Dimethylarginine:Pyruvate Aminotransferase in Rats

PURIFICATION, PROPERTIES, AND IDENTITY WITH ALANINE:GLYOXYLATE AMINOTRANSFERASE 2*

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Dimethylarginine:pyruvate aminotransferase, which plays a role in the metabolism of dimethylarginines, has been purified to homogeneity from rat kidney. The enzyme has a molecular weight of approximately 200,000 and an isoelectric point at about pH 6.3. The enzyme consists of four similar subunits having a molecular weight of about 50,000. The enzyme catalyzes the effective transaminations of guanidinoethyl-L-arginine (N⁰⁰,N⁰⁰-dimethyl-L-arginine, N⁰⁰,N⁰⁰'-dimethyl-L-arginine and N⁰⁰-monomethyl-L-arginine) and the α-amino group of L-ornithine accompanying by the known alanine:glyoxylate aminotransferase activity with the ratios of their specific activities remaining constant during the purification steps. The physicochemical and immunological properties of the purified enzyme were shown to be identical with those of the isozyme of alanine:glyoxylate aminotransferase (EC 2.6.1.44), designated as alanine:glyoxylate aminotransferase 2 (Noguchi, T. (1987) in Peroxisomes in Biology and Medicine (Fahimi, H. D., and Sies, H., eds) pp. 234–243, Springer-Verlag, Heidelberg). The distribution profiles in tissues and the negative response to glucagon treatment further supported the identity of the two enzymes. The present data show that alanine:glyoxylate aminotransferase 2 functions in dimethylarginine metabolism in vivo in rats.

N⁰⁰,N⁰⁰-Dimethyl-L-arginine (DMA)¹ and N⁰,N⁰'-di methyl-L-arginine (DM'A) are known to occur widely in tissue proteins methylated post-translationally (1), and these amino acids are released in body fluids after in vivo protein breakdown (2). Recently, we showed for the first time that both the amino acids were catabolized by a pathway forming the corresponding α-keto acids, α-keto-(N,N-dimethylguanidino)valeric acid (DMGV) and α-keto-(N,N'-dimethylguanidino)valeric acid (DM'GV), and that a part of DMA was degraded via an alternative pathway leading to the formation of citrullin and dimethylamine (3), in which the unique enzyme, DMA dimethylaminohydrolase, was shown to participate (4). The excretion of DMGV and DM'GV in urine was found to be markedly affected by the administration of a vitamin B₆ antagonist, 1-amino-L-proline (5). This fact suggested that pyridoxal 5'-phosphate-dependent enzyme(s) (presumably aminotransferase(s)) might be responsible for the formation of these α-keto acids (3).

An aminotransferase isolated from rat kidney was shown to catalyze the effective transamination between dimethylarginines and pyruvate and formed DMGV and DM'GV. This aminotransferase also catalyzed the transamination between L-alanine and glyoxylate. Although various aminotransferases which utilize pyruvate or glyoxylate as the amino acceptor have been purified from mammalian tissues, there was no known aminotransferase utilizing dimethylarginines as the amino donor. Recently, some of the aminotransferases utilizing pyruvate (or glyoxylate), such as serine:pyruvate aminotransferase (EC 2.6.1.51), phenylalanine:pyruvate aminotransferase (EC 2.6.1.58), histidine:pyruvate aminotransferase (EC 2.6.1.58), kynurenine:glyoxylate aminotransferase (EC 2.6.1.7) and asparagine:oxo-acid aminotransferase (EC 2.6.1.14) have been suggested to be identical with the isozyme of alanine:glyoxylate aminotransferase (EC 2.6.1.44), designated as alanine:glyoxylate aminotransferase 1 (AGT1) (6, 7). Furthermore, it was suggested that the activity of alanine:2,5-dioxovalerate aminotransferase (EC 2.6.1.43) (9) might be the property of another isozyme, designated as alanine:glyoxylate aminotransferase 2 (AGT2) (6, 10). We attempted, therefore, to clarify whether the dimethylarginine:pyruvate aminotransferase activity in rat kidney corresponds to that of the known aminotransferases that utilize pyruvate or glyoxylate as the amino acceptor. In the present report, we describe the purification and properties of dimethylarginine:pyruvate aminotransferase from rat kidney and the identity of the purified enzyme with AGT2 (6, 10). The identity was deduced by a comparison of physicochemical, enzymological, and immunological properties.

EXPERIMENTAL PROCEDURES AND RESULTS²

Copurification of Dimethylarginine:Pyruvate Aminotransferase with Alanine:Glyoxylate Aminotransferase—The dimethylarginine:pyruvate aminotransferase activity was always accompanied by alanine:glyoxylate aminotransferase activity in all the chromatographic procedures. The ratio of the specific activities remaining constant throughout the purification (Table I). On native polyacrylamide gel electrophoresis (PAGE), the purified enzyme gave a single protein band and both the dimethylarginine:pyruvate aminotransferase and al-

¹ Portions of this paper (including "Experimental Procedures," part of "Results," Figs. 2-5, 7, 8, 10, and 11, and Tables I, IV-VIII) are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are included in the microfilm edition of the Journal that is available from Waverly Press.

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³ The abbreviations used are: DMA, N⁰,N⁰-dimethyl-L-arginine; DM'A, N⁰,N⁰'-dimethyl-L-arginine; DMGV, α-keto-(N,N-dimethylguanidino)valeric acid; DM'GV, α-keto-(N,N'-dimethylguanidino)valeric acid; PAGE, polyacrylamide gel electrophoresis.
amino:glyoxylate aminotransferase activities were shown to coincide with this protein band (Fig. 1).

**Physicochemical Properties of Dimethylarginine:Pyruvate Aminotransferase**—The molecular weight of the enzyme was determined to be 197,000 ± 5,000 (n = 3) by Sephacryl S-300 gel filtration (Fig. 2) and 179,000 ± 3,000 (n = 3) by analytical ultracentrifugation. On sodium dodecyl sulfate-PAGE, the denatured enzyme also gave a single protein band with a molecular weight of 50,000 (Fig. 2), suggesting that the enzyme consisted of four similar subunits. By chromatofocusing, denatured enzyme also gave a single protein band with an estimated isoelectric point of the enzyme was estimated to be around pH 6.3 (Fig. 3). The enzyme showed an absorption spectrum of a typical pyridoxal 5'-phosphate-dependent enzyme and the phosphate content of the enzyme was determined to be 4.1 ± 0.1 (n = 3) mol/mol of the enzyme, based on the molecular weight of the enzyme as 200,000.

**Effect of pH**—The aminotransferase activity of the enzyme was investigated as a function of pH over the range from 6.5 to 12.0. The enzyme exhibited optimum activity at about 10 for the transamination between dimethylarginines and pyruvate, at about 9.5 for the transamination between L-alanine and glyoxylate, and at about 10.5 for the transamination of the α-amino group of L-ornithine and pyruvate, respectively (Fig. 5).

**Substrate Specificity**—The relative activities of the transamination for various amino acids were determined with both pyruvate and glyoxylate as amino acceptor. The aminotransferase specificity of the enzyme is shown in Table II. Pyruvate, glyoxylate, oxaloacetate, and α-ketobutyrate could serve as good amino donors, whereas α-ketovalerate was inactive in the presence of both DMA and L-alanine.

**Transamination of L-Ornithine**—L-Ornithine was found to act as a good amino donor for pyruvate and glyoxylate. The product of the transamination was investigated using a reaction system containing L-[U-14C]ornithine (2 μCi/μmol) and pyruvate. The reaction products were fractionated using an amino acid analyzer as described earlier (3) and monitored by the radioactivity and the absorption of the adduct with o-aminobenzaldehyde (13). As shown in Fig. 6, the radioactive product with an absorption at 443 nm emerged at the elution position coincident with that of 4α-pyrrolidone-5-carboxylic acid, but not with that of 4α-pyrrolidone-5-carboxylic acid. No other radioactive compound was detected except for unreacted L-ornithine. The radioactive product isolated by preparative thin layer chromatography (25) was shown to react with o-aminobenzaldehyde to form an adduct having an absorption spectrum identical with that obtained from au-
the amino donors and 2 transamination between DMA and pyruvate. The $V_{\text{max}}$ value for the transamination between L-lysine with pyruvate, respectively, and that for pyruvate with DMA about eight times that for the transamination between DMA for the transamination between L-alanine and glyoxylate was shown to be irreversible in the same manner as that between L-alanine and glyoxylate, whereas the transamination of DMA (or DM'A) with pyruvate was reversible and the $K_m$ value, [DM'GV] /[L-alanine]/[DM'A] [pyruvate], was estimated to be 0.8 ± 0.2 (n = 5) at pH 10.0 and 37°C. In each of the above transamination reactions including the $\alpha$-transamination\(^1\) of L-ornithine, Lineweaver-

### Kinetic Properties

The $K_m$ values for DMA and DM'A were determined to be 9.7 and 13.7 mM in transamination with pyruvate, respectively, and that for pyruvate with DMA and DM'A were estimated to be 2.4 and 2.9 mM, respectively (Table IV). In the transamination between L-alanine and glyoxylate, the $K_m$ values for L-alanine and glyoxylate were 7.8 and 2.9 mM, respectively. In these transamination reactions, the $K_m$ values were estimated to be around 10 mM for the amino donors and 2 mM for the amino acceptors, respectively, except that the $K_m$ values in the transamination of L-ornithine with pyruvate were found to be 70 mM and 4.1 mM for L-ornithine and pyruvate, respectively. The $K_m$ value for L-ornithine was about seven times that for DMA, but the $V_{\text{max}}$ value for this transamination reaction was twice that for the transamination between DMA and pyruvate. The $V_{\text{max}}$ value for the transamination between L-alanine and glyoxylate was about eight times that for the transamination between DMA and pyruvate. The transamination between DMA (or DM'A) and glyoxylate was shown to be irreversible in the same manner as that between L-alanine and glyoxylate, whereas the transamination of DMA (or DM'A) with pyruvate was reversible and the $K_m$ value, [DM'GV] /[L-alanine]/[DM'A] [pyruvate], was estimated to be 0.8 ± 0.2 (n = 5) at pH 10.0 and 37°C. In each of the above transamination reactions including the $\alpha$-transamination\(^1\) of L-ornithine, Lineweaver-

### Transamination between DMA (or DM'A) and pyruvate (or glyoxylate) was competitively inhibited by the addition of L-alanine and L-ornithine, and that between L-alanine and glyoxylate was inhibited in the presence of DMA, DM'A, and L-ornithine (Fig. 7), suggesting that all the transamination reactions were catalyzed at the same active site of the enzyme.

### Inhibitors

Phenylhydrazine, hydroxylamine, and L-amino-L-proline (5) inhibited both the dimethylarginine:pyruvate and L-alanine:glyoxylateaminotransferase activities equivalently (Table V). The enzyme inhibited by such compounds was restored to some extent by the treatment with excess amounts of pyridoxal 5'-phosphate. Moreover, the enzyme was also inhibited by several SH-blocking reagents (e.g. HgCl₂ and p-chloromercuribenzoate) (Table V). The inhibition by SH-blocking reagents was not reversed by the addition of excess amounts of an amino donor and/or acceptor.

### Immunological Properties

Antiserum against the purified enzyme prepared from the enzyme-injected rabbit was shown to produce a single connecting band of precipitin between dimethylarginine:pyruvateaminotransferase and AGT2 isolated from rat kidney and liver mitochondria (10), by Ouchterlony double diffusion analyses (Fig. 8). The complete reactivity of the above antibody with the isozymes of alanine:pyruvateaminotransferase and AGT2 were immunologically identical. The immunotitration of the three activities of the enzyme, dimethylarginine:pyruvateaminotransferase, alanine:glyoxylateaminotransferase, and ornithine:pyruvateaminotransferase activities, with the purified IgG antibody specific for dimethylarginine:pyruvateaminotransferase resulted in equivalent losses of all the activities (Fig. 9). The reactivity of the above antibody with the isozymes of alanine:glyoxylateaminotransferase was also examined by an immunoblotting method. The antibody was shown to react with mitochondrial AGT2 prepared from rat kidney, and also

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\(^{1}\) In this paper, we used the following description: $\alpha$-transamination for the transamination of the amino group of L-ornithine and $\delta$-transamination for the transamination of the $\delta$-amino group of L-ornithine.
to recognize only AGT2 even in crude extracts of both kidney and liver (Fig. 10).

Effect of Glucagon—In the liver of rats injected with glucagon, the transamination activity between L-alanine and glyoxylic acid was markedly increased compared with that of control rats, whereas the dimethylarginine:pyruvate aminotransferase activity remained constant (Table VI). This fact showed that the dimethylarginine:pyruvate aminotransferase activity was not associated with the glucagon-inducible alanine:glyoxylate aminotransferase isozyme, namely, AGT1 (10).

Distribution of Dimethylarginine:Pyruvate Aminotransferase in Rat Tissues—In the purification of the enzyme, we used the tissue samples which had been freshly removed from rats, immediately frozen and stored at −80°C until use. After homogenization of the stored sample, most of the dimethylarginine:pyruvate aminotransferase activity was recovered in the cytosol fraction, and the remainder was found in the particulate fractions precipitated at 10,000 × g. The subcellular distribution of the enzyme was, therefore, re-examined using freshly prepared kidney sample. Approximately 80% of both the dimethylarginine:pyruvate aminotransferase and alanine:glyoxylate aminotransferase activities in kidney sample were located in 10,000 × g-precipitate fraction (particulate fraction, presumably mitochondria) (Table VII), indicating that the freeze-thawing of the tissue sample resulted in the breakdown of the particles and also in the solubilization of the mitochondrial enzymes into the 10,000 × g-supernatant fraction as reported by Kobayashi et al. (30). In liver homogenates, half of the alanine:glyoxylate aminotransferase activity was recovered in the 10,000 × g-supernatant fraction, whereas about 80% of the dimethylarginine:pyruvate aminotransferase activity was located in the particulate fraction. The alanine:glyoxylate aminotransferase activity in the 10,000 × g-supernatant fraction of liver homogenate may be largely due to peroxisomal AGT1 (8, 30). The subcellular distribution of dimethylarginine:pyruvate aminotransferase in kidney was also examined by centrifugation in a sucrose density gradient. The sedimentation pattern in a sucrose density gradient for post-nuclear supernatant fraction is shown in Fig. 11. The dimethylarginine:pyruvate aminotransferase activity and alanine:glyoxylate aminotransferase activity showed nearly identical distribution with glutamate dehydrogenase activity as the mitochondrial marker. Distribution of the dimethylarginine:pyruvate aminotransferase activity in tissues is shown in Table VIII together with the amount of the enzyme protein determined by the enzyme immunoassay using the antibody specific for dimethylarginine:pyruvate aminotransferase. Both the enzyme activity and enzyme protein were detected only in kidney and liver homogenates. The enzyme activity and enzyme protein concentration in rat kidney were found to be higher than those in liver homogenate. This observation was fairly consistent with the report on the difference in the distribution of AGT2 between kidney and liver (29, 30).

DISCUSSION

Dimethylarginine:pyruvate aminotransferase responsible for dimethylarginine metabolism in rats was isolated and characterized. The enzyme was demonstrated to catalyze the effective transamination between guanidino-N-methylated L-arginine (e.g. DMA, DM’A, and N’-monomethyl-L-arginine) and N’-nitro-L-arginine and pyruvate (or glyoxylate), in addition to the transamination of the α-amino group of L-ornithine. The enzyme was also shown to have alanine:glyoxylate aminotransferase activity. The ratio of the dimethylarginine:pyruvate aminotransferase activity to alanine:glyoxylate aminotransferase activity in rat kidney was constant during the purification steps. Both activities were shown to coincide with a single protein band by a native PAGE of the purified enzyme. Moreover, all the transamination reactions of the enzyme were shown to be catalyzed at the same active site.

In the present experiment, we have shown that dimethylarginine:pyruvate aminotransferase and AGT2 activities also support the immunological identity of both enzymes.

It is known that the alanine:glyoxylate aminotransferase activity in rat liver is increased by treatment of rats with glucagon; the increased activity is due to the induction of peroxisomal AGT1 and not to mitochondrial AGT2 (10). In liver of the glucagon-injected rats, the dimethylarginine:pyruvate aminotransferase activity was shown to remain constant, while the alanine:glyoxylate aminotransferase activity was significantly increased. In contrast, both the activities in kidney were hardly affected by the glucagon treatment. These observations also support the idea that dimethylarginine:pyruvate aminotransferase activity is associated with AGT2, not with glucagon-inducible AGT1, since almost all the alanine:glyoxylate aminotransferase activity in rat kidney is due to mitochondrial AGT2 (29, 31).

In previous work (3), we demonstrated that DMGV and DM’GV, the transamination products of DMA and DM’A, were detected only in rat kidney and liver, and both the metabolites were accumulated mainly in kidney rather than in liver. In the present experiment, we have shown that dimethylarginine:pyruvate aminotransferase occurs only in the particulate fractions of kidney and liver, especially in mitochondria in kidney, and predominates in kidney rather than in liver, by the enzyme immunoassay (Table VIII). This fact is fairly consistent with the reports that AGT2 in rats predominates in kidney rather than in liver and is located mainly in mitochondria (6, 7, 10, 29). Accordingly, it may be concluded that dimethylarginines are catabolized by dimethylarginine:pyruvate aminotransferase, namely AGT2, mainly in rat kidney and liver mitochondria. In practice, we proved that this system is actively working in vivo by the use of radioactive DMA and DM’A (3).

The physiological roles of rat kidney mitochondrial AGT2 have not been well defined compared with that of AGT1, since the contribution of mitochondrial AGT2 to the glyoxylate detoxification in rats in vivo is suggested to be much less than that of AGT1 (6, 32). Only an α-amino-γ-butyrglyoxylate aminotransferase activity has been recognized as a potential activity of AGT2 (33). The present result that AGT2 has the dimethylarginine:pyruvate aminotransferase activity adds a new metabolic role to AGT2 in rats. Furthermore, the data shown in the present experiment provide the first evidence
for transamination of the α-amino group of L-ornithine in mammals. However, the $K_a$ value for L-ornithine is $70 \mu M$ indicating a relatively low affinity of this substrate for the enzyme. Although transamination of the α-amino group of L-ornithine may be another function of AGTZ, the significance of this activity remains, at the present time, to be clarified more systematically in relation to L-ornithine β-aminotransferase (EC 2.6.1.13) which utilizes cysteine acceptor and is present exclusively within mitochondria of both liver and kidney (13). Our report provides useful knowledge about the diversity of the functions and the evolutionary changes of the isozymes of alanine:glyoxylate aminotransferase, especially AGTZ, in mammalian species (6).

REFERENCES


SUPPLEMENTARY MATERIALS

DIHYDROPYRUVATE:PYRUVATE AMINOTRANSFERASE IN MAMMALS

EXPERIMENTAL PROCEDURES

Materials – 6- and 2-Dihydroxy-L-lysine (DHL) were obtained from Sigma Chemical Co. (St. Louis, MO). N-Oxalyl-D,L-alanine (Oxalylglycine) was custom synthesized (1). Pyroglutamate was custom synthesized (2). Pyroglutamyl-L-lysine was prepared from DHL, according to the literature (3). L-lysine, N-ethylmaleimide, N-acetyl-L-lysine, and N-acetyl-L-lysine (Boc) were purchased from Sigma Chemical Co. (St. Louis, MO). N-Boc-D,L-alanine was purchased from Novabiochem (San Diego, CA). All other chemicals and reagents were purchased from the usual sources.

Growth media – The composition of the growth medium for mouse keratinocytes and mouse fibroblasts was described previously (1). The standard medium contained 10% fetal bovine serum in DME (Dulbecco's Modified Eagle's Medium) supplemented with 50 U/ml penicillin, 50 μg/ml streptomycin, 10 μg/ml fungizone, and 10 ng/ml human recombinant GMCSF (granulocyte-macrophage colony-stimulating factor).

In vitro incubations – Cultures were incubated at 37°C, 5% CO₂ in a humidified atmosphere. The medium was changed every 3 days.

Immunohistochemical study – Sections were prepared as described previously (1). Tissues were fixed in buffered formalin and paraffin embedded. Sections were cut at 4 μm and stained with hematoxylin and eosin.

Immunofluorescence – Sections were incubated with a rabbit polyclonal antibody to DHL, then with a fluorescein isothiocyanate (FITC)-conjugated secondary antibody. The slides were viewed under a fluorescence microscope.

Immunoprecipitation – Cells were lysed in a buffer containing 25 mM Tris-Cl, 150 mM NaCl, 0.5% NP-40, and protease inhibitors. The lysate was cleared by centrifugation and the supernatant was used as the sample.

Immunoblotting – Proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to a nitrocellulose membrane. The membrane was incubated with the primary antibody, then with a horseradish peroxidase-conjugated secondary antibody. The bands were visualized using a chemiluminescent detection reagent.
**Dimethylarginine-Pyruvate Aminotransferase in Rats**

**Materials and Methods**
- **Purification of Dimethylarginine-pyruvate aminotransferase from rat kidney**
- **Effect of inhibitors on the dimethylarginine-pyruvate aminotransferase and alanine-pyruvate aminotransferase activities**

**Results**
- Purification of Dimethylarginine-pyruvate aminotransferase:
  - Elution was carried out at 0.4 M NaCl.

**Effect of inhibitors on the dimethylarginine-pyruvate aminotransferase and alanine-pyruvate aminotransferase activities**
- Female rats of two strains (average body weight, 75 g) were divided into two groups.
- One group was treated with dimethylarginine (100 mg/kg) and the other was used as a control.

**Discussion**
- The results were summarized in Table I.

**Table I**

<table>
<thead>
<tr>
<th>Purification step</th>
<th>Total protein (mg)</th>
<th>Dimethylarginine-pyruvate aminotransferase</th>
<th>Alanine-pyruvate aminotransferase</th>
<th>Ratios (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude extract</td>
<td>10,650</td>
<td>219</td>
<td>0.020</td>
<td>100</td>
</tr>
<tr>
<td>Acetone insol.</td>
<td>2,415</td>
<td>156</td>
<td>0.049</td>
<td>53</td>
</tr>
<tr>
<td>DEAE-carbonate</td>
<td>128</td>
<td>46</td>
<td>0.032</td>
<td>83</td>
</tr>
<tr>
<td>Chromatofocusing</td>
<td>5.7</td>
<td>29</td>
<td>0.16</td>
<td>82</td>
</tr>
</tbody>
</table>

**Table II**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration (mM)</th>
<th>Relative activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dimethylarginine-pyruvate aminotransferase</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Alanine-pyruvate aminotransferase</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Glycine</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Serine</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Threonine</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>100</td>
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<td>Glutamic acid</td>
<td>100</td>
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**Table III**

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Glutamine treatment</th>
<th>Enzyme activity (units/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dimethylarginine-pyruvate aminotransferase</td>
<td>Alanine-pyruvate aminotransferase</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.02 ± 0.04</td>
<td>0.03 ± 0.09</td>
</tr>
<tr>
<td>Liver</td>
<td>0.04 ± 0.04</td>
<td>0.05 ± 0.04</td>
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**Table IV**

<table>
<thead>
<tr>
<th>Kinetic properties of Dimethylarginine-pyruvate aminotransferase</th>
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<tbody>
<tr>
<td><strong>Transamination</strong> (mM)</td>
</tr>
<tr>
<td>-------------------------</td>
</tr>
<tr>
<td>Glycine</td>
</tr>
<tr>
<td>Alanine</td>
</tr>
</tbody>
</table>

**Table V**

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Glutamine treatment</th>
<th>Enzyme activity (units/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dimethylarginine-pyruvate aminotransferase</td>
<td>Alanine-pyruvate aminotransferase</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.31 ± 0.04</td>
<td>0.16 ± 0.29</td>
</tr>
<tr>
<td>Liver</td>
<td>0.03 ± 0.01</td>
<td>0.04 ± 0.07</td>
</tr>
</tbody>
</table>

**Table VI**

<table>
<thead>
<tr>
<th><strong>Zinc content (mg/g)</strong></th>
<th>0.12 ± 0.01</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney</td>
<td>0.04 ± 0.01</td>
</tr>
<tr>
<td>Liver</td>
<td>0.04 ± 0.01</td>
</tr>
</tbody>
</table>

**Discussion**
- The results were summarized in Table I.

**References**
- Shock et al. (1978).

**Acknowledgments**
- This work was supported by grant No. 04-02-01-44 from the Russian Foundation for Basic Research.
TABLE VII  
Distribution of the enzyme in rat tissues  

<table>
<thead>
<tr>
<th>Tissue†</th>
<th>Enzyme activity (units × 10^3/mg protein)</th>
<th>Enzyme content (µg/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney</td>
<td>1.66 ± 0.26a</td>
<td>1.12 ± 0.42</td>
</tr>
<tr>
<td>Liver</td>
<td>0.31 ± 0.02</td>
<td>0.12 ± 0.04</td>
</tr>
</tbody>
</table>

† The following tissues tested do not contain the enzyme activity and the enzyme proteins: heart, lung, spleen, pancreas, stomach, intestine, and brain.

a Mean ± SD (n=5)  

Fig. 4. Absorption spectra of dimethylarginine/pyruvate aminotransferase. Spectra of the enzyme (1.76 mg/ml) were measured in 10 mM sodium phosphate buffer, pH 7.5 at 25°C. A, spectrum in the buffer; B, spectrum in the presence of 10 mM DMA.

Fig. 5. Effect of pH on the activity of dimethylarginine/pyruvate aminotransferase. The enzyme activities were assayed as described under EXPERIMENTAL PROCEDURES, except that sodium phosphate buffer was used at pH 5.5-6.5 and HEPES/NaOH buffer at pH 7.5-8.5. A, dimethylarginine/pyruvate aminotransferase activity; B, alanine/pyruvate aminotransferase activity; C, ornithine/pyruvate aminotransferase activity.

Fig. 6. Ouchterlony double diffusion with antiserum against dimethylarginine: pyruvate aminotransferase. The center well contained dimethylarginine: pyruvate aminotransferase antiserum; well A, the purified enzyme; well B, kidney mitochondrial alanine:pyruvate aminotransferase 2; well C, liver mitochondrial alanine:pyruvate aminotransferase 3.
Fig. 10. Immunoblotting with anti-dimethylarginine:pyruvate aminotransferase antibody. Purified dimethylarginine:pyruvate aminotransferase (A) (lane 1) and partially purified kidney dimethylarginine:pyruvate aminotransferase 2 (B) (lane 2) and partially purified liver dimethylarginine:pyruvate aminotransferase 2 (B) (lane 3) were electrophoresed in 12% PAGE in the presence of SDS and 2-mercaptoethanol as described under EXPERIMENTAL PROCEDURES. Half (A) of the gel was used for protein staining and the other half (B) for immunostaining after electrophoretic blotting as described under EXPERIMENTAL PROCEDURES.

Fig. 11. Subcellular distribution of dimethylarginine:pyruvate aminotransferase in rat kidney. The post-nuclear fraction prepared from rat kidney was subjected to sucrose density gradient centrifugation as described under EXPERIMENTAL PROCEDURES. DMAT, dimethylarginine:pyruvate aminotransferase activity (○); AGT, alanine:glutamate aminotransferase activity (■); GDH, glutamate dehydrogenase activity (▲).

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