CHAPTER 1

TISSUE AND CELLULAR DISTRIBUTION OF L-ALANINE:4,5-DIOXOVALERATE TRANSAMINASE IN RATS AND EFFECT OF AGE OF ITS ACTIVITY
SUMMARY

The formation of 5-aminolevulinic acid, the first committed precursor of heme is now considered to be mediated by alanine:DOVA transaminase in addition to the conventional pathway by ALA synthetase. In the present investigation rat liver mitochondria was found to contain alanine:DOVA transaminase which catalyzes the synthesis of ALA via a transamination reaction and its capacity to synthesize ALA was greater than that of ALA synthetase from the same mitochondria.

We examined the presence of alanine:DOVA transaminase in the different tissues of adult rats, and the enzyme activity was found to be widely distributed. The maximal enzyme activity of alanine:DOVA transaminase was observed in kidney, followed by liver. Moreover, the presence of this enzyme was also detected from the tissues of heart, brain, testis, adrenal gland and spleen in less quantity as compared to kidney and liver. Further, the subcellular distribution of alanine:DOVA transaminase in liver was examined in the different fractions, e.g., peroxisomal, mitochondrial, lysosomal and cytosplasmic using different enzymes as markers. Alanine:DOVA transaminase activity was found to be associated only with mitochondrial fraction. Its presence had been further localized inside the mitochondria.
Though, the developmental aspects of heme biosynthesis has been reported previously, information on the activity of alanine:DOVA transaminase in different tissues of newborn, young and adult rats is lacking. This prompted us to carry out the present study, in which the basal level of hepatic and heart alanine:DOVA transaminase activity decreased significantly as a function of age. But no alteration was observed in brain and skeletal muscle.

Our findings suggest the important observation that rat mitochondrial alanine:DOVA transaminase has greater capability to synthesize ALA than ALA synthetase and its activity is regulated in a tissue specific manner, a time dependent decrease is a general feature of aging animals.

**INTRODUCTION**

All cells in higher animals are capable of heme biosynthesis, either continually throughout the life or some stage of their development. The heme is utilized in the synthesis of various heme proteins including the mitochondrial and microsomal cytochromes which are essential to cellular growth and function. ALA is the first committed precursor of porphyrin and heme (4). In other words, the specific step of the biosynthesis of all tetrapyrroles, is the formation of ALA. In animal tissues and in some bacterial...
systems ALA is known to be formed via the classical pathway using succinyl CoA and glycine as precursors which are condensed by the enzyme ALA synthetase (4,132). A major site for control of the hepatic heme biosynthetic pathway is proved to be at the level of ALA formation (43-45). Though ALA is an obligatory precursor in the biosynthesis of chlorophyll, ALA synthetase has not yet been demonstrated in green plants. It now seems established that plants directly from ALA from the intact skeleton of 5-C precursors (17,19,32) by the enzyme which catalyzes a transamination reaction between alanine and 4,5-dioxovaleric acid. As seen in the plant system, an alternate biosynthetic pathway of ALA by alanine:DOVA transaminase has been demonstrated recently in the bovine liver (21). In addition, a recent report of biosynthesis of porphyrins and heme from C DOVA in hepatocytes (23) further suggests its important role on the regulation of heme biosynthesis. Though extensive studies have been done on the regulatory role of ALA synthetase on heme biosynthesis, very little is known about the presence of alanine:DOVA transaminase in the mammalian system and its physiological significance. Since ALA formation is known to regulate the heme biosynthetic pathway (43-45) the enzyme alanine:DOVA transaminase might have some important role in the regulation of heme biosynthesis.
Although developing erythrocytes account for most of the heme produced by higher animals, the liver is the second major locus of heme synthesis. Hepatic heme synthesis may also be stimulated in response to the inducers of microsomal cytochrome P-450 which may be controlled by different factors, e.g., age, nutritional status (160-162). Since developmental aspects of hepatic heme biosynthesis at the level of different enzymatic steps, e.g., ALA synthetase, (160,163), uroporphyrinogen I synthetase and heme oxygenase (164) are reported, the changes of alanine:DOVA transaminase activity during aging may enlighten the control mechanism of heme biosynthesis.

In this chapter, we report the presence of alanine:DOVA transaminase not only in liver but also in the other mammalian tissues. Subcellular distribution of this enzyme has been studied and seen to be localized inside the mitochondria. Furthermore, an attempt has been made to study the changes of alanine:DOVA transaminase activity during aging.

RESULTS

Alanine:DOVA Transaminase Activity in Various Tissues of Rat

The enzyme activity was measured in different organs
of adult rat (Table I). The maximal specific activity was obtained in the kidney, followed by liver, heart, brain, testis, spleen, adrenal gland, and skeletal muscle. Our study gives the first report of the presence of this enzyme in tissues other than liver and kidney. This may be correlated with the formation of hemeproteins in different tissues.

As alanine:DOVA transaminase was shown to be present in the mammalian system, the comparative studies of the activity of 5 ALA synthetase and alanine:DOVA transaminase were done. The capacity of hepatic alanine:DOVA transaminase to synthesize ALA was studied and appeared to be far greater than the capacity of ALA synthetase from the same source (Table II). We were able to detect ALA formation by transaminase reaction, even in the crude preparations easily by simple colorimetric assay, whereas the yield of ALA formation by ALA synthetase was very low. This has been supported by other workers (134) and they recommended radiochemical assay for ALA synthetase. Thus, the experimental evidence conclusively suggest the greater capacity of alanine:4,5 DOVA transaminase to form ALA than by ALA synthetase.
### TABLE I

**TISSUE DISTRIBUTION OF ALANINE:4,5-DIOXO-VALERATE TRANSAMINASE ACTIVITY IN HOMOGENATE**

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Specific activity&lt;sup&gt;a&lt;/sup&gt; n mole/mg Protein/30 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain</td>
<td>2.08 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Heart</td>
<td>1.78 ± 0.02</td>
</tr>
<tr>
<td>Liver</td>
<td>52.08 ± 0.97</td>
</tr>
<tr>
<td>Kidney</td>
<td>72.67 ± 0.70</td>
</tr>
<tr>
<td>Adrenal gland</td>
<td>1.85 ± 0.02</td>
</tr>
<tr>
<td>Spleen</td>
<td>1.35 ± 0.10</td>
</tr>
<tr>
<td>Testis</td>
<td>2.90 ± 0.15</td>
</tr>
</tbody>
</table>

<sup>a</sup> Values are the mean ± SEM of triplicate determinations on at least 6 animals.
TABLE II

ALA SYNTHETASE AND L-ALANINE:4,5-DIOXOVALERATE TRANSAMINASE ACTIVITIES IN RAT LIVER HOMOGENATE

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Specific activity&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>μ mole of ALA formed/g tissue wet wt/1 hr</td>
</tr>
<tr>
<td>ALA Synthetase</td>
<td>0.381 ± 0.01</td>
</tr>
<tr>
<td>DOVA Transaminase</td>
<td>6.860 ± 0.03</td>
</tr>
</tbody>
</table>

<sup>a</sup> Values are the mean ± SEM of triplicate determinations on at least 6 animals.
Cellular Distribution of Alanine:DOVA Transaminase:

A representative sedimentation in a sucrose density gradient for the homogenate from rat liver is presented in Fig. 6. The peroxisomes and mitochondria were separated, the peroxisomes marked by catalase (E.C.1.11.1.6) were at a density of about 1.23 g/ml, and the mitochondria, marked by glutamate dehydrogenase (E.C.1.4.1.2) at a density about 1.21 g/ml. Acid phosphatase (E.C.3.1.3.2) as a lysosomal marker was distributed over a broad density range with a peak of about 1.22 g/ml (not shown in figure) and ALA dehydratase (E.C.4.2.1.24) as a cytoplasmic fraction, at a density of about 1.09 g/ml. Alanine:4,5-dioxovalerate transaminase activities showed distribution profile identical with that of glutamate dehydrogenase. The hemogenate activity of glutamate dehydrogenase and alanine:4,5-dioxovalerate transaminase activity were recovered in the mitochondrial fraction. These results show that this transaminase is associated with the mitochondria.

Localization of Alanine:4,5-Dioxovalerate Transaminase in Mitochondria

Experiments were undertaken to localize L-alanine; 4,5-dioxovalerate transaminase within the mitochondria. Liver tissue was homogenized and the mitochondria were separated by the method indicated in Materials and Methods.
Fig. 6: SUBCELLULAR DISTRIBUTION OF ALANINE:

4,5-DIOXOVALERATE TRANSAMINASE IN

RAT LIVER: The post nuclear supernatant was prepared from rat liver and subjected to sucrose density gradient centrifugation as described in the text. ○—○, alanine: DOVA transaminase; □—□, ALA dehydratase; ●—●, glutamate dehydrogenase; △—△ catalase.
The cytosol was devoid of enzyme activity. The mitochondria were sonicated and freeze-thawed and centrifuged in Beckman Ultra Centrifuge at 105,000 x g for 1 hr. The enzyme activity appeared only in the supernatant fraction indicating that the enzyme is not membrane bound.

Age Dependent Variation in the Activity of Alanine: DOVA Transaminase

The basal activities of mitochondrial alanine: DOVA transaminase from different tissues of newborn, young and adult rats are represented in Table III. It is clear from the results that the activity was decreased significantly in liver and heart as a function of age. A decrease of approximately 43% in young rat liver and with a further significant decrease of 55% in adult rat was seen. Similar type of drop in activity 32% and 46% was observed in heart of young and adult rats respectively. The depletion of this enzyme activity in brain is not significant. In contrast to liver and heart, the activity of kidney alanine: DOVA transaminase remains unchanged.

DISCUSSION

The data represented here report the presence of high activity of mammalian L-alanine:4,5-dioxovalerate transaminase which catalyses the formation of 5-aminolevulinic acid via a transamination reaction in liver and
TABLE III

ALANINE:4,5-DIOXOVALERATE TRANSAMINASE ACTIVITY IN MITOCHONDRIA OF VARIOUS TISSUES OF AGING RAT

<table>
<thead>
<tr>
<th>Age</th>
<th>Liver</th>
<th>Kidney</th>
<th>Heart</th>
<th>Skeletal muscle</th>
<th>Brain</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-4 day</td>
<td>265.755 ± 17.34</td>
<td>580.00 ± 32.4</td>
<td>43.92 ± 1.08</td>
<td>1.98 ± 3.0</td>
<td>17.7 ± 1.6</td>
</tr>
<tr>
<td>Young</td>
<td>154.89 ± 17.8</td>
<td>606.89 ± 26.01</td>
<td>29.56 ± 0.54</td>
<td>1.66 ± 1.07</td>
<td>14.2 ± 1.8</td>
</tr>
<tr>
<td>Adult</td>
<td>121.75 ± 14.8</td>
<td>644.25 ± 10.87</td>
<td>23.84 ± 2.02</td>
<td>1.98 ± 0.08</td>
<td>15.2 ± 2.0</td>
</tr>
</tbody>
</table>

a Values are the mean ± SEM of triplicate determinations on at least 6 animals.

Numbers in the parentheses are percent difference of enzyme activity.
kidney of rats, however, other tissues, e.g., brain, heart, testis, spleen, skeletal muscle and adrenal gland contain this enzyme in less quantity. The product of the transamination reaction is ALA, the precursor of porphobilinogen and tetrapyrroles and heme. In addition we were able to demonstrate greater efficacy of the enzyme alanine:DOVA transaminase to synthesize ALA in rat liver than by ALA synthetase. These observations suggest that alanine:DOVA transaminase might have physiological significance in mammalian tissue to synthesize heme, necessary for the formation of hemeproteins.

In the light of the present findings on the activity of this enzyme in different tissues, it seems that this pathway is highly active in kidney and liver. This can be explained on the fact that these tissues have high content of cytochrome P-450, a major hemeprotein, with rapid turnover rate (62,165), and would require more active heme biosynthesis for the maintenance of normal hepatic concentrations. Similarly, less enzyme activity in brain is in agreement with earlier observation that brain heme and cytochrome P-450 concentrations are much lower than that of hepatic level (166). The neural tissue has been shown to accumulate heme for the biosynthesis of mitochondrial hemeprotein, which have longer half lives than cytochrome P-450 (62). The presence of
alanine:DOVA transaminase in testis and adrenal gland further supports the idea that this enzyme is involved in the formation of heme protein, as cytochrome P-450 is required for hydroxylation of adrenal and testicular steroids (167,168). The existence of this enzyme in skeletal muscle may be justified as the myoglobin synthesis requires heme.

The present study also indicates the effect of age on the activity of DOVA:transaminase in liver, brain and heart whereas in skeletal muscle and kidney it remains same. Similar changes with age have been observed in the case of ALA synthetase (163). Furthermore, the initiation of the erythropoiesis in fetus and new born was accompanied by the appearance of red foci in the liver and earlier observations established the fact that liver acts as erythropoietic organ during development (169). The elevation of hepatic alanine:DOVA transaminase activity may be correlated with observed induction of erythropoiesis in the liver of neonatal rats. In this connection, the correlation of induction of ALA synthetase with erythropoiesis in fetal mouse liver (170), rat liver (171) and human fetal liver (172) may be recalled. In future, we propose to study the effect of erythropoietin on the activity of alanine:DOVA transaminase in kidney and liver.
From this study, two important concepts regarding the regulation of alanine:DOVA transaminase are apparent. The first is that alanine:DOVA transaminase may be regulated in a tissue specific manner. Secondly, a significant decrease in the activity of this enzyme is a general feature of the aging rat in liver. Our observation is comparable to the developmental alteration of hepatic enzymes responsible for heme biosynthesis, e.g., ALA synthetase (160,163) and heme oxygenase (164). Thus, the patterns of heme biosynthesis in various tissues may be altered to varying degrees by factor such as age.

The findings of this investigation, further suggest that alanine:DOVA transaminase in brain, skeletal muscle and kidney is not subject to the same control mechanisms operative in liver and heart. Thus, the pattern of heme biosynthesis in various tissues are altered to a varying degrees by factors such as nutritional status, age or the presence of inducer.

Though the present study suggests only the importance of alanine:DOVA transaminase in heme biosynthesis, further studies are required to clarify its physiological significance and its regulatory role on heme biosynthesis.