importance of subcellular particles is clearly shown by the fact that no inactivation of tyrosine aminotransferase occurs in a homogenate incubated at pH7.4 after centrifugation at 13000*g* (Table 1). However, neither nuclei nor debris can be responsible for inactivation of tyrosine aminotransferase, since they are removed by centrifugation at 750*g* (Table 1).

The rate of inactivation of tyrosine aminotransferase is increased by 10mM-cysteine (Table 1), a known activator of the lysosomal tyrosine aminotransferase-inactivating system previously described (Auricchio & Liguori, 1971). It is likely that in homogenates at both acidic and neutral pH values tyrosine aminotransferase is inactivated by the lysosomal system. Cysteine activation also requires particle integrity since it disappears after addition of Triton X-100 (Table 1). On the basis of all of these results we conclude that tyrosine aminotransferase is inactivated in neutral homogenates by a system present in subcellular particles sedimenting at about 13000*g* and working in a sequestered acidic environment. This system is most likely the lysosomal system previously described (Auricchio & Liguori, 1971), and the fact that the integrity of particles and a sequestered acidic pH environment are necessary for inactivation of tyrosine aminotransferase suggests that this process occurs inside the lysosomes. The finding by others that degradation of tyrosine aminotransferase does not occur in homogenates at neutral pH values (Schimke *et al.*, 1965) can be explained in the light of our results, as a consequence of a drastic homogenization. The same explanation is valid for the observation that degradation of total protein did occur in acidic homogenates and was absent in neutral homogenates (Schimke *et al.*, 1965). This result suggests to us that not only degradation of tyrosine aminotransferase but also general proteolysis take place inside the lysosomes and requires the integrity of these particles.

Purification of tyrosine aminotransferase-inactivating system

By studying the pH-dependence of the rate of inactivation of tyrosine aminotransferase in crude acidic homogenates, we observed two pH optima (Auricchio & Liguori, 1971), suggesting the presence in liver of two different tyrosine aminotransferase-inactivating enzymes. The elution pattern of activity from the Sephadex column confirms that there are two enzymes involved in inactivation of tyrosine aminotransferase. On the basis of our characterization these enzymes are identified with the lysosomal cathepsins B and B_1. The purified activities inactivating tyrosine aminotransferase and cathepsins B and B_1 have the following common features: they hydrolyse benzoyl-L-arginineamide (Otto, 1967), are eluted from a Sephadex G-100 column with elution volumes corresponding to molecular weights of 50000 and 25000 (Otto, 1967) and are stimulated by cysteine (Otto, 1967; Barrett, 1969). The proteolytic activity of these proteins indicates that inactivation of tyrosine aminotransferase is a degradative process.

Since tyrosine aminotransferase is stable in the presence of trypsin, chymotrypsin, and bacterial proteinase (Schimke *et al.*, 1965) we conclude that inactivation of tyrosine aminotransferase in liver is a very specific process. Cathepsin B_1 *in vitro* inactivates three other enzymes (Otto, 1967) and thus may have a special role in enzyme degradation.

Because NaF did not inhibit inactivation of tyrosine aminotransferase by purified cathepsin preparation or by crude homogenate it is unlikely that a phosphatase is responsible for inactivation of tyrosine aminotransferase. It should be emphasized that the inactivation of tyrosine aminotransferase in homogenates seems to be completely due to the lysosomal system. In fact, after drastic homogenization, Triton X-100 addition and removal of particles sedimenting at 13000*g*, the rate of inactivation of tyrosine aminotransferase is not only decreased, but is completely stopped.

Inactivation of tyrosine aminotransferase in liver slices

Experiments with slices were done to test if the results obtained by using homogenates also apply to intact liver tissue. Preliminary experiments showed that inactivation of tyrosine aminotransferase is unaffected by drastic inhibition of protein synthesis (Fig. 2). Therefore tyrosine aminotransferase synthesis does not contribute to the enzymic activity in slices. Further, inactivation of tyrosine aminotransferase in slices appears to be under the same control mechanisms as described for hepatoma cells. Indeed, the rate of inactivation of tyrosine aminotransferase decreased on addition of an amino acid mixture to the incubation medium of slices. The same effect has been shown to occur in hepatoma cells (Auricchio *et al.*, 1969).

Cysteine, which increases activity of the partially purified lysosomal inactivating system as well as the inactivation process in neutral homogenates, also enhances the rate of inactivation of tyrosine

amino-transferase in slices (Fig. 4). This finding suggests that inactivation in slices is due to the same lysosomal system responsible for the inactivation in the homogenate and supports a role for lysosomes in protein degradation *in vivo*.

3,3′,5-Tri-iodo-L-thyronine decreases the rate of inactivation of tyrosine aminotransferase in slices (Fig. 5*a*) as well as in homogenates (Fig. 5*b*). Thyroid hormones increase protein and RNA synthesis (Roodyn *et al.*, 1965; Tata & Widnell, 1966). The finding that 3,3′,5-tri-iodo-L-thyronine also inhibits inactivation of tyrosine aminotransferase in homogenates and in slices suggests that 3,3′,5-tri-iodo-L-thyronine, like insulin, can act as a 'pleiotypic activator' (Hershko *et al.*, 1971).

Both inhibition of inactivation of tyrosine aminotransferase by 3,3′,5-tri-iodo-L-thyronine and activation by cysteine thus occur in homogenates as well as in slices. This indicates that the same control mechanism is at work in whole tissue and in homogenates and that leakage of enzyme from slices, if occurring at all, can only be of collateral significance. Therefore in the study of the control of the biochemical mechanisms of protein degradation one can use, besides intact cells, homogenates, a considerable advantage from an experimental point of view.

A general conclusion is that the findings reported in the present paper strongly suggest that lysosomes are the site for intracellular protein degradation and that hormones can be involved in the control of this process.

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