# A STUDY OF A TYROSINE AMINOTRANSFERASE INACTIVATING SYSTEM IN RAT LIVER HOMOGENATE

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## 1. Introduction

Tyrosine aminotransferase (EC 2.6.1.5) undergoes unusually rapid turnover in vivo in both rat liver [1–3] and hepatoma (HTC) cell cultures [4]. The mechanism responsible for tyrosine aminotransferase (TAT) degradation has not yet been identified probably because of the difficulty of reproducing TAT degradation in homogenates. In fact homogenization of rat liver [5] and of hepatoma (HTC) cells (F. Auricchio, unpublished observations) stops TAT degradation. In order to characterize and purify the system(s) responsible for TAT degradation in vivo, conditions have been sought under which TAT can also be degraded in vitro.

In the present study we have demonstrated that TAT inactivation proceeds in rat liver homogenates when an acid buffer is used. This inactivation is due to a thermolabile protein system which is probably composed of two components. The system seems to be localized in lysosomes and is strongly activated by –SH donors. A preliminary "rough" purification of this system is also reported. Triamcinolone, a glucocorticosteroid that induces TAT formation in rat liver [6] does not change the level of this system.

## 2. Materials and methods

Male Wistar rats were used. Induction of rat liver TAT by triamcinolone (9α-fluoro-16α-hydroxyprednisolone diacetate) was obtained as previously described [7]. Rat livers were homogenized in an all-glass Dounce homogenizer. The buffer used in most experiments was a citrate buffer, pH 5, obtained by mixing 51.5 volumes of 0.2 M $Na_2HPO_4$ and 48.5 volumes of 0.1 M citric acid. "Stable" TAT was prepared following preliminary purification steps (including heat treatment) according to a previously published procedure [7].

The fractionation of rat liver homogenate by differential centrifugation was performed in the following way: the livers were gently homogenized in 0.25 M sucrose with a Potter–Elvehjem homogenizer. The homogenate was filtered through several layers of cheese cloth and centrifuged at 755 *g* for 10 min. The resultant pellet was labeled "nuclear fraction". The supernatant was centrifuged at 12.100 *g* for 10 min; this latter pellet represents the mitochondrial–lysosomal fraction. The supernatant was centrifuged at 105,000 *g* for 90 min. The pellet was labeled "microsomal fraction".

TAT activity was assayed and its units calculated as previously reported [7]. The protein content was measured with the biuret reagent. Acid α-glucosidase activity was measured as reported in a previous paper [8].

## 3. Results and discussion

Fig. 1 shows that incubation of liver homogenates of triamcinolone-treated rats, at 37° in citrate buffer, pH 5, induces an inactivation of TAT. The half-time of inactivation is about 4.5 hr. This value is within the range of the values obtained for TAT degradation in vivo [1–4].

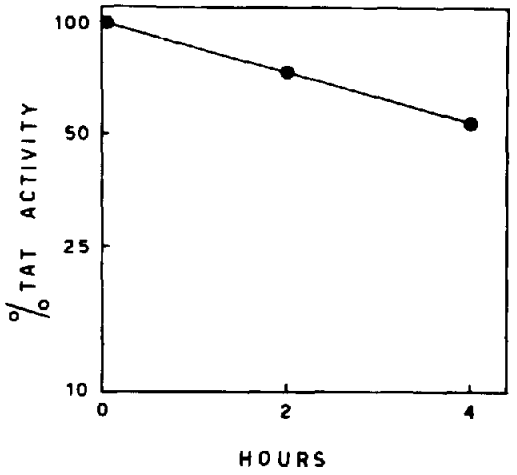


Fig. 1. 4 rats were killed 10 hr after triamcinolone treatment. The livers were homogenized in 5 volumes of citrate buffer, pH 5, and incubated at 37°. At the indicated intervals TAT activity was assayed in the incubation mixture. TAT specific activity at 0 time was 0.3 units/mg protein.

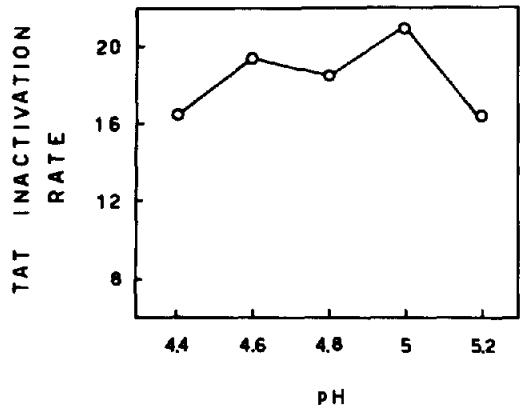


Fig. 2. pH-dependence of TAT inactivation rate in liver homogenate of triamcinolone-treated rats. Experimental conditions, except for pH values, were identical to those of the experiment presented in fig. 1. For further details see text.

No TAT inactivation was observed under identical experimental conditions, when the buffer used was changed to 0.05 M potassium phosphate buffer pH 7.6, with $10^{-3}$ M EDTA and $10^{-3}$ M dithiothreitol.

Homogenization of rat livers in citrate buffers of different pHs, followed by incubation at 37° for 8 hr, results in TAT inactivation curves of the type presented in fig. 1. However, different slopes are obtained for different pHs. The rate of inactivation of TAT was calculated from these curves in arbitrary units and plotted against the pH values of the buffers used in the incubation (fig. 2). This plot shows that, in our experimental conditions, TAT inactivation is not due to acid denaturation, because if this were the case, one would expect the efficiency of TAT inactivation to progressively increase with the decrease of the pH.

Furthermore, TAT inactivation may be due to the action of two components with different pH optima (fig. 2). It should be pointed out that it seems likely that several enzymes are involved in the lysosomal degradation of globin [9]. When liver homogenate of triamcinolone-treated rats was incubated in citrate buffer, pH 4.5, at two different temperatures (37° and 4°), inactivation of TAT after 8 hr was of about 40 and zero percent of the initial activity respectively (table 1).

TAT inactivation rate in liver homogenate of triamcinolone-treated rats is identical to that of the homogenate of livers of the control animals. Furthermore the TAT degradation rate in vivo is similar for both the livers of rats treated and not treated with glucocorticosteroids [1–3]. This fact, also, suggests that in our experimental conditions TAT inactivation is due to the same system which is involved in TAT degradation in vivo.

We have performed a preliminary purification of TAT from rat liver according to a previously published procedure, which up to now is the only one giving pure rat liver TAT [7]. The partially purified TAT was incubated in citrate buffer, pH 5, at 37° for 8 hr. During this time the TAT preparation is stable (table 1). This fact confirms that TAT inactivation in the experiments previously presented is not due to a pH-dependent denaturation of the enzyme.

When "stable" TAT (e.g. partially purified TAT, free, from the inactivating system) is incubated in citrate buffer in the presence of equal amounts of rat liver homogenate from rats treated or not with triamcinolone its activity is lost. The TAT inactivation rate is very similar in both cases. Therefore, we conclude that treatment with triamcinolone, an inducer of TAT [6], does not change the levels of the TAT inactivating system.

Heating of rat liver homogenate at 67° for 7 min

Table 1
Inactivation of rat liver TAT incubated under different conditions in citrate buffer.

| Condition | Time of incubation (hr) | % Inactivation |
|---|---|---|
| Homogenate[a], pH 4.5 | | |
| 37° | 8 | 40 |
| 4° | 8 | 0 |
| "Stable" TAT[b], pH 5, 37° | 8 | 0 |
| "Stable" TAT[c], pH 5, 37° | | |
| + homogenate | 4 | 67 |
| + heated homogenate | 4 | 0 |
| "Stable" TAT[d] + purified inactivating system, pH 5, 37° | 2 | 37 |
| + 10 mM cysteine | 2 | 90 |
| + 10 mM dithiotreitol | 2 | 92 |

[a] Protein concentration: 20 mg/ml, TAT specific activity: 0.2 units/mg protein.
[b] Protein content: 10 mg/ml, TAT specific activity: 8 units/mg protein.
[c] "Stable" TAT: 2 units/ml, homogenate protein: 20 mg/ml. TAT specific activity of the homogenate: 0.012 units/mg protein.
[d] "Stable" TAT: 1 unit/ml, inactivating system protein: 1 mg/ml.

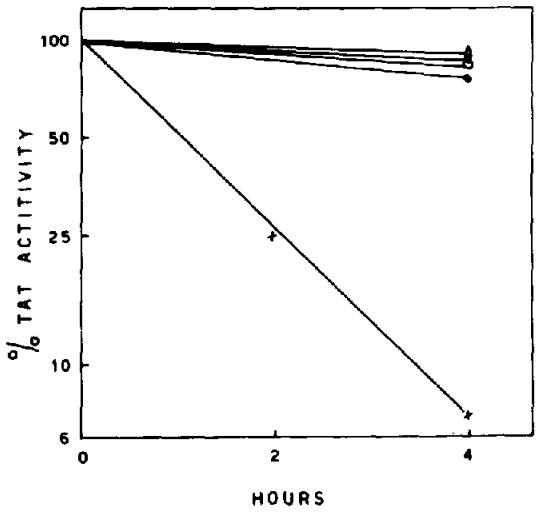


Fig. 3. "Stable" TAT inactivation by liver subcellular fractions. Homogenate was fractionated by differential centrifugation (see text). Fractions were frozen, thawed and dialized overnight against citrate buffer, pH 5, at 4° and subsequently diluted with the same buffer to the same protein concentration. The single fractions were separately incubated with "stable" TAT in citrate buffer, pH 5, at 37°. One ml of mixture contained 8 mg of subcellular fraction protein and 2.2 units of "stable" TAT. At the indicated intervals TAT activity was measured in the incubation mixtures.
Symbols: ○ homogenate, ● nuclear fraction, x mitochondrial-lysosomal fraction, △ microsomal fraction, □ 105,000 *g* supernatant.

completely destroys the capacity of the liver to inactivate pure TAT (table 1).

When rat liver homogenate was fractionated by differential centrifugation and equal amounts of protein from the different fractions were incubated in citrate buffer with identical amounts of "stable" TAT, the fraction containing mitochondria and lysosomes was by far the most active in inactivating "stable" TAT (fig. 3). The purification factor of the TAT inactivating system in the mitochondrial–lysosomal fraction over the crude homogenate was identical (7 fold) to that of the acid α-glucosidase, an enzyme which is present in the lysosomes [10]. This fact together with the acid pH optimum of the TAT inactivating system strongly suggests that this system is localized in the lysosomes even if the present experiments do not exclude the possibility of a mitochondrial localization. When the subcellular fraction containing the TAT-inactivating system is frozen, thawed, dialyzed overnight against citrate buffer (pH 5, at 4°), and centrifuged at 10,000 rpm for 30 min, the system is found in the supernatant and it is purified about 30 fold over the homogenate.

When the purified inactivating system was incubated with "stable" TAT in citrate buffer pH 5, in the presence of 10 mM cysteine or 10 mM dithiothreitol, TAT inactivation was strongly increased (table 1).

In conclusion, from our experimental data we report the existence of a TAT inactivating system in the rat liver homogenate which is likely to be responsible, at least in part, for TAT degradation in vivo. The preliminary characterization and purification here reported will probably help in the elucidation of the complex mechanism responsible for protein degradation in mammalian cells.

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