The Effect of Growth Hormone and Thyroxine on the Amount of L-Arginine:Glycine Amidinotransferase in Kidneys of Hypophysectomized Rats

PURIFICATION AND SOME PROPERTIES OF RAT KIDNEY TRANSAМИDINASE*

(Received for publication, February 12, 1979)

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The initial step in creatine biosynthesis involves the transfer of an amidine group from arginine to glycine to form guanidinoacetic acid and is catalyzed by the enzyme L-arginine-glycine amidinotransferase (transamidinase). A procedure for the purification of rat kidney transamidinase by chromatography with DEAE-cellulose and phenyl-Sepharose is described in this report. Two fractions of enzyme, designated as α- and β-transamidinase, were obtained with chromatography on DEAE-cellulose. The two fractions were individually purified to homogeneity as judged by their migration as a single band in native and sodium dodecyl sulfate gel electrophoresis and by sedimentation equilibrium experiments. No significant differences in the properties of the two fractions have been found. The minimum molecular weights, determined by sodium dodecyl sulfate gel electrophoresis, were 42,000 and 44,000. The apparent molecular weights, determined by sedimentation equilibrium, were 82,600 and 83,300. The amino acid compositions and specific activities of the two fractions were similar. The pI values were 6.9 to 7.3. The Km values were 2.8 and 2.4 mM for arginine and 3.0 and 3.1 mM for glycine. The α and β fractions were identical immunologically. Monospecific antibodies to the purified transamidinase have been prepared.

Growth hormone and thyroxine have been reported previously to be necessary for rat kidney transamidinase activities (Van Pilsum, J. F., Carlson, M., Boen, J. R., Taylor, D., and Zakis, B. (1970) Endocrinology 87, 1237-1244). The amounts of transamidinase protein in kidneys from three groups of experimental rats have been determined by immunotitration experiments. Hypophysectomized rats had 30 and 33%, respectively, of the activities and amounts of immunoprecipitable transamidinase protein found in kidneys of sham-hypophysectomized rats. Hypophysectomized rats that had received nine daily injections of growth hormone and thyroxine had similar amounts of transamidinase activity and transamidinase protein as sham-hypophysectomized rats. Growth hormone and thyroxine are concluded to be necessary for maintaining the levels of transamidinase protein in rat kidney.

Creatine has a major role in vertebrate energy metabolism. Phosphocreatine is used to maintain ATP levels in muscle and other tissues whenever ATP utilization exceeds its production from metabolic fuels. Evidence also has been presented that phosphocreatine is coupled with mitochondrial electron transport (1). Thus, vertebrate energy metabolism may be influenced by alterations in the activities of the enzymes involved in creatine synthesis.

The first reaction in the biosynthesis of creatine is the transfer of the amidine group of arginine to the amino group of glycine to form guanidinoacetic acid (2). This reaction is catalyzed by the enzyme L-arginine-glycine amidinotransferase, EC 2.1.4.1, commonly called transamidinase. The transamidinase activities found in kidney and pancreas are manyfold greater than those found in other tissues of the rat and various mammals (3-5). Rat kidney transamidinase activities have been found to be altered greatly in a variety of dietary and hormonal states (6-17). The requirement of growth hormone and thyroxine for transamidinase activities in rat kidney was considered of interest in view of the lack of knowledge on the action of these two hormones at the molecular level. Kidneys from hypophysectomized or thyroidectomized rats had only 15 to 20% of the transamidinase activities found in kidneys of intact rats. Thyroidectomized rats injected with thyroxine (13) or hypophysectomized rats injected with bovine growth hormone (10) had kidney transamidinase activities similar to those found in intact rats. The transamidinase activities have been concluded to be dependent on the simultaneous presence of both growth hormone and thyroxine (13). The possibilities of growth hormone and thyroxine inducing alterations in enzyme amount or structure could be investigated only if the purified enzyme was available. Attempts made in our laboratory to isolate rat kidney transamidinase by the method of Conconi and Grazi (18) for hog kidney transamidinase were not successful.

An isolation procedure for rat kidney transamidinase is described in this report. Some properties of the enzyme have been determined and monospecific antibodies to the enzyme were prepared for the purpose of quantitating the amount of the enzyme protein in rat kidney. Transamidinase activities and the amounts of transamidinase protein were determined in kidneys from sham-hypophysectomized rats, hypophysectomized rats, and hypophysectomized rats injected with...
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growth hormone and thyroxine. A good correlation between the amounts and the activities of transamidinase in the kidneys of the experimental animals was found.

EXPERIMENTAL PROCEDURES

Materials

The L-thyroxine and Trizma (2-amino-2-hydroxymethyl-1,3-propanediol) base were purchased from Sigma Chemical Co., St. Louis, MO. The bovine growth hormone was NIH preparation B-18. DEAE-cellulose (Whatman DE-52, preswollen) was purchased from Reeves Angel, Clifton, N.J. Sephadex G-150 and phenyl-Sepharose were purchased from Pharmacia Fine Chemicals, Inc., Piscataway, N.J. Ninhydrin was purchased from EM Laboratories, Detroit, MI. Freund's adjuvants were purchased from Difco Laboratories, Detroit, MI. The proteins used for molecular weight standards, as well as other chemicals, were the best grade available from commercial sources.

Animals

The rats used as a source of kidney for the enzyme isolation were purchased from Holtzman, Inc., Madison, WI. All rats were housed individually and were allowed to consume a purified diet (19) and tap water ad libitum at least 2 weeks prior to surgery.

The hypophysectomized rats were purchased from Hormone Assay Laboratories, Chicago, II., and were received on the day of the surgery. The rats had access to one-fourth an orange on the day of their arrival and were given an intraperitoneal injection of 0.5 ml of a suspension of 1.0 mg of cortisone in physiological saline (0.9% NaCl solution) on the day after their arrival. All rats were housed and fed as described above for the duration of the experiment. The temperature of the animal room was maintained at 78°C and the lights were on in the daytime and off at night. The survival rate of hypophysectomized rats maintained as described has been excellent. The rats were weighed on the 3rd day after their surgery and again 2 weeks later to determine the success of the hypophysectomy and health of the animals. One-half of the hypophysectomized rats received nine daily injections, subcutaneous in the dorsal neck region, of 200 μg of bovine growth hormone, and 0.3 μg of thyroxine/100 g of body weight and the other half of the rats received physiological saline injections. Hypophysectomized and sham-hypophysectomized rats were selected for killing on the basis of uniform weight gain.

New Zealand white rabbits were purchased from Oak Crest Rabbitry, Hanover, MN.

General Methods

Transamidinase activities were determined by a modification of the procedure reported previously (20). Two hundred microliters of the arginine/glycine substrate mixture and 200 μl of the enzyme solutions were incubated at 37°C for 1 h and the enzyme reaction was terminated by the addition of 0.6 ml of 0.6 N perchloric acid. Three milliliters of the ninhydrin color reagent was added to the mixture of enzyme, substrates, and perchloric acid and the color reaction was done as previously reported. The transamidinase activities were expressed as micromoles of ornithine produced per h. Protein concentration was determined by the method of Lowry et al. (21) and by absorbance at 280 nm with bovine serum albumin as the reference protein. Disc gel electrophoresis was performed in 7% acrylamide, pH 6.0, according to the method of Davis (22). Sodium dodecyl sulfate gel electrophoresis was performed by the method of Weber and Osborn (23). The Kₐ and Vₐₐₜₜ of the enzyme were determined by the method of Van Winkle and Vavra (24). The carbohydrate content was determined by gas-liquid chromatography. The amino acid composition of the enzyme was determined by a modification of the method of Crestfield et al. (25) in which 6 μm guanidine HCl was used in the preparation of the reduced carboxymethylated protein. The tryptophan content of the enzyme was determined by the method of Simpson et al. (26). The isoelectric points were determined with 5% polyacrylamide plates containing 0.1% urea and pH 3 to 10 Ampholines, purchased from LKB. Electrophoresis was for 3 h at 30 watts constant voltage with a LKB Multifold flat plate electrophoresis apparatus and a LKB 2103 power supply. The pH of the gels was determined with a surface glass electrode, LKB model 2177-111.

Sedimentation Equilibrium

The molecular weights were determined in a Spinco model E ultracentrifuge equipped with a duralamium AN-D rotor and double sector cells with 3-mm column. The sedimentation equilibrium experiments were done by the meniscus depletion technique of Yphantis (27). The enzyme solutions were concentrated by ultrafiltration to 1.3 mg/ml and thoroughly dialyzed against 27.5 mM Tris-HCl, 5 mM EDTA, buffer pH 7.4. The centrifuge temperature was 15.3°C and the speed of 20,410 rpm was maintained with a mechanical speed control and measured with a digital tachometer. The recordings were made with a Rayleigh interferometer and spectroscopic KK-G plates. Equilibrium was considered to have been attained when no fringe shift was observed on photographs taken after intervals of several hours. The measurements were made with a Nikon comparator with 50X optics. The data were computed and plotted on a Hewlett-Packard 9100B calculator. The partial specific volumes were estimated from the amino acid composition (28).

Purification of Rat Kidney Transamidinase

The rats were killed by decapitation, their kidneys removed, cleaned, and pooled. A 20% homogenate in cold-distilled water was made with a Waring Blender. The homogenates were stored at -15°C for 2 to 3 months with only slight loss of transamidinase activities. All steps of the purification were performed at 4°C.

Step 1—The 20% kidney homogenate was thawed and centrifuged at 35,000 × g for 30 min at 4°C. Approximately 180 ml of the supernatant solution was applied to a DEAE-52 cellulose column, 2.4 × 100 cm, which had been previously equilibrated with 27.5 mM Tris-HCl buffer, pH 7.4, containing 5 × 10⁻⁶ mol of EDTA/liter (Buffer I). The flow rate through the column was maintained at 1 ml/min with a peristaltic pump and 18-ml fractions were collected. Chromatography was performed with 2 liters of Buffer I and 3.6 liters of Buffer II, with a gradient of 0 to 0.6 M NaCl. Two series of fractions containing transamidinase activity were pooled and concentrated and were designated as a- and β-transamidinase, respectively. The fractions of elute in each series with similar specific activities, based on absorbance at 280 nm, were pooled and dialyzed against Buffer I for 18 h. The dialyzed fractions were concentrated to volumes of approximately 60 ml with a model 12 Amicon ultrafilter equipped with a PM 10 membrane.

Step 2—Approximately 60 ml of the dialyzed enzyme solution and 70 ml of Buffer I were applied to a phenyl-Sepharose column, 1.6 × 10 cm, equilibrated with Buffer I. The flow rate of the column was maintained at 0.5 ml/min and 3-ml fractions were collected. The enzyme was eluted with 70 ml of Tris (5 mM), glycine (38 mM) buffer, pH 8.3. Fractions with similar specific activities, based on absorbance at 280 nm, were pooled and dialyzed against Buffer I.

Preparation of a Monospecific Antibody to Purified Transamidinase

One milliliter of transamidinase, 0.5 mg/ml, was emulsified with an equal volume of Freund's complete adjuvant and injected subcutaneously in multiple sites on the back of the rabbit. Two weeks later, a second injection was given. Anti-transamidinase was detected 4 weeks after the first injection. Booster injections with enzyme and incomplete Freund's adjuvant were given at 2-week intervals. The rabbit was bled from an ear artery 7 days after the booster injection. Ouchterlony gel double immunodiffusion analysis of the antiserum was performed by the technique of Kabat and Meyer (29). Immunoelectrophoresis of the serum was done as described by Ouchterlony and Nilsson (30).

All transamidinase was purified by affinity chromatography according to the method of Cuatrecasas (31). Five milligrams of enzyme in 10 ml of Buffer I was allowed to react with 3 ml of CNBr-activated Sepharose for 18 h at 4°C. The enzyme–resin complex was transferred to column (1.2 × 10 cm) and washed with 200 column volumes of 0.01 M phosphate buffer, pH 7.4, containing 0.15 mol/liter of NaCl (Buffer II). Five milliliters of serum was dialyzed against Buffer II and transferred to the column. The flow rate of all solutions through the column was 0.3 ml/min and 2-ml fractions were collected. Anti-transamidinase was eluted with 2 μM NaSCN and dialyzed successively in 0.01 M phosphate buffer, pH 7.4, containing 0.15 mol/liter of NaCl, respectively. The affinity column was re-equilibrated with Buffer II and was reused four to five times.
Effect of Growth Hormone and Thyroxine on Rat Transamidinase

**Immunotitration of Transamidinase**

The kidneys from each experimental group were pooled, homogenized, and centrifuged as described above. The 35,000 x g supernatants of the kidney homogenates, referred to as the kidney supernatant, were used for the immunotitration experiments, and the determination of transamidinase activities and total protein.

**Determination of the Equivalence Point of Transamidinase with Anti-transamidinase—**Solutions of the kidney supernatants of similar protein concentrations and enzyme activities were prepared by diluting the kidney supernatants from the hypophysectomized rats and the other two experimental groups of rats with 0.1 M phosphate buffer, pH 7.4, and phosphate buffer containing bovine serum albumin, respectively. One-half milliliter of the diluted supernatants was mixed with 100 μl of purified anti-transamidinase (0.67 mg/ml). The samples were incubated at 25°C for 1 h followed by 14 h at 4°C. The immunoprecipitates were collected by centrifugation at 425 x g for 20 min at 4°C and washed twice in 0.15 M NaCl. Transamidinase activities of the supernatant solutions and the saline washes were determined. No detectable transamidinase activities were found in the saline washes.

**Quantitation of Transamidinase Protein—**The kidney supernatants were diluted 1:20 with Buffer I. One-half milliliter aliquots of the diluted supernatant solutions were mixed with varying volumes of anti-transamidinase and Buffer I to make a total volume of 1 ml. Incubation and collection of the immunoprecipitates was done as described above.

With both procedures described above, kidney supernatant solutions were incubated under the same conditions without the antibody as a control. Nonspecific binding of the antibody was determined by incubating anti-transamidase with a solution from the DEAE-cellulose column devoid of transamidinase activity (Fraction 33, Fig. 1). No visible protein precipitate was detected after centrifugation of the mixtures of protein and antibody. The absence of a protein precipitate was confirmed using the method of Ganschow and Schimke (32), in which the protein was dissolved in 0.1 M NaOH and absorbance measured at 280 nm. The values obtained with the kidney supernatants from the sham-hypophysectomized rats were used as the control reference points.

**RESULTS**

**Purification of Transamidinase—**A plot of the transamidinase activities and the absorbance at 280 nm of the fractions of eluate from the DEAE-cellulose column is shown in Fig. 1. A 32-fold purification of both the α and β fractions was obtained with a total yield of 31% of the enzyme activity. A plot of transamidinase activities and the absorbance at 280 nm of the fractions of eluate from the phenyl-Sepharose column is shown in Fig. 2. Similar results were obtained with the β fraction of transamidinase. Approximately 150-fold purification of the α and β fractions of the enzyme were obtained with a total yield of 10% of the enzyme activity. Seven milligrams of enzyme was obtained from 50 g of rat kidneys. A summary of the purification procedure is given in Table I.

**Homogeneity—**The specific activities of the enzyme fractions from the phenyl-Sepharose column were approximately constant and were not increased by gel filtration on Sephadex G-150. One band of protein was observed after disc gel electrophoresis of transamidinase in 7% polyacrylamide gels at pH 8.9 and in 5% polyacrylamide gels containing sodium dodecyl sulfate (Fig. 3). The gels were scanned at 620 nm and a single Gaussian distribution of protein was observed. Plots of the ln fringe displacement versus r² from the sedimentation equilibrium experiments fitted to a least squares line were linear over the entire fluid column, indicating homogeneity.

**Properties—**The apparent molecular weight of transamidinase, as determined by sedimentation equilibrium, was 83,300 for α-transamidinase and 82,600 for β-transamidinase. With the use of marker proteins in sodium dodecyl sulfate electrophoresis, the molecular weight of the single band was calculated to be 44,000 for α-transamidinase and 42,000 for β-transamidinase (Fig. 4). Therefore, transamidinase appears to be a dimer with a molecular weight of 83,000.

**Fig. 1.** Chromatography of the 35,000 x g supernatant of rat kidney homogenate on DEAE-cellulose. Experimental conditions are described in the text. Transamidinase activity (■), absorbance at 280 nm (○), and molarity of NaCl (△) are shown.

**Fig. 2.** Chromatography of rat kidney transamidinase on phenyl-Sepharose. Experimental conditions are described in the text. Transamidinase activity (■) and absorbance at 280 nm (○) are shown. The arrow on the abscissa indicates the point of application of the Tris/glycine buffer to the column.

**TABLE I**

<table>
<thead>
<tr>
<th>Step Procedure</th>
<th>Volume</th>
<th>Protein</th>
<th>Enzyme Activity (units)</th>
<th>Specific Activity (units/mg)</th>
<th>Purification Factor</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Homogenate</td>
<td>245</td>
<td>10,500</td>
<td>1366</td>
<td>0.13</td>
<td>1</td>
<td>100</td>
</tr>
<tr>
<td>2 Supernatant</td>
<td>180</td>
<td>4,000</td>
<td>915</td>
<td>0.23</td>
<td>1.8</td>
<td>67</td>
</tr>
<tr>
<td>3 DEAE-cellulose</td>
<td>150(α)</td>
<td>41</td>
<td>168</td>
<td>4.1</td>
<td>32</td>
<td>12</td>
</tr>
<tr>
<td>4 Phenyl-Sepharose</td>
<td>420(β)</td>
<td>63</td>
<td>258</td>
<td>4.1</td>
<td>12</td>
<td>19</td>
</tr>
<tr>
<td>5 Phenyl-Sepharose</td>
<td>8.5(β)</td>
<td>2.5</td>
<td>180</td>
<td>18.0</td>
<td>138</td>
<td>7</td>
</tr>
</tbody>
</table>

The amino acid composition of α- and β-transamidinase is shown in Table II. No significant differences in the composition were detected. The partial specific volumes of the enzyme, determined from the amino acid composition, were 0.735 ml/g and 0.734 ml/g for α- and β-transamidinase, respectively. A sample of a mixture of the α and β fractions of transamidinase was found to contain less than 0.4% carbohydrate.
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B - respectively, and the $V_{\text{max}}$ was 0.39 μmol of ornithine/min/mg of protein. The $K_m$ values for β-transamidinase were 2.4 mM and 3.1 mM for arginine and glycine, respectively. The $V_{\text{max}}$ of the β fraction, corrected for the loss of the enzyme activity with time after its isolation, was 0.37 μmol of ornithine/min/mg protein. The kinetic parameters of α- and β-transamidinase are therefore considered to be similar. The properties of CY- and β-transamidinase are summarized in Table I.

Anti-rat Kidney Transamidinase Antisera—Rabbits were

Two bands of protein of equal intensity were obtained with both α- and β-transamidinase in the isoelectric focusing experiments in 6 M urea. The two bands are thought to represent the two subunits of transamidinase. The pI values were 7.0 and 7.15 for α-transamidinase and 6.9 to 7.3 for β-transamidinase.

The kinetic parameters of α- and β-transamidinase were determined by incubation of the enzymes with constant and varying amounts of the two individual substrates, respectively. Substrate concentrations near the $K_m$ values were used because saturating levels of glycine were found to inhibit the enzyme in the presence of low amounts of arginine. Hanes-Woolf plots of the data obtained with β-transamidinase are shown in Fig. 5. The intersecting plots of $[S]/V$ versus $[S]$ are evidence that the enzyme catalysis is a bi-substrate ping-pong mechanism (33). Similar kinetic plots were obtained for α-transamidinase. The $K_m$ values for α-transamidinase were calculated to be 2.8 mM and 3.0 mM for arginine and glycine, respectively, and the $V_{\text{max}}$ was 0.39 μmol of ornithine/min/mg of protein. The $K_m$ values for β-transamidinase were 2.4 mM and 3.1 mM for arginine and glycine, respectively. The $V_{\text{max}}$, of the β fraction, corrected for the loss of the enzyme activity with time after its isolation, was 0.37 μmol of ornithine/min/mg protein. The kinetic parameters of α- and β-transamidinase are therefore considered to be similar. The properties of α- and β-transamidinase are summarized in Table I.

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Anti-rat Kidney Transamidinase Antisera—Rabbits were
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FIG. 5. Hanes-Woolf plots of $S/V$ versus $S$ obtained by incubation of transamidinase with varying arginine concentrations at constant glycine concentrations (left) and with varying glycine concentrations at constant arginine concentrations (right): Two hundred microliters of enzyme solution, containing 0.021 mg of transamidinase (specific activity 12.7 units/mg) was incubated with 0.2 ml of the substrate mixtures. The incubation and color reaction was done as described in the text. The inset in the right panel is a plot of the slope of the lines (obtained from the $S/V$ versus $S$ plots) versus the reciprocal of the concentrations of arginine and glycine. The intercept on the ordinate is $-1/K_{m}$ for arginine and glycine.

TABLE III
Some properties of $\alpha$- and $\beta$-transamidinase

<table>
<thead>
<tr>
<th></th>
<th>$\alpha$</th>
<th>$\beta$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apparent molecular weight</td>
<td>83,300</td>
<td>82,600</td>
</tr>
<tr>
<td>Subunit molecular weight</td>
<td>44,000</td>
<td>42,000</td>
</tr>
<tr>
<td>$K_{m}$ for arginine (mm)</td>
<td>2.8</td>
<td>2.4</td>
</tr>
<tr>
<td>$K_{m}$ for glycine (mm)</td>
<td>3.0</td>
<td>3.1</td>
</tr>
<tr>
<td>$V_{max}$ (umol ornithine/min/mg protein)</td>
<td>0.39</td>
<td>0.37</td>
</tr>
<tr>
<td>pI</td>
<td>7.0, 7.2</td>
<td>6.9-7.3</td>
</tr>
<tr>
<td>Partial specific volume (ml/g)</td>
<td>0.735</td>
<td>0.734</td>
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</tbody>
</table>

FIG. 6. Ouchterlony double immunodiffusion of anti-transamidinase and serial dilutions of the 35,000 × $g$ supernatant from kidney homogenates of intact rats. The center well contained 5 $\mu$l of rabbit antiserum to transamidinase (left) or 5 $\mu$l of anti-transamidinase purified according to the procedure described in the text (right). Well 1 contained 5 $\mu$l of 35,000 × $g$ rat kidney supernatant and Wells 2 to 6 contained 5 $\mu$l of 1:1, 1:2, 1:4, and 1:16 dilutions, respectively, of the kidney supernatant.

immunized against purified $\alpha$- and $\beta$-transamidinase as described under "Experimental Procedures." Double diffusion analysis of the harvested antiserum versus serial dilutions of rat kidney homogenate showed two precipitin lines (Fig. 6). Therefore, the antibody was purified as described under "Experimental Procedures." Only one precipitin line was observed in double diffusion (Fig. 6) and immunoelctrophoresis analyses of the purified anti-transamidinase. Purified anti-transamidinase was allowed to diffuse against transamidinase at various stages of the purification (Fig. 7). The smooth fusion of the precipitin lines without spur formation has been interpreted to indicate that the enzyme had identical antigenic properties at the various stages of the purification. Transamidinase from different stages of the purification procedure was immunotitrated with constant amounts of purified anti-transamidinase (Fig. 8). The equivalence point was similar for the different preparations of the enzyme. No transamidinase activity was detected when the immunoprecipitate was resuspended and assayed. Precipitin lines obtained from the diffusion of purified $\alpha$- and $\beta$-transamidinase against antibodies to $\alpha$- and $\beta$-transamidinase showed smooth fusion, indicating that the $\alpha$ and $\beta$ fractions of transamidinase were immunologically identical. The diffusion of anti-$\alpha$-transamidinase against $\alpha$- and $\beta$-transamidinase is shown in Fig. 9. These data support the purity and specificity of the anti-transamidinase preparation and establish its usefulness in precipitating transamidinase protein from rat kidney supernatant solutions.

FIG. 7. Immunodiffusion analysis of anti-rat transamidinase and different preparations of transamidinase. The center well contained 8 $\mu$l of purified anti-transamidinase. Wells 1 and 2 contained 5 $\mu$l of 35,000 × $g$ rat kidney supernatant from homogenates of intact rats. Wells 3 and 4 contained 5 $\mu$l of the $\alpha$ fraction of transamidinase from the DEAE-cellulose column and Wells 5 and 6 contained 5 $\mu$l of the purified $\alpha$ fraction of the enzyme.

FIG. 8. Quantitative immunoprecipitation of kidney transamidinase from 35,000 × $g$ supernatant solution from intact rats (O—O) and from a sample of transamidinase eluted from the column of DEAE-cellulose (C—C). Varying amounts of the solutions of transamidinase were incubated with 0.1 ml of anti-transamidinase. Experimental conditions are described in the text.
The effect of growth hormone and thyroxine on transamidinase activities and amounts of transamidinase protein and total protein in kidneys of hypophysectomized rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Transamidinase activity</th>
<th>Antibody required to immunoprecipitate enzyme activity</th>
<th>Total kidney protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham-hypophysectomized</td>
<td>0.44 (100%)</td>
<td>195 (100%)</td>
<td>19.0 (100%)</td>
</tr>
<tr>
<td>Hypophysectomized</td>
<td>0.13 (30%)</td>
<td>65 (33%)</td>
<td>17.8 (93%)</td>
</tr>
<tr>
<td>Hypophysectomized +</td>
<td>0.43 (88%)</td>
<td>155 (79%)</td>
<td>16.6 (83%)</td>
</tr>
<tr>
<td>growth hormone and</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>thyroxine</td>
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FIG. 10. Immunotitration of kidney transamidinase from sham-hypophysectomized rats (○), hypophysectomized rats (□), and hypophysectomized rats injected with growth hormone and thyroxine (●). Constant amounts (0.1 ml) of anti-transamidinase were incubated with varying amounts of the 35,000 × g supernatants from the kidney homogenates. The transamidinase activities of the diluted supernatant solutions are shown on the abscissa. Experimental conditions are described in the text. Each symbol represents the mean of duplicate analyses on a pool of kidneys from six rats.

Table IV

The effect of growth hormone and thyroxine on transamidinase activities and amounts of transamidinase protein and total protein in kidneys of hypophysectomized rats

FIG. 11. Quantitative immunoprecipitation of kidney transamidinase from sham-hypophysectomized rats (○), hypophysectomized rats (□), and hypophysectomized rats injected with growth hormone and thyroxine (●). Varying amounts of anti-transamidinase were incubated with a constant volume of the 35,000 × g supernatants of rat kidney homogenates. Experimental conditions are described in the text. Each symbol represents the mean of duplicate analyses on a pool of kidneys from six rats.

had 98% of the kidney transamidinase activities of the sham-hypophysectomized rats. These data confirm previous reports on the effect of hypophysectomy on rat kidney transamidinase activities (10, 13).

Varying amounts of kidney supernatant solutions were immunotitrated with constant amounts of transamidinase. Extrapolation of the titration curves in Fig. 10 to the abscissa indicated that identical equivalence points were observed in the different experimental groups. In each case, a given quantity of antibody precipitated identical amounts of catalytic activity. The amount of antibody required to precipitate all the transamidinase activity from constant volumes of kidney supernatant solutions from the three experimental groups is illustrated in Fig. 11 and summarized in Table IV. The amount of antibody required to precipitate transamidinase activity from the different kidney preparations was proportional to the enzyme activities in the respective groups of rats. It may be inferred from these two immunochromic measurements of transamidinase that changes in transamidinase catalytic activity following hypophysectomy and injection of growth hormone and thyroxine into hypophysectomized rats are a result of proportionate alterations in the quantity of immunologically identical transamidinase protein. The total protein, however, in the supernatants from the kidneys of the three experimental groups of rats was considered to be similar (Table IV).

DISCUSSION

Transamidinase was purified with a simple, two-step procedure which involved DEAE-cellulose and phenyl-Sepharose chromatography. Two fractions of transamidinase were obtained with chromatography of the kidney supernatant solution on DEAE-cellulose. The purified α- and β-transamidinase had a variety of similar properties; apparent molecular weights, subunit molecular weights, amino acid compositions, specific activities, kinetic parameters, isoelectric points, and antigenic properties. All attempts to vary the conditions of DEAE-cellulose chromatography such as changes in the type, pH, or conductivity of the buffer to obtain a single fraction of transamidinase have been unsuccessful to date. Two fractions of rat kidney transamidinase were also obtained by chromatography of rat kidney supernatants on DEAE-Sephadex.

The α- and β-transamidinase reacted identically with the anti-transamidinase prepared from the α or β fractions of the enzyme. Therefore, the total transamidinase of rat kidney could be quantitated by immunotitration with the antibody to either fraction of enzyme. Evidence that this is true is the fact that the transamidinase activity of rat kidney supernatants was completely immunoprecipitated with anti-α-
transamidinase in all three experimental groups of rats (Fig. 11).

The α and β fractions were considered to be homogeneous for two reasons: 1) migration as a single band in native and sodium dodecyl sulfate electrophoresis, 2) linear plot of ln c versus r² in sedimentation equilibrium experiments. Purified transamidinase was used to elicit an antibody to transamidinase which was purified from serum by affinity chromatography. The monospecificity of the anti-transamidinase was demonstrated in Ouchterlony double diffusion and immunoelectrophoresis experiments. Quantitative precipitin experiments have been interpreted to indicate that transamidinase can be precipitated similarly from enzyme preparations at different stages of the purification procedure, and therefore can be used to quantitate transamidinase protein in kidneys of experimental rats.

Some comparisons in the properties of rat and hog kidney transamidinase may be made. Hog kidney transamidinase had $K_m$ values of 1.8 and 2.3 mM for arginine and glycine, respectively (34), which are similar to the $K_m$ values for rat kidney transamidinase listed in Table II. The specific activity of hog kidney transamidinase is ~4-fold greater than rat kidney transamidinase. The native molecular weights of hog and rat kidney transamidinase are 100,000 and 83,000, respectively. Hog kidney transamidinase was not retained on DEAE-cellulose (18) under conditions similar to those used in the procedure described in the present report. Hog kidney transamidinase was retained and chromatographed on columns of cellulose phosphate, whereas no retention of the rat kidney transamidinase on this resin has been observed. Both the hog and rat transamidinase appear to catalyze the formation of guanidinoacetic acid with a reaction mechanism involving two half-reactions with binary complexes between the substrate and enzyme (34).

Kidney transamidinase from sham-hypophysectomized, hypophysectomized, and hypophysectomized rats injected with growth hormone and thyroxine appears to be immunologically identical in all three experimental supernatants; 0.05 unit of growth hormone and thyroxine appears to be immunologically identical in all three experimental groups of rats (Fig. 67.2).

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The effect of growth hormone and thyroxine on the amount of L-arginine:glycine amidinotransferase in kidneys of hypophysectomized rats. Purification and some properties of rat kidney transamidinase.

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