WHAT DETERMINES THE HALF-LIFE OF PROTEINS IN VIVO? SOME EXPERIENCES WITH ALANINE AMINOTRANSFERASE OF RAT TISSUES

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Received July 7, 1969

SUMMARY

The half-life of alanine aminotransferase in rat muscle is about 20 days, compared with that of the same enzyme in liver of about three days. The thermal denaturation rate of the enzyme in vitro gives a calculated half-life of about 400 days at body temperature. A liver lysosomal preparation effectively inactivated the enzyme under conditions in which it was otherwise quite stable. From our results we conclude that turnover of alanine aminotransferase in vivo is a reflection of an active (enzymatic) process which does not depend on prior thermal denaturation.

INTRODUCTION

The problem of the factors which determine the relative rates of turnover of specific proteins in cells of higher organisms has been the object of increasing attention in recent years. It has become apparent that alterations in tissue levels of enzyme can be brought about not only through effects on their rates of formation, but also on their rates of removal (1,2). Attempts to understand the mechanism of control of turnover are hampered by a lack of basic knowledge of the factors which determine tissue half-lives of specific proteins, or in fact of the whole process of in vivo protein degradation (3). A priori, two distinct types of process suggest themselves as potential determining factors of the relative rate of turnover of a protein in a tissue. The first is the thermal stability of the protein

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under the chemical and topological conditions in which it exists in the cell, and the second is the susceptibility of the native protein to degradative attack. In some special cases a measured tissue half-life may be a reflection of a secretion rate, e.g., serum albumin in liver cells. In an attempt to obtain a better understanding of the turnover process, we have initiated a search for the factors which may be involved in the turnover of alanine aminotransferase in rat tissues.

METHODS

Assay of alanine aminotransferase and definition of units were as previously described (4). Rat liver lysosomes were prepared by the method of Tappel (5).

RESULTS

Half-Life in Muscle - The half-life of alanine aminotransferase in rat liver has been determined both by the perturbation of steady state method (6) and by the radioactivity disappearance method (7). A value of 3.0 - 3.5 days can be taken for both the normal and glucocorticoid-induced enzyme.

On the other hand, in rat muscle the half-life of the enzyme is about 20 days (Fig. 1). The muscle and liver enzymes are identical in electrophor-

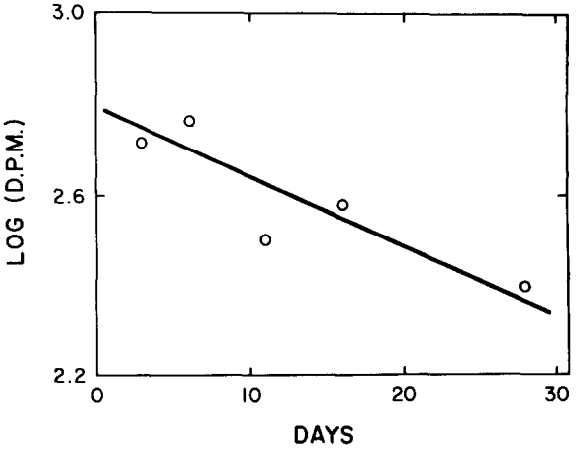

Fig. 1. Half-Life of alanine aminotransferase in rat muscle. ^{3}H-(4,5)-L-Leucine (50 μc) was injected intraperitoneally, and three animals were sacrificed at each time shown. The muscles from both back legs were removed, and alanine aminotransferase was purified through the ammonium sulfate step as described for the liver enzyme (4). The enzyme was then precipitated with rabbit antiserum prepared by injection of the crystalline enzyme (4) and counted. Values of radioactivity are per total tissue.

etic mobility (8), immunochemically (8,9), and in pH and salt solubility (8). In addition, the heat stability in vitro of the enzyme from the two sources is indistinguishable (8). Thus is appears that the enzyme is the same in liver and muscle, so that the difference in tissue half-life must be attributed to differences in cellular environment.

Thermal Stability in Vitro - An Eyring plot (10) of the first order decay constant of alanine aminotransferase at a number of temperatures is reproduced in Fig. 2. From these data an enthalpy of activation for the inactiva-

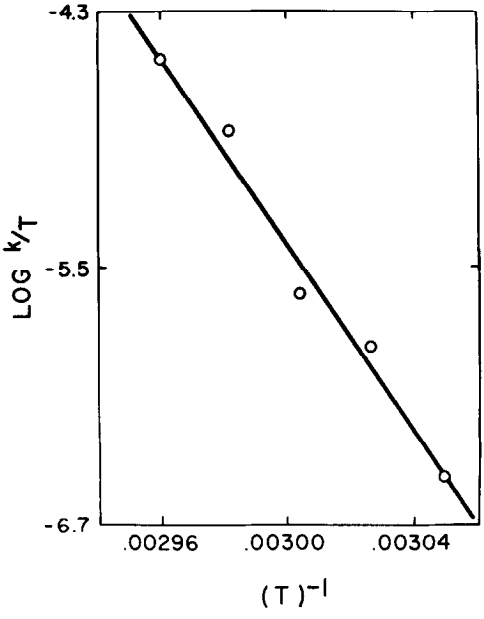

Fig. 2. Eyring plot of alanine aminotransferase inactivation as a function of temperature. The enzyme was purified from rat liver through the ammonium sulfate step as previously described (4) and was in 5 mM potassium phosphate and 0.5 mM EDTA, pH 7.3. Protein concentration was 2.4 mg/ml. Values of k are the first order inactivation constants at the temperatures indicated and are in $(sec)^{-1}$. Values of T are in degrees Kelvin.

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tion reaction of 99.5 Kcal/mole was obtained. The free energy of activation and the entropy of activation were calculated to be 22.4 Kcal/mole at 60° and 225 e.u., respectively.

The value of the inactivation rate constant, k, at the body temperature of the rat, 37.5°, can be calculated from Fig. 2 by extrapolation. The value obtained is 1.8×10^{-3} $(day)^{-1}$, and the thermal half-life, there-

fore, at this temperature is about 400 days ($t_{1/2} = (\ln 2)/k$). This figure is 2 orders of magnitude higher than the half-life of the enzyme in liver and more than an order of magnitude higher than that in muscle. A number of metabolites were tested as possible labilizers of the enzyme, but none were found, nor was its thermal stability affected when tested after a 1000-fold dilution in buffer.

Lysosomal Inactivation - The inactivation of alanine aminotransferase bya lysosomal preparation from liver is shown in Fig. 3. It may be seen that under these conditions in which the enzyme is quite stable, the lysosomal preparation was highly effective in bringing about inactivation of the enzyme. Incubation of the enzyme with lysosomes in the absence of a sulfhydryl compound, but otherwise under identical conditions, led to no inactivation. This result seems to exclude a non-enzymatic labilization of alanine aminotransferase by some component of the lysosomal preparation.

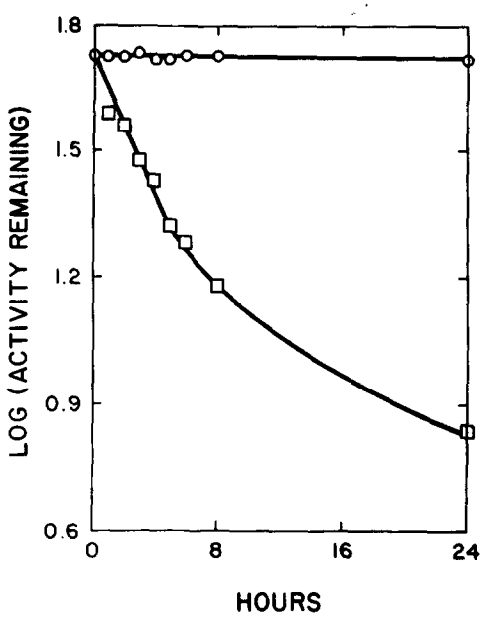

Fig. 3. Lysosomal inactivation of rat liver alanine aminotransferase. The incubation mixture contained in a final volume of 3.0 ml: 2.5 ml of citrate-phosphate buffer, pH 5.0 (11), 120 μmoles of mercaptoethanolamine, 6 μmoles of EDTA, 6 mg of Triton X-100, 54 units of crystalline alanine aminotransferase (0.14 mg) (4) and 0.40 mg of lysosomal protein (squares). Circles: no lysosomes.

DISCUSSION

These results point to an active inactivation process as the determining factor in the tissue turnover of alanine aminotransferase. Other possible explanations of the differences in stability of the enzyme in liver, in muscle and in the test tube, such as the presence of undiscovered labilizing factors in the tissues, etc., could be proposed and debated, but are unnesessary in view of the ability of lysosomes to inactivate the enzyme.

This conclusion seems to be at variance with that of Coffey and de Duve (12), who found examples of proteins which were chemically stable and resistant to lysosomal attack under their incubation conditions, and who therefore concluded that, "...denaturation appears to be a prerequisite to the digestion of a protein by the lysosomal enzymes." On the other hand, their results with peroxidase were analogous to those reported here for alanine aminotransferase in that inactivation was greater in the presence of lysosomal preparations than in their absence. Since both peroxidase and alanine aminotransferase contain prosthetic groups, the possibility exists that the lysosomal inactivation rates of these enzymes reflect initial attacks on the prosthetic groups, rather than proteolysis. Yet even this sort of process must be visualized as enzymatic, in light of the results at hand, and thus would remain within the scope of our conclusion that the cell is capable of an active inactivation process which does not depend on prior thermal denaturation, even though "digestion", in the strict sense of proteolysis, may be a subsequent event. On the other hand, we do not gainsay the possibility that for other proteins thermal denaturation in the lysosomal milieu precedes enzymatic attack. This may well be the significance of the apparent high acidity within the lysosome.

We do not attempt to relate quantitatively at this time the lysosomal inactivation rate of alanine aminotransferase to its specific removal rate *in vivo*, since the relevance of the state of the lysosomes (intact or disrupted) and the effect on the rate of the presence of other cell proteins as competitive substrates must first be evaluated.

If, as de Duve and Wattiaux have suggested (13), incorporation of cell substance into the lysosome is by an engulfment process, or in some other manner which cannot differentiate among the protoplasmic constituents, it is necessary to seek another basis for the variability in relative turnover

rates characteristic of individual cell components. A simple unidirectional sequence

$$C \xrightarrow{k_1} C' \xrightarrow{k_2} \text{degradation products}$$

where C represents the mean concentration of a component outside the lysosomal compartment and C' represents the mean concentration inside the lysosomal compartment, cannot account for variations in half-lives among tissue components even on the basis of variations in the degradation rate constant, k_2, since at steady state the flow through the pathway per unit concentration of C depends only on k_1, which is the same for all components.[1]

On the other hand, if a step in the opposite sense of step 1 is included, a feasible system results.

$$C \underset{k_2}{\overset{k_1}{\rightleftharpoons}} C' \xrightarrow{k_3} \text{degradation products}$$

Step 2, like step 1, need not differentiate among cell components, and the specificity of relative turnover rates could now reside entirely in step 3, for which only the usual variation in rates among substrates of degradative enzymes, or in thermal stability, need be invoked.

If we define the specific removal rate of a component, k_{sp}, as the fraction which is removed from the extralysosomal compartment per unit time, it can be shown that

$$k_{sp} = k_1 \cdot \frac{k_3}{k_2 + k_3}$$

If $k_3 \gg k_2$, i.e., the rate of intralysosomal degradation is very rapid relative to leakage back to the extralysosomal compartment,, k_{sp} becomes k_1, which is the specific rate of lysosomal ingestion and is the upper limit for the specific removal rate of a component from a cell by this process, regardless of how rapidly it is degraded intralysosomally (and thus a lower limit for the half-life is set at $t_{1/2} = (\ln 2)/k_1$). On the other hand when k_2 is of

[1] We thank Dr. K. Paigen for pointing this out to us.

the order of or greater than k_3, k_{sp} depends upon k_3, so that under these conditions variations in the specific removal rates of cell components, or variability in that of a particular component, can arise from differences in and effects on the specific degradation rate within the lysosome.

This analysis is quite general and rather primitive, yet it appears to be a useful framework within which questions of the determining factors in the turnover of cell constituents can be considered.

ACKNOWLEDGEMENT

This work was supported by a grant from the National Institutes of Health (AM-08873).

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