Role of Coenzyme in Aminotransferase Turnover*

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The role of coenzyme in determining intracellular content of pyridoxal enzymes was assessed by analyzing effects of pyridoxine deficiency on the rapidly degraded, readily dissociable tyrosine aminotransferase (EC 2.6.1.5) and the slowly degraded, nondissociable alanine aminotransferase (EC 2.6.1.2) of rat liver. Synthesis of the tyrosine enzyme was reduced, leading to a decreased amount of this enzyme, much of which was present as active apoenzyme. Synthesis of alanine aminotransferase was unchanged but much of this enzyme was present as an inactive apoenzyme which retained immunological reactivity. Degradation rates of both enzymes (1/2 about 1.5 h, tyrosine aminotransferase; about 3 days, alanine aminotransferase) were not changed in pyridoxine deficiency. Hence, interaction with coenzyme is not a significant determinant in intracellular degradation of these aminotransferases. Coenzyme dissociation and intracellular stability probably reflect structural features of the proteins which determine both properties.

It is well known that enzyme ligands, especially substrates and coenzymes, are effective in preventing inactivation of their specific enzymes in vitro. The proposal was made some years ago that enzyme-ligand interactions may also be determinants of rates of enzyme degradation in vivo (1, 2), and this concept was substantiated in the demonstration by Schimke et al. (3) that substrate tryptophan stabilized tryptophan oxygenase against degradation in rat liver. Enzyme-coenzyme interactions, specifically those involving pyridoxal phosphate, have been postulated as playing a comparable role in degradation of pyridoxal enzymes. This view has received considerable impetus from the discovery by Katunuma and his colleagues of proteases which specifically cleave the apoenzyme forms of pyridoxal enzymes (4, 5), as well as from the correlation found by Litwack and Rosenfield (6) between rates of dissociation of coenzyme to yield unstable apoenzymes which may be rate limiting for degradation of pyridoxal enzymes, differential rates of dissociation then being responsible for the observed heterogeneity of degradation rates in vivo.

We have assessed the role of coenzyme in turnover of two aminotransferases in rat liver, the l-tyrosine- and l-alanine-2-oxoglutarate aminotransferases (EC 2.6.1.5 and 2.6.1.2, respectively). These are similar enzymes with respect to size, subunit composition, coenzyme content, and localization in the soluble fraction of cytoplasmic protein (7, 8), as well as catalytic function. Both are inducible by glucocorticoids (9, 10). However, they differ in properties particularly relevant to the current topic. Tyrosine aminotransferase is degraded very rapidly in vivo while alanine aminotransferase degradation is slow. Pyridoxal phosphate is readily dissociated from tyrosine aminotransferase; moderate purification yields an active apoenzyme which readily reassociates with coenzyme (11). The coenzyme of alanine aminotransferase is essentially non-dissociable (7). In this assessment we have analyzed the effect of pyridoxine deficiency on the various parameters determining amount and activity levels of these enzymes.

EXPERIMENTAL PROCEDURES

Animals - Male weanling rats of the Sprague-Dawley strain were maintained on regular laboratory chow and water ad libitum (normal) or on a pyridoxine-deficient diet (Nutritional Biochemicals) for 12 ± 2 weeks (B6-deficient). Growth of the animals on the deficient diet ceased in the latter phases of treatment, and other signs of pyridoxine deficiency were apparent.

Isotopic-Immunochemical Procedures - Preparation of homogeneous tyrosine and alanine aminotransferases and elicitation of specific antisera were as described by Stiles et al. (8), and methods for partial purification of extracts and immunoprecipitation have been detailed before (8, 12). Briefly, aliquots of liver soluble fractions taken at intervals after isotope administration and equivalent to 5 g of liver were supplemented with the appropriate carrier enzyme and passed over 2.0 ml column of DEAE-cellulose. After elution, each was heat-treated and precipitated with a slight excess of the respective antibody. Recovery of both enzymes was consistently between 70 and 76% and has been corrected for in the data presented. Control precipitations to estimate nonspecific radioactivity in the immune precipitates were also done (12) and small corrections were made for this factor as well.

Assays - Tyrosine aminotransferase was assayed as described before (11); the unit of activity is 1 µmol/min. Proteins were determined by the biuret procedure (14). Electrophoresis on sodium dodecyl sulfate-polyacrylamide gels was by the method of Weber and Osborne (15).

RESULTS AND DISCUSSION

Tyrosine Aminotransferase - Direct assay of crude soluble fractions of liver revealed that pyridoxine deficiency caused a 40% decrease in tyrosine aminotransferase activity, assayed in the usual way with added coenzyme (Table I). Assays without added pyridoxal phosphate show that the bulk of the enzyme in livers of normal rats is present as holoenzyme, whereas in...
Immunological analysis of tyrosine aminotransferase synthesis in normal and B$_6$-deficient rats

Data are the means ± standard error for six rats in each group. The animals were fasted for 18 h and received 100 µCi of $[^3H]$leucine/100 g of body weight 15 min before death. Aminotransferase activities were assayed in 105,000 × g supernatant fractions in the presence (total activity) or absence (holoenzyme activity) of pyridoxal phosphate.

<table>
<thead>
<tr>
<th>Status</th>
<th>Aminotransferase activity (units/mg protein)</th>
<th>% holoenzyme</th>
<th>Radioactivity in:</th>
<th>Relative rate of aminotransferase synthesis (B/A)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total enzyme</td>
<td>Holoenzyme</td>
<td>Total soluble protein (cpm/mg protein)</td>
<td>Aminotransferase (B) (cpm/5 g liver)</td>
</tr>
<tr>
<td>Normal</td>
<td>41.2 ± 2.25</td>
<td>36.8 ± 2.46</td>
<td>4548 ± 240</td>
<td>5472 ± 833</td>
</tr>
<tr>
<td>B$_6$-deficient</td>
<td>24.4 ± 2.82</td>
<td>13.3 ± 2.47</td>
<td>5470 ± 510</td>
<td>3091 ± 498</td>
</tr>
</tbody>
</table>

B$_6$-deficient animals nearly half is in the apoenzyme form, confirming an earlier report (16). When the rate of synthesis of tyrosine aminotransferase was determined by pulse labeling and immune precipitation we found this parameter to be reduced to 46% of normal in the B$_6$-deficient rats, essentially in accord with the decrease in total enzyme. Direct "chase" analyses of the rate of tyrosine aminotransferase degradation showed this parameter to be unchanged, the enzyme being degraded with the usual half-life of 1.5 h in both normal and B$_6$-deficient rats (Fig. 1). Specificity of our immune precipitation methods with this enzyme has been documented before (8, 17). Hence, coenzyme deficiency results in a significant reduction in tyrosine aminotransferase levels, a reduction clearly attributable to reduced synthesis of the enzyme. Much of this enzyme is free of coenzyme in the B$_6$-deficient state, but its degradation nevertheless proceeds at the normal rate.

Alanine Aminotransferase—Activity levels of alanine aminotransferase were similarly reduced in B$_6$ deficiency (Table II). In agreement with Segal and Matsuzawa (13), we could detect no effect of added coenzyme in assays of this enzyme in crude extracts under a variety of incubation conditions, i.e., all of the active enzyme is present as holoenzyme.

The specificity of immune precipitations of this enzyme is illustrated in Fig. 2, which shows results of electrophoresis in denaturing gels of labeled alanine aminotransferase precipitated by antibody from liver extracts of normal and deficient rats. In both cases only minor contamination by unrelated, labeled protein is apparent and more than 90% of the radioactivity is present in the 57,000-dalton subunits of the enzyme.

In contrast to the results with tyrosine aminotransferase, the rate of synthesis of alanine aminotransferase was not significantly changed in pyridoxine deficiency (Table II). Analysis of the rate of degradation showed that this parameter was also not altered significantly in the livers of B$_6$-deficient rats (Fig. 3). The half-life observed in these measurements (3.2 to 3.4 days) is in agreement with that reported by Segal and Kim (10) and is comparable to that of the total soluble proteins, which also remains unchanged in B$_6$ deficiency (Fig. 3). These measurements are apparently not markedly influenced by isotope reutilization since the same rate of degradation of soluble hepatic proteins was determined by Kim and Kim (18) using the poorly reutilized precursor, guanidino-labeled arginine.

There is an apparent discrepancy in these data, in that total active enzyme was reduced by 40% but rates of both synthesis and degradation were normal. This was resolved by the immunotitration analyses presented in Fig. 4. Here it is apparent that the livers of B$_6$-deficient rats contain a significant fraction of alanine aminotransferase in a form that is recognized by antibody but enzymically inactive. By this assay the alanine aminotransferase content of extracts from deficient animals

![Fig. 1. Effect of B$_6$ deficiency on the rate of tyrosine aminotransferase degradation. The data are the average of two animals at each point. Rats were fasted for 18 h before isotope administration. At zero time each rat was given intraperitoneally 100 µCi of $[^3H]$leucine/100 g of body weight. Solid circles, normal; open circles, B$_6$-deficient rats.](http://www.jbc.org/)

![Fig. 2. Polyacrylamide-sodium dodecyl sulfate gel electrophoresis of radioactive immunoprecipitates formed with specific anti-alanine aminotransferase antibody. Rats were fasted for 18 h and received 100 µCi of $[^3H]$leucine/100 g of body weight 1 h before they were decapitated and liver extracts (105,000 × g soluble fractions) were prepared. Radioactive alanine aminotransferase was selectively precipitated by specific anti-aminotransferase after partial purification as described under "Experimental Procedures." Electrophoresis was carried out at 8 mA/gel for 3½ h. A, normal; B, B$_6$-deficient rats.](http://www.jbc.org/)
Role of Coenzyme in Aminotransferase Turnover

4960

TABLE II

Immunochemical analysis of alanine aminotransferase synthesis in normal and B<sub>6</sub>-deficient rats

<table>
<thead>
<tr>
<th>Status</th>
<th>Enzyme (units/mg protein)</th>
<th>Antigen (antigen units)</th>
<th>Radioactivity (cpm/mg protein)</th>
<th>Relative rate of aminotransferase synthesis (B/A)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>0.212 ± 0.041</td>
<td>0.217 ± 0.01</td>
<td>7780 ± 666</td>
<td>0.353 ± 0.021</td>
</tr>
<tr>
<td>B&lt;sub&gt;6&lt;/sub&gt;-deficient</td>
<td>0.121 ± 0.014</td>
<td>0.191 ± 0.017</td>
<td>8370 ± 691</td>
<td>0.315 ± 0.067</td>
</tr>
</tbody>
</table>

Data are the means ± standard error for five rats in each group. The animals were fasted for 18 h and received 100 µCi of [3H]leucine/100 g of body weight 1 h before death. Aminotransferase enzyme activity and antigen activity were assayed in 105,000 x g supernatant fractions as described under "Experimental Procedures" and in the legend to Fig. 4.

Since the enzymically inactive alanine aminotransferase is fully active as antigen it is apparent that our immunochemical analyses of rates of degradation (and of synthesis) include this form as well as the enzymically active form. The inactive form, apparently complete except lacking coenzyme (cf. Fig. 9), constitutes 40% of the total enzyme in extracts from deficient rats. An alteration in rate of degradation (or of synthesis) of this fraction of the enzyme would be readily detected by these procedures, and hence, we conclude that alanine aminotransferase apoenzyme is both synthesized and degraded at the same rate as the active holoenzyme.

Pyridoxine deficiency lowers the rate of tyrosine aminotransferase synthesis, and thereby the amount of this enzyme, but does not influence synthesis of alanine aminotransferase or of the total soluble proteins. Tyrosine aminotransferase synthesis is controlled by a variety of hormonal regulators (19) and it is probable that the effect we observe reflects partial endocrine dysfunction owing to prolonged vitamin deficiency.

These data clearly exclude the possibility that coenzyme binding is a significant determinant of rates of degradation of the two aminotransferases and, in our view, cast considerable doubt on the validity of the concept that enzyme-coenzyme interactions are generally significant in intracellular protein turnover. Coenzymes do protect a number of enzymes against inactivation in vitro (4, 5, 20), including tyrosine aminotransferase (21), but it is apparent that extrapolation of such effects to intracellular processes is not warranted.

While differences in coenzyme binding cannot account for the differential rates of degradation of these enzymes, it is likely that these properties are interrelated in that they are reflections of structural features which determine both. It is of interest that alanine aminotransferase appears to be highly hydrophobic, as it is insoluble in low concentrations of (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> and can readily be purified by hydrophobic chromatography on valine-Sepharose. But by these criteria tyrosine aminotransferase is not. Intracellularly, the tyrosine enzyme could be considered, then, to be a highly flexible structure undergoing continuous conformational "breathing," a situation encouraging both facile coenzyme dissociation and rapid degradation, while the tight structure of the hydrophobic enzyme permits neither of these.

REFERENCES


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Role of Coenzyme in Aminotransferase Turnover

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