A COMPARISON OF THE PROTEOLYTIC SUSCEPTIBILITY OF SEVERAL RAT LIVER ENZYMES

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Summary:

The susceptibility of five enzymes to proteolytic attack has been compared in rat liver extracts. The results show that enzymes with short in vivo half-lives were especially vulnerable to proteolytic attack, as measured by loss of activity in vitro, in contrast to the long-lived enzymes which were resistant to attack. Furthermore, there was a good correlation between the relative rates of inactivation in vivo and in vitro only with specific proteases such as trypsin and chymotrypsin, not with non-specific proteases such as pronase and subtilisin. This suggests that proteolytic enzymes with some degree of specificity are involved in the degradation of intracellular enzymes.

There are marked differences in the rates at which specific rat liver proteins are degraded, as evidenced by their in vivo half-lives (1). The mechanisms involved in intracellular protein degradation, however, are just beginning to be explored and are not understood. It appears that the rates of degradation of individual enzymes are unrelated to their thermal labilities in vitro (2,3). Specific inactivating proteins or intracellular proteases have been suggested to be responsible for controlling turnover, but these proteins have not been identified or characterized (1). Lysosomes are involved in the digestion of exogenous proteins taken into cells by pinocytosis; however, to date there is no evidence that degradation of intracellular proteins is carried out by lysosomal enzymes under normal physiological conditions.

In the present study several rat liver enzymes with known in vivo half-lives were compared with regard to their susceptibility to proteolytic attack in vitro. The aim was to assess the importance of this property, vulnerability to proteases, in determining the in vivo longevity of proteins in rat liver tissue. The enzymes that were compared and their in vivo half-lives ($t_{1/2}$) are as follows:

	$t_{1/2}$*	References
Lactate dehydrogenase	3.5 - 16 days	3,4
Arginase	4 - 5 days	5
Catalase	1.1 - 2.2 days	6,7
Serine dehydratase	3 - 20 hours	8,9
Tyrosine aminotransferase	1.5 - 11.7 hours	10,11

*Range of *in vivo* half-lives reported in the literature.

These *in vivo* half-lives must be considered estimates since the values vary with different investigations and they depend on techniques used (3), diet (9) and hormones administered (11). All the above enzymes are considered to be "soluble" in the cell, with the exception of catalase. Catalase activity is associated with peroxisomes to a certain extent (7); however, upon homogenization and centrifugation (20,000 x *g* for 20 min), about 50% of the activity is found in the supernatant fractions (12). It has not been established whether this supernatant enzyme is different from the peroxisome catalase or if it is due to leakage from peroxisomes occurring *in vivo* or during preparation of tissue fractions.

Methods

Male Holtzman rats (250-400 g) were killed by decapitation and livers perfused with 0.9% NaCl. Livers were then homogenized in a glass tube fitted with a teflon pestle with 0.15 M KCl (1 g liver + 3 ml KCl), and centrifuged at 50,000 x *g* for 80 minutes. The resulting supernatant fraction was adjusted to pH 7 with NaOH. These extracts were either used immediately or stored in the freezer. All activities were stable for at least two weeks.

Serine dehydratase was assayed by the method of Greenberg (13), tyrosine aminotransferase by a method described by Diamondstone (14). Catalase activity was determined by Beers and Sizer's method (15), arginase activity by the Van Slyke and Archibald method (16). Lactate dehydrogenase was assayed by the method described by Kornberg (17).

Results and Discussion

To assess the vulnerability of the liver enzymes to proteases, the extracts were incubated with one of several proteases, and aliquots of the in-

cubation mixtures were assayed for enzyme activities at various intervals. The effects of incubations with trypsin and chymotrypsin on the rat liver enzyme activities are shown in Fig. 1. It can be seen that: (a) arginase and lactate dehydrogenase activities are not affected during the two hour incubation period; (b) tyrosine aminotransferase and serine dehydratase show marked decays of activity, and (c) catalase activity falls off at a rate intermediate between the long- and short-lived enzymes. The order of vulnerability corresponds to the order of in vivo half-lives. Thus there appears to be a correlation between this in vitro property and in vivo half-lives.

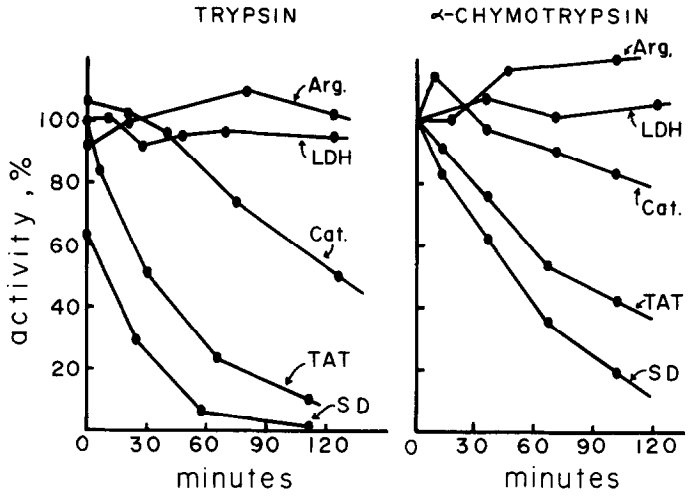

Fig. 1. The effect of trypsin and chymotrypsin on enzyme activities in liver extracts. Extracts were incubated at 37°C with trypsin or chymotrypsin (91 μg/ml) in 45 mM potassium phosphate buffer (pH 8). The system contained 3 ml of extract and had a final volume of 3.3 ml. Trypsin (Type III:2X crystallized from bovine pancreas) and chymotrypsin (Type II:3X crystallized from bovine pancreas) were purchased from the Sigma Chemical Company. Aliquots of the incubation mixtures were removed at various times for enzyme assays. Activities are expressed as percent of the activity in a buffered extract which did not contain trypsin or chymotrypsin. The enzymes assayed were: arginase (Arg), lactate dehydrogenase (LDH), catalase (Cat), tyrosine aminotransferase (TAT) and serine dehydratase (SD).

Several control experiments were performed: (1) It was found that the enzymatic activities were stable for two hours if incubated in the absence of trypsin and chymotrypsin; (2) All activities were stable when trypsin inhibitor (soybean) was mixed with trypsin prior to addition. When trypsin inhibitor

was added to extracts 30 minutes after trypsin, it prevented further tryptic inactivation. These results demonstrate that an active trypsin molecule is responsible for the decay of enzyme activities; (3) Trypsin and chymotrypsin were inactive during enzyme assays, except in the case of serine dehydratase. In most cases, aliquots taken from incubation mixtures were diluted 50- to 100-fold for enzyme assays and this diluted the proteases to the extent that no significant proteolytic activity remained. Trypsin inhibitor was added to serine dehydratase assay tubes since the dehydratase activity decreased during the assay when inhibitor was not added. The 40% drop in serine dehydratase activity at time zero of incubation in Fig. 1 is due to a very rapid inactivation of the dehydratase when trypsin is added at 4°C, not to inactivation by trypsin during the subsequent assay of enzymatic activity.

The effects of pronase and subtilisin on the five enzyme activities are shown in Fig. 2. With these non-specific proteolytic enzymes; (a) lactate dehydrogenase activity decays quite rapidly; (b) arginase activity is appar-

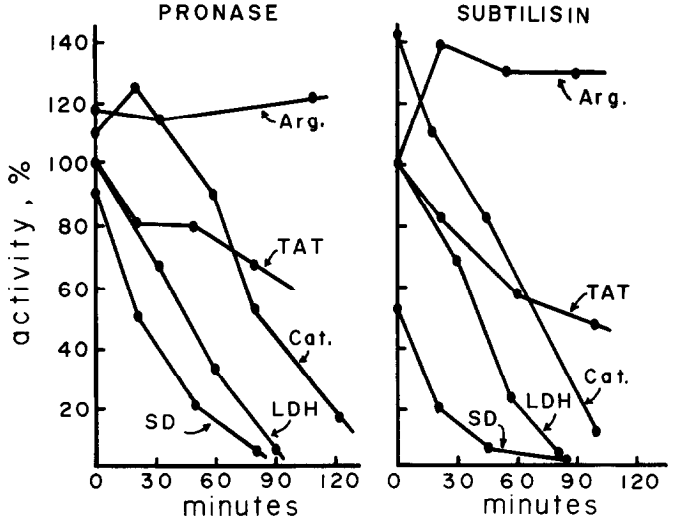

Fig. 2. The effect of pronase and subtilisin on enzyme activities in liver extracts. Extracts were incubated at 37°C with pronase or subtilisin in phosphate buffer at pH 7.4. The concentrations of extract, proteases and buffer were the same as those described in Fig. 1. Pronase, a non-specific protease from Streptomyces griseus (Type VI:Fungal) and subtilisin, a bacterial protease from B. subtilis, were purchased from the Sigma Chemical Company. Activities are expressed as percent of the activity in a buffered liver extract which contained no added proteases.

ently activated and then stable for the two hour incubation; (c) catalase is also activated initially and then activity decays dramatically; (d) serine dehydratase activity decreases markedly and immediately, and (e) tyrosine aminotransferase activity is affected to a moderate extent by both proteases. Thus, the order of in vitro vulnerability to these proteases is unrelated to the in vivo order of lability of these enzymes, in contrast to the results with trypsin and chymotrypsin.

Ligands are expected to modify the sensitivity of some enzymes to proteases, and the extent to which ligands stabilize or labilize the enzymes may have a regulatory function in vivo. The ligand which we have tested in this in vitro system is pyridoxal 5'-phosphate (PLP) which is a cofactor for both serine dehydratase and tyrosine aminotransferase. The Km for the former enzyme is 24 μM (18) and for the latter 0.24 μM (19). The effect of PLP on the rate of trypsin inactivation of the enzymes is shown in Fig. 3. The addition of PLP had no effect on inactivation of lactate dehydrogenase, arginase or catalase. The two enzymes requiring PLP as a cofactor were rendered less vulnerable to inactivation.

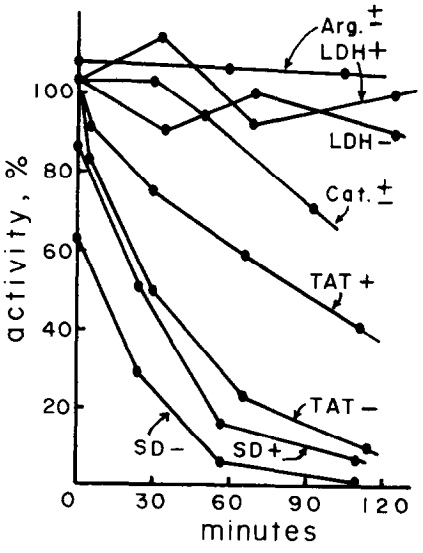

Fig. 3. The effect of pyridoxal 5'-phosphate (PLP) on trypsin inactivation of enzyme activities in liver extracts. Extracts were incubated with trypsin, as described in Fig. 1, with (+) and without (-) 40 μM PLP.

The data presented here suggest that proteolytic susceptibility is an important property in the determination of half-lives, and that proteases with some degree of specificity may initiate the inactivation and degradation of intracellular protein. Although trypsin and chymotrypsin are not present in liver cells, intracellular proteases having similar specificities may well be. For instance, cathepsins B and C, isolated from beef spleen, have specificities resembling those of trypsin and chymotrypsin, respectively (20).

The molecular weight of proteins (or subunits) has been suggested as a determinant of degradation rates. Dehlinger and Schimke (21) have found that "greater degradation rates are associated with greater molecular weights" of proteins (or subunits). However, this does not appear to be an important parameter for the enzymes studied here. The enzymes in order of increasing *in vivo* rates of degradation have the following M.W.'s (22): lactate dehydrogenase, 140,000 (4 subunits); arginase, 138,000 (4 subunits); catalase, 250,000 (4 subunits); serine dehydratase, estimated M.W. 21,000 - 63,000 (subunits unknown); tyrosine aminotransferase, 91,000 (3 - 4 subunits).

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