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INVOLVEMENT OF LYSOSOMES IN SUBSTRATE STABILIZATION OF TRYPTOPHAN-2,3-DIOXYGENASE IN RAT LIVER

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SUMMARY : Administration of tryptophan or hydrocortisone to rats caused a several-fold increase in tryptophan-2,3-dioxygenase activity in the liver. Highly purified lysosomes were isolated from livers of tryptophan- or hydrocortisone-treated animals as well as the control rats. Immunoblotting of lysosomal proteins with anti-tryptophan-2,3-dioxygenase showed 48 kDa band, corresponding to the subunit molecular weight of the enzyme. The relative amount of the immuno-reactive substance in the lysosomes from hydrocortisone-treated rats was 3 times higher than the control while the value in the lysosomes from tryptophan-treated rats was essentially the same as in the control. These results indicate that administration of tryptophan renders cytosolic tryptophan-2,3-dioxygenase less vulnerable to lysosomal uptake and causes an accumulation of the enzyme in the cytosol.

Tryptophan-2,3-dioxygenase (EC 1.13.1.12, TO) has attracted considerable interest as an enzyme under metabolic regulation. It has long been recognized that administration of glucocorticoid hormone or tryptophan to a rat leads to increased levels of TO in the liver as a result of increased rates of enzyme synthesis by the hormone as well as decreased rates of enzyme degradation by the substrate (1,2). The mechanism of hormonal regulation of the enzyme synthesis has consequently been extensively studied (3,4) but the function of tryptophan in decreasing the rates of enzyme degradation in vivo has remained unknown. The present report provides evidence that the lysosomal system is responsible for degradation of TO in vivo and the administration of tryptophan reduces the rate of sequestration of TO into lysosomes.

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Abbreviation. TO, tryptophan-2,3-dioxygenase.

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MATERIALS AND METHODS

Animals: Male Wistar rats weighing about 230 g each were used in all experiments. They were fed ordinary lab chow and water ad libitum, but were fasted overnight prior to use. Hydrocortisone sodium succinate (6 mg) or tryptophan (100 mg in 7 ml of 0.9% NaCl) was administered intraperitoneally at 0 and 4 h, and the animals were sacrificed at 6 h. The control animals received injections of 0.9% NaCl at the same times before sacrifice.

Assay of TO activity: The sample of frozen liver tissue was homogenized in 99 volumes of 20 mM potassium phosphate buffer (pH 7.0) containing 2.5 mM tryptophan and 2 μM methemoglobin with a Potter-Elvehjem homogenizer. The enzyme activity in the homogenate was assayed by the method of Seglen and Jervell (5), and one unit of activity was defined as the amount forming 1 μmol of kynurenine/h at 37℃. Protein was measured by the method of Lowry et al.(6).

Preparation of soluble proteins and lysosomes from rat liver: The livers were homogenized in 4 volumes of cold 0.25 M sucrose with a Potter-Elvehjem homogenizer. The homogenate was centrifuged for 15 min at 3,000 x g and the supernatant fluid therefrom recentrifuged at 104,000 x g for 1 h to obtain the soluble protein fraction. Highly purified lysosomes were isolated by the method developed in our laboratory (7) with some modifications (8).

Immunoblot analysis: Electrophoresis of soluble and lysosomal proteins, and immunoblot assay of TO were carried out as described previously (8) using the antibody against rat-liver TO (3). The relative intensities of the immunoreactive bands were measured by scanning the filter at 600 nm in a Shimazu CS-900 Scanning Spectrometer.

RESULTS

Administration of tryptophan or hydrocortisone to rats caused an increase in TO activity in the liver, reaching levels about 5 times those of control animals in 6 h (Table 1). Immunoblot analysis of the soluble rat-liver proteins using antibody against TO showed a major 48 kDa band and a minor band of 30 kDa (Fig. 1). The major 48 kDa band represents the subunit of TO whose molecular weight was reported to be 47,796 daltons (9). The minor 30 kDa band probably represents impurity in the antibody preparation which is unrelated to TO. The intensities of the immunostained 48 kDa bands in the samples from tryptophan- and hydrocortisone-treated animals were clearly higher than those from the untreated controls, indicating that the increased measurable TO activity by tryptophan- or hydrocortisone-

Table 1

Effect of tryptophan or hydrocortisone administration on TO activity in rat liver

Treatment	TO activity (units/g liver)
Control	6.5 ± 1.0
Tryptophan	34.4 ± 5.6
Hydrocortisone	32.4 ± 6.5

Values are the means ± S.D. of 4 animals.

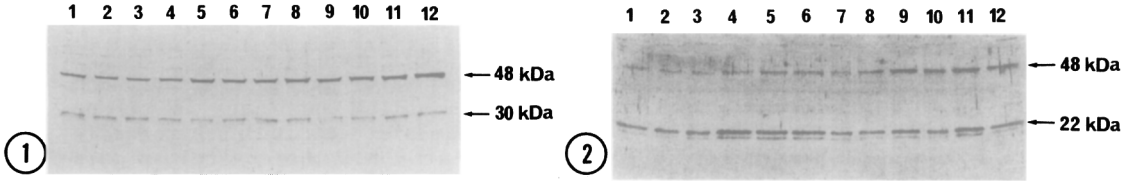

Fig. 1. Immunoblotting of soluble proteins with anti-TO antibody. Soluble proteins (10 µg) from the livers of control (lanes 1-4), tryptophan-treated (lanes 5-8) and hydrocortisone-treated (lanes 9-12) rats were subjected to sodium dodecylsulfate-polyacrylamide gel electrophoresis and immunoblotted with anti-TO antibody.

Fig. 2. Immunoblotting of lysosomal proteins with anti-TO antibody. Lysosomal proteins (7 µg) from the livers of control (lanes 1-4), tryptophan-treated (lanes 5-8) and hydrocortisone-treated rats were immunoblotted as in Fig. 1.

treatments was associated with an increase in amount of immunologically reactive protein. This result confirms the findings of previous studies (2,3).

Lysosomes were isolated from livers of tryptophan- or hydrocortisone-treated animals as well as control rats. The lysosomal proteins were then subjected to immunoblot analysis using TO-antibody. The immunoblotting showed two bands at 48 kDa and 22 kDa (Fig. 2). The 48 kDa band represents TO subunit which was also recognized in the immunoblotting of soluble proteins (Fig. 1). The 22 kDa band may represent relatively stable intralysosomal degradative intermediate of TO, but this possibility needs further investigation. In order to estimate the relative amounts of TO in the lysosomes, the immunostained bands in Fig. 2 were scanned by a scanning spectrometer. Typical densitometric tracings are shown in Fig. 3.

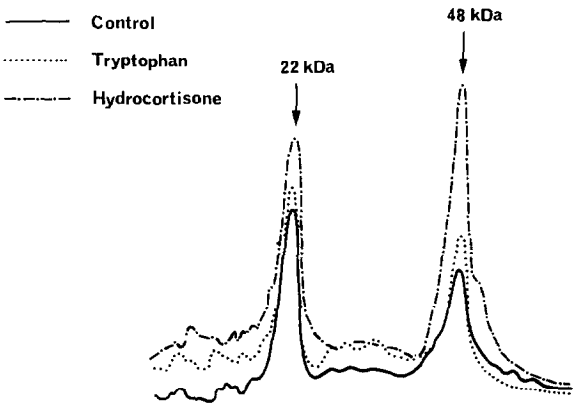

Fig. 3. Densitometric tracings of the immunoblotted lysosomal proteins. The ordinate shows the density of the bands, seen in Fig. 2, and the abscissa shows the direction of migration in the gel (from right to left).

Measurements of the areas of 48 kDa bands in such tracings, taking the mean of control samples as arbitrary 100, gave the relative values of 100 ± 27 for control group (lanes 1-4 in Fig. 2), 130 ± 28 for tryptophan group (lanes 5-8 in Fig. 2) and 290 ± 20 for hydrocortisone group (lanes 9-12 in Fig. 2).

DISCUSSION

Subsequent to the demonstration of substrate-stabilization of TO *in vivo*, Schimke and co-workers (10) attempted to answer the following questions. (a) What is the nature of the system that degrades TO *in vivo*, and can this degradation be reproduced *in vitro* ? (b) What is the function of tryptophan in stabilizing TO *in vivo* ? They found that the rapid loss *in vivo* of TO could not be duplicated in liver homogenates and the simplest system capable of this degrading action involved the use of liver slices. They also found that tryptophan exerted a marked stabilizing effect on purified TO, whether in the presence of heat, organic solvents, urea, or proteolytic enzymes. However, a number of differences were encountered in the stability properties of TO *in vivo* and in various systems *in vitro*. The basic questions posed above have remained unanswered.

The immunoblot analysis of lysosomal proteins showed the presence of TO subunit in the lysosomes, indicating that the lysosome is the site of degradation of TO *in vivo*. This conclusion is consistent with the earlier suggestion of Rudek et al. (11) who observed the presence of TO in the rat-liver lysosomes after treatment with Triton WR-1339.

Semiquantitative estimation of the relative amounts of TO within liver lysosomes yielded the values of 100, 130 and 290 for control, tryptophan-treated and hydrocortisone-treated animals, respectively. Since the lysosomes were isolated without use of protease inhibitors, these values should represent the steady state concentrations of TO in the lysosomal compartment. The level of TO in the lysosomes from hydrocortisone-treated rats was almost 3 times that of control animals. Considering that the cytosolic TO level in the liver of the hormone-treated rats was several times higher than that of control rats, the increased TO level in the lysosomes can be understood as the result of an increased sequestration of TO into lysosomes. Administration of tryptophan to rats caused an increase in the cytosolic TO level to almost the same extent as the hormone treatment. However, the lysosomal TO level remained essentially unchanged. We therefore conclude that administration of tryptophan renders the

cytosolic TO molecules less vulnerable to lysosomal uptake and causes an accumulation of TO in the cytosol.

The nature of the change in TO molecule imparted by the presence of tryptophan which accounts for resistance to lysosomal uptake remains to be elucidated. It has been shown that stabilization of purified TO occurs without altering its gross structure as determined by sedimentation and immunological criteria (10). As a general feature of intracellular protein degradation, Dice and Goldberg (12) found a correlation between protein charge and degradation rates; proteins with low isoelectric points tend to be degraded faster than those with neutral or basic isoelectric points. Segal and co-workers (13) subsequently found a correlation between turnover rates and hydrophobicity of soluble rat liver proteins; the more hydrophobic a protein is, the faster the protein is degraded. We recently reported that these general rules of protein degradation can be accounted for by the selectivity of the lysosomal digestive system (14). It is therefore possible that binding of tryptophan to TO brings about some subtle change in the conformational state of the enzyme in such a manner that hitherto exposed acidic or hydrophobic domain tends to be buried, making the molecules less liable to lysosomal recognition. The binding of substrate and cofactor to enzyme molecules appears to be important in imparting stability against lysosomal proteolysis. As another example of such phenomenon, we recently found that the increased rate of degradation of cytosolic aspartate aminotransferase in the liver of pyridoxine-deficient rats was associated with the increased rate of sequestration of the enzyme into lysosomes (15).

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