The Cofactor-mediated Regulation of Apoenzyme Levels in Animal Tissues

I. THE PYRIDOXINE-INDUCED RISE OF RAT LIVER TYROSINE TRANSMINASE LEVEL IN VIVO*

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It has so far not been possible to evaluate fully the extent to which the synthesis of apoenzymes, in animal organs, may be regulated by cellular concentrations of the substrate or cofactor moiety of the same enzyme system. A few examples of decreased apoenzyme levels have been reported to occur in tissues of animals maintained for some weeks on a diet deficient in the appropriate vitamin or substrate (1). However, during the protracted time period required for such nutritional studies, complex physiological alterations may occur which befoul the factors directly responsible for the control of specific enzyme synthesis. More suitable systems for study were provided in a few instances when a single large dose of substrate evoked a rapid response in enzyme level. This is best exemplified by the several fold rise in the level of mammalian liver tryptophan pyrrolase within a few hours after tryptophan administration (2). In connection with observations (3, 4) on the role of the heme cofactor in this process, it has been suggested (5) that the enzyme-mediated control of apoenzyme levels, or "cofactor induction," may be a relatively frequent phenomenon. The present study, a preliminary report of which has appeared (6), indicates that the level of liver apotyrosine-α-ketoglutarate transaminase amounts can occur in 4 hours. This response in intact (nonadrenalectomized) rats was slightly higher.

This response in intact (nonadrenalectomized) rats was slightly higher. Thus, an approximately 3-fold increase and that two 100-mg doses elicited the highest response. It was preferable to measure reaction rates instead of estimating the product at the end of the incubation period only, since the variation in the endogenous (zero time) p-hydroxyphenylpyruvate content was estimated as previously described (9). It was preferable to measure reaction rates instead of estimating the product at the end of the incubation period only, since the variation in the endogenous (zero time) p-hydroxyphenylpyruvate content of the different experimental livers may introduce a significant error. The results are expressed as micromoles of p-hydroxyphenylpyruvate formed per g of liver per minute.

RESULTS

The tyrosine-α-ketoglutarate transaminase activity of liver extracts as a function of added pyridoxal phosphate is shown in Fig. 1. It may be seen that maximal activity is obtained at $10^{-3}$ M concentration of pyridoxal phosphate. Neither higher amounts of pyridoxal phosphate nor preliminary incubation and homogenization in the presence of pyridoxal phosphate elicited greater activity in normal liver extracts. Therefore, the activity measured with excess pyridoxal phosphate is assumed to reflect the apotyrosine-α-ketoglutarate transaminase levels of the liver. It may be seen that liver extracts obtained from a rat after pyridoxine administration exhibit a significantly higher tyrosine-α-ketoglutarate transaminase activity than do normal liver extracts supplemented with optimal amounts of pyridoxal phosphate.

Table I depicts a series of experiments on the response of tyrosine-α-ketoglutarate transaminase levels, estimated with excess pyridoxal phosphate, to varying doses of pyridoxine. It may be seen that at least 100 mg were necessary to obtain a significant increase and that two 100-mg doses elicited the highest response. Thus, an approximately 3-fold increase in apotyrosine-α-ketoglutarate transaminase amounts can occur in 4 hours. This response in intact (nonadrenalectomized) rats was slightly higher.

Table II shows the effects of puromycin, an inhibitor of protein synthesis (10) and induced enzyme formation (11, 12), and of actinomycin, an inhibitor of ribonucleic acid synthesis (13), on the cofactor induction of tyrosine-α-ketoglutarate transaminase, in vivo. Previous experiments (12), carried out under similar conditions, showed that these substances inhibited the incorpor-
poration of precursors into liver protein and RNA, respectively. It may be seen that puromycin does not influence the normal tyrosine-α-ketoglutarate transaminase level but largely prevents the pyridoxine-induced rise, whereas actinomycin is ineffective. In contrast, as already reported (12), the hormonal induction of this enzyme is inhibited by both agents.

Attempts at estimating the pyridoxal phosphate concentration in the liver following pyridoxine administration did not lead to conclusive results. The increases in the total amount of pyridoxal phosphate were small and variable. This difficulty is presumably associated with the fact that much of it is tightly bound to various proteins. However, the decrease in the degree of stimulation of tyrosine-α-ketoglutarate transaminase activity by added pyridoxal phosphate (cf. Table III) indicates that the amount of available pyridoxal phosphate increased after pyridoxine administration. This was tested 1 hour after pyridoxine administration, before the total obtainable activity, i.e., the level of apotyrosine transaminase, rose significantly. The decreased pyridoxal phosphate requirement is detectable at a later time as well (cf. Fig. 1). It may also be seen in Table III that puromycin, which prevented the accumulation of apotyrosine-α-ketoglutarate transaminase (cf. Table II), does not interfere with this saturation process.

**DISCUSSION**

The present studies indicate that cofactors of an enzyme system in vivo, in addition to their obvious function, may have an important role in regulating the amount of protein moiety of the same system. It is unlikely that the pyridoxine-induced increase of tyrosine transaminase levels represents the activation of existing enzyme since the activity, both before and after pyridoxine treatment, was measured in the presence of excess added pyridoxal phosphate. Puromycin, an inhibitor of protein synthesis and induced enzyme formation (10-12), interferes with the induction of tyrosine transaminase, but does not prevent the increase in available pyridoxal phosphate that occurs after pyridoxine treatment. Thus, in addition to the increase in cofactor

<table>
<thead>
<tr>
<th>Pyridoxine administered (per 100 g of body weight)</th>
<th>Induction period</th>
<th>Tyrosine transaminase activity (with 10^{-4} M pyridoxal phosphate)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0</td>
<td>0.82 ± 0.05</td>
</tr>
<tr>
<td>10 mg × 3</td>
<td>6</td>
<td>0.92 ± 0.10</td>
</tr>
<tr>
<td>100 mg</td>
<td>2</td>
<td>1.30 ± 0.20</td>
</tr>
<tr>
<td>50 mg × 2</td>
<td>4</td>
<td>1.52 ± 0.09</td>
</tr>
<tr>
<td>100 mg × 2</td>
<td>4</td>
<td>2.53 ± 0.05</td>
</tr>
<tr>
<td>100 mg × 3</td>
<td>6</td>
<td>2.30 ± 0.16</td>
</tr>
</tbody>
</table>

**Table II**

**Effect of inhibitors on induced rise of liver tyrosine-α-ketoglutarate transaminase activity in adrenalectomized rats in vivo**

The time between the first injection and the enzyme assay was 4 hours in each case. Puromycin (3.5 mg per 100 g) and pyridoxine (100 mg per 100 g) were given at 0 and 2 hours; cortisone (1 mg per 100 g) and actinomycin (0.07 mg per 100 g) were given at zero time. Each value given is a mean (± standard error) of results on six to eight rat livers.

<table>
<thead>
<tr>
<th>Substances administered</th>
<th>Tyrosine transaminase activity (with 10^{-4} M pyridoxal phosphate)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0.90 ± 0.06</td>
</tr>
<tr>
<td>Puromycin</td>
<td>1.10 ± 0.25</td>
</tr>
<tr>
<td>Actinomycin</td>
<td>0.92 ± 0.15</td>
</tr>
<tr>
<td>Pyridoxine</td>
<td>2.53 ± 0.05</td>
</tr>
<tr>
<td>Cortisone</td>
<td>2.55 ± 0.10</td>
</tr>
<tr>
<td>Pyridoxine + puromycin</td>
<td>1.12 ± 0.13</td>
</tr>
<tr>
<td>Pyridoxine + actinomycin</td>
<td>2.40 ± 0.10</td>
</tr>
<tr>
<td>Cortisone + puromycin</td>
<td>1.51 ± 0.18</td>
</tr>
<tr>
<td>Cortisone + actinomycin</td>
<td>1.52 ± 0.12</td>
</tr>
</tbody>
</table>

**Table III**

**Pyridoxal phosphate requirement of rat liver tyrosine-α-ketoglutarate transaminase during early phase of induction**

Rats were killed 1 hour after the administration of the indicated substances, and the tyrosine-α-ketoglutarate transaminase activity of each liver extract was estimated without (−) and with (+) 10^{-4} M added pyridoxal phosphate. Each value is a mean (± standard error) of results on six individual livers.

<table>
<thead>
<tr>
<th>Substances administered (per 100 g of body weight)</th>
<th>Tyrosine transaminase activity (with 10^{-4} M pyridoxal phosphate)</th>
<th>Stimulation by pyridoxal-P (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0.40 ± 0.04</td>
<td>110</td>
</tr>
<tr>
<td>Pyridoxine, 100 mg</td>
<td>0.80 ± 0.05</td>
<td>12</td>
</tr>
<tr>
<td>Pyridoxine, 100 mg + puromycin, 3.5 g</td>
<td>0.74 ± 0.06</td>
<td>24</td>
</tr>
</tbody>
</table>

**Table I**

**Effect of pyridoxine administration on liver apotyrosine-α-ketoglutarate transaminase levels in adrenalectomized rats**

The interval between repeated pyridoxine administrations was 2 hours. The time between the first pyridoxine treatment and killing of the rats is shown under "Induction period." The tyrosine-α-ketoglutarate transaminase activity of fresh liver extracts was estimated in the presence of 10^{-4} M pyridoxal phosphate. The values given are means (± standard error) of results on 8 to 10 individual livers.

![Figure 1](http://www.jbc.org)
level, an intact protein-synthesizing machinery is necessary. This finding further suggests that pyridoxine administration stimulates the rate of apotyrosine transaminase synthesis de novo rather than cause the activation of latent enzyme. The observed 3-fold rise in enzyme level occurs within 4 hours. That the system responsible for the synthesis of apotyrosine transaminase can respond at such a rate to suitable stimuli has been demonstrated by the direct measurements of the cortisone-induced accumulation of the same enzyme (14).

The need for using large doses of pyridoxine in these studies probably results from the rapid excretion of this substance and the inefficiency of its conversion to pyridoxal phosphate. A quantity of 100 mg was necessary to decrease significantly the requirement of liver tyrosine transaminase activity for added pyridoxal phosphate. This saturation process precedes the increase in total enzyme amount. A similar time sequence of events was observed during the substrate induction of rat liver tryptophan pyrrolase. Much of this dissociable heme enzyme (15) exists in normal liver as free apoenzyme which is converted to holoenzyme shortly after the administration of tryptophan (3). This process is followed by an increase in the total, immunochemically detectable (16) amount of enzyme. This observation suggested that a high (normal) concentration of apotryptophan pyrrolase in the cytoplasm may inhibit the release of the same protein from the enzyme-forming site, whereas its conversion to holotryptophan pyrrolase may facilitate this process and result in higher tryptophan pyrrolase levels (3). Such a hypothesis may be applicable to the now observed cofactor induction of tyrosine transaminase, although a more direct action of the cofactor on the enzyme-forming site, or on the rate of degradation of the enzyme, has not been excluded.

It has been previously shown (12) that the cortisone-induced elevation of liver tryptophan pyrrolase and tyrosine transaminase is inhibited by actinomycin, whereas the substrate induction of tryptophan pyrrolase is not. The insensitivity of the pyridoxine-induced elevation of tyrosine transaminase to actinomycin (cf. Table II) completes this picture and lends further support to the existence of the previously discussed (5, 12) two major regulatory processes. One of these, the hormonal process, may depend on an increase in the amount of certain RNA species, whereas cofactor induction may involve a faster rate of functioning of an unchanged number of synthetic sites.

SUMMARY

The tyrosine-α-ketoglutarate transaminase activity of liver extracts, assayed in the presence of excess pyridoxal phosphate, was increased by the parenteral administration of pyridoxine to adrenalectomized rats. A 3-fold rise can occur in 4 hours. The administration of puromycin, an inhibitor of protein synthesis, prevents the pyridoxine-induced rise in the amount of tyrosine-α-ketoglutarate transaminase. The results suggest that coenzyme levels, in addition to regulating the activity of existing enzyme, may influence, in vivo, the amount of the protein moiety of appropriate enzyme systems.

REFERENCES

The Cofactor-mediated Regulation of Apoenzyme Levels in Animal Tissues: I.
THE PYRIDOXINE-INDUCED RISE OF RAT LIVER TYROSINE
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