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STUDIES ON TYROSINE-α-KETOGLUTARATE TRANSAMINASE FROM BOVINE THYROID AND LIVER TISSUE

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SUMMARY

1. This paper describes the purification and characteristics of tyrosine-α-ketoglutarate transaminase (L-tyrosine:2-oxoglutarate aminotransferase, EC 2.6.1.5) from bovine thyroid tissue. The most active preparations appeared to be more than 80% homogeneous on the basis of ultracentrifugal and immunoelectrophoretic analysis.

2. Bovine thyroid tyrosine-α-ketoglutarate transaminase was compared with the same enzyme isolated from bovine liver and found to be similar with respect to heat tolerance, pH optima, substrate affinity, and response to most inhibitors. Immunologically the proteins appeared to be identical.

3. The only difference between the two enzymes was the effect of diiodotyrosine. Diiodotyrosine did not inhibit thyroid transaminase and would partially protect it against inhibition by the sulfhydryl inhibitor, *p*-chloromercuriphenylsulfonic acid. On the other hand, diiodotyrosine inhibited liver transaminase 50% at 2.5 mM concentration and did not protect it from the inhibitory effect of *p*-chloromercuriphenylsulfonic acid.

4. Both thyroid and liver transaminase utilized tyrosine and monoiodotyrosine as substrates but used diiodotyrosine poorly.

INTRODUCTION

It has been shown that the α-keto analogue of diiodotyrosine will react with free or thyroglobulin bound diiodotyrosine to form thyroxine[1-3]. The α-keto analogue of monoiodotyrosine will also react with diiodotyrosine to form 3,5,3'-triiodothyronine, but this reaction procedes at a much slower rate[3]. The studies reported here were initiated in an attempt to demonstrate an enzyme activity in thyroid tissue which would produce the α-keto analogues of monoiodotyrosine and diiodotyrosine. RIVLIN, HOLLANDER AND ASPER[4] reported that tyrosine-α-ketoglutarate transaminase was present in thyroid tissue[4]. Tyrosine-α-ketoglutarate transaminase

Abbreviation: PCMPS, *p*-chloromercuriphenylsulfonic acid.

(L-tyrosine:2-oxoglutarate aminotransferase, EC 2.6.1.5)[5] was the only catalytic activity found in the thyroid which would produce the α-keto analogues of iodinated tyrosines in reasonable amounts. Peroxidase will produce small amounts of these compounds.

Tyrosine transaminase from bovine thyroid tissue was compared with tyrosine transaminase from bovine liver tissue in an attempt to show differences which would suggest that the enzymes from the two tissues had different metabolic roles.

METHODS AND MATERIALS

Assay methods

The enzymes were routinely assayed by incubation at 38° with 10 μmoles of L-tyrosine, 0.6 μmole of α-ketoglutarate, 10 μg of pyridoxal phosphate, 100 μmoles potassium phosphate (pH 7.25), and water to a final volume of 1.1 ml. After an appropriate interval the reaction was stopped by the addition of 0.08 ml of trichloroacetic acid (1 g/ml), and 0.6-ml aliquots of the clear mixture after centrifugation were assayed for *p*-hydroxyphenylpyruvate by a modification of the Briggs reaction[6].

When doing transaminase assays on crude liver preparations it was necessary to add 5 mmoles of diethyldithiocarbamate to inhibit *p*-hydroxyphenylpyruvate oxidase. Diethyldithiocarbamate was not necessary when working with the thyroid because the oxidase appeared to be present in small amounts (see below). Proteins were determined by the biuret reaction[7], but with very dilute solutions the more sensitive LOWRY procedure[8] was used. Both methods were standardized with bovine serum albumin (Armour Laboratories).

Antiserum studies

Rabbit antiserum against bovine thyroid tyrosine-α-ketoglutarate transaminase was produced by injecting adult rabbits with 5 mg of purified enzyme mixed with Freund's adjuvants three times at two-week intervals. Ouchterlony double immunodiffusion studies were done on prepared Immuno-Plates made by the Hyland Laboratories.

Ultracentrifugation

Sedimentation experiments were conducted in a Spinco Model E ultracentrifuge equipped with a phase plate as the schlieren diaphragm and a rotatable light source for Rayleigh optics. Sedimentation velocity was determined on purified transaminase after dialysis against 0.1 M potassium phosphate buffer (pH 7.4). The ultracentrifugal studies were conducted by Mr. ROGER WADE, Department of Biochemistry, University of Washington.

Materials

The amino acids, α-ketoglutarate, pyridoxal phosphate, *p*-hydroxyphenylpyruvic acid, and *p*-chloromercuriphenylsulfonic acid (PCMPS) were obtained from Sigma Chemical Company. Propylthiouracil was obtained from the Mann Research Laboratories. Hydroxylapatite was prepared by the method of TISELIUS, HJORTEN AND LEVLA[9].

Preparation of the enzyme

Bovine thyroids were obtained frozen from local slaughter houses. Fat and connective tissues were removed from the glands, and the thyroids were passed twice through a meat grinder. All subsequent steps were carried out at 4°. Ground thyroid was mixed with an equal volume of a solution containing 0.15 M KCl, 0.1 mM EDTA, and 10 mM Tris–HCl (pH 6.5) and homogenized in a Virtis high-speed homogenizer for 20 sec at full speed. This solution was centrifuged at 1000 × *g* for 20 min in an International P–R 2 refrigerated centrifuge to remove cellular debris and nuclei. The supernatant was centrifuged at 90 000 × *g* for 20 min in a Spinco Model L preparatory centrifuge. The red supernatant solution was diluted with an equal volume of water and the pH raised to 7.4 with KOH. Hydroxylapatite in 0.01 M potassium phosphate (pH 7.4), 100 ml of packed solid per liter of diluted supernatant, was added and the mixture was stirred for 1 h on ice. The hydroxylapatite was precipitated by centrifugation at 1000 × *g* for 5 min and washed until free of apparent hemoglobin with 0.05 M potassium phosphate (pH 7.4). The transaminase was removed from the hydroxylapatite by repeated washings with 0.3 M potassium phosphate (pH 7.4). The combined washes were cut with solid ammonium sulfate at 50% and 90% saturation and the precipitate at 90% saturation was dialysed against 2 changes of water 1 h each and 0.01 M potassium phosphate (pH 6.0) for 8 h. The protein at pH 6.0 was placed in a column of CM-Sephadex A50 equibrated with 0.01 M phosphate (pH 6.0). After washing the column thoroughly with 0.01 M and 0.05 M buffer the enzyme was removed with 0.1 M phosphate (pH 6.0). The enzyme was concentrated by precipitation with 90% saturation of ammonium sulfate then dialysed against 2 changes of water 1 h each and 0.01 M potassium phosphate (pH 7.4) for 8 h. This dialysed enzyme was placed on a column of hydroxylapatite equibrated in 0.01 M phosphate (pH 7.4). After the column was thoroughly washed with 0.1 M phosphate (pH 7.4) the active protein was removed with 0.2 M phosphate (pH 7.4).

Tyrosine–α-ketoglutarate transaminase was isolated from bovine liver tissue in the same manner except that the hydroxylapatite adsorbtion step was unnecessary because the enzyme is present in much larger quantities in the liver. The liver supernatant after centrifugation at 90 000 × *g* was precipitated with $(NH_4)_2SO_4$ at 50 and 90% saturation, dialysed against water and 0.05 M potassium phosphate (pH 6.0), then applied to CM-Sephadex. The remainder of the purification was as described for the thyroid enzyme.

RESULTS

Table I shows the recovery of thyroid transaminase during the purification procedure. Approximately 80% of the activity is found in the supernatant after centrifugation at 90 000 × *g*. That portion of the enzyme attached to the particulate fraction (90 000 × *g* residue) was relatively firmly attached to the particles and could not be extracted by aqueous solutions. Sonication, repeated freezing and thawing, or acetone powdering could solubilize this portion of the enzyme. 100-fold purification was obtained by the hydroxylapatite gel step which removed the major portion of the hemoglobin present. The purest enzymes from the hydroxylpapatite column were approximately 10 000-fold increased in activity and were quite unstable. The

TABLE I

ACTIVITY OF THYROID TYROSINE-α-KETOGLUTARATE TRANSAMINASE AT DIFFERENT STAGES OF PURIFICATION

Fraction	*Total protein*	*Specific activity**	*Total activity*
Whole homogenate	—	—	1150
1 000 × *g* supernatant	313	0.0031	970
90 000 × *g* supernatant	222	0.0036	800
90 000 × *g* residue	2.3	0.026	60
0.3 M phosphate eluate from hydroxylapatite gel	1.26	0.38	480
CM-Sephadex, 0.1 M phosphate eluate	0.042	6.9	290
Hydroxylapatite, 0.2 M phosphate eluate	0.004	35.0	160

* μmoles *p*-hydroxyphenylpyruvate formed per mg protein per h.

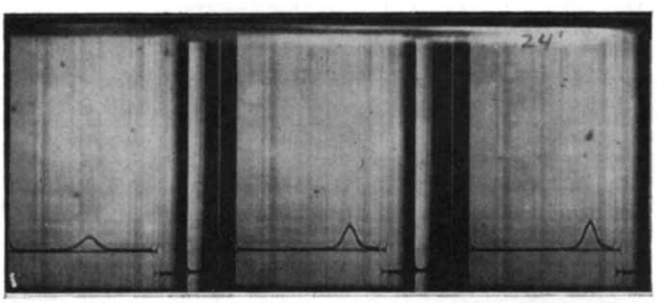
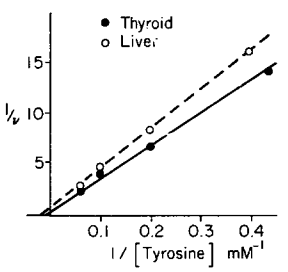


Fig. 1. Sedimentation pattern of the purified enzyme. Photographs were taken after 24, 32 and 76 min centrifugation at 59 924 rev./min. Sedimentation proceeds from right to left. The enzyme preparation contained 3.0 mg/ml specific activity 35 μmoles substrate transaminated per mg protein per h and had been previously dialysed against 0.1 M potassium phosphate buffer (pH 7.4).



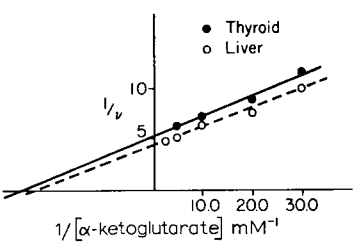


Fig. 2. Lineweaver–Burk plot comparing the effect of varying tyrosine concentration on *p*-hydroxyphenylpyruvate production by liver and thyroid transaminase. The standard assay described in the text was used and tyrosine concentration varied between 2.5 mM and 10 mM.

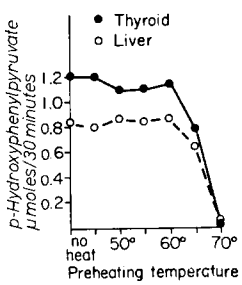
Fig. 3. Lineweaver–Burk plot comparing the effect of varying α-ketoglutarate concentration on *p*-hydroxyphenylpyruvate production by liver and thyroid transaminase. The standard assay was used and α-ketoglutarate concentration was varied between 0.03 and 0.2 mM.

activity of the most highly purified enzymes decreased by 50% in 3–4 days at −20°.

The specific activity of tyrosine-α-ketoglutarate transaminase in bovine liver homogenate and a lesser degree of increase in activity was achieved in purification of this enzyme (about 1000-fold).

Moving boundary ultracentrifugation of the most purified preparation of the thyroid transaminase showed a single major component calculated to have a Svedberg constant (s^0_{20w}) of 6.28. Fig. 1 shows the sedimentation pattern of the ultracentrifugation. This purified enzyme was used for injection in the immunological studies reported later. Because the purest enzyme was very unstable, the less purified enzyme eluted from CM-Sephadex was used for substrate specificity, inhibitor, and other studies.

Fig. 2 is a Lineweaver–Burk plot which compares the effect of varying tyrosine concentration on the activity of the 2 enzymes. In neither instance was it possible to reach the concentration of tyrosine which would affect maximum activity because tyrosine is not sufficiently soluble at pH 7.25. Fig. 3 is a Lineweaver–Burk plot of the effect of varying α-ketoglutarate concentration on the activity of the two enzymes. The K_m values for this substrate are similar for the 2 enzymes, 0.42 mM for thyroidal and 0.38 mM for liver enzyme. Concentrations of α-ketoglutarate over 5.0 mM were inhibitory to both enzymes under the conditions of this assay. Fig. 4 illustrates the effect of preheating the enzymes to a variety of temperatures for 5 min prior to assay. Both enzymes withstood preheating to 60° without injury and both were completely inactivated by heating to 70° for 5 min. If α-ketoglutarate, 0.1 mM, and a reducing agent, such as cysteine, 0.1 mM, were present during the heating process neither enzyme was inactivated by heating to 70° for 5 min.



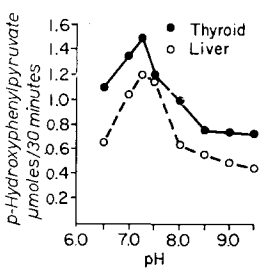


Fig. 4. The effect of preheating liver and thyroid transaminase on catalysis. The enzymes were heated to the indicated temperature for 5 min and 0.3 mg of enzyme protein was tested for activity in the standard assay described under METHODS.

Fig. 5. The effect of pH on liver and thyroid transaminase activity. The enzymes, 0.3 mg of protein, were assayed by the standard method except that the buffer was varied. Potassium phosphate buffer was used between pH 6.5–8.0, Tris–HCl between 7.0–8.5 and KOH–glycine between 8.0–9.5.

Fig. 5 compares the effect of pH upon the catalytic activity of liver and thyroid transaminase. Potassium phosphate was used between pH 6.5 and 8.0, Tris–HCl between 7.0 and 8.5 and glycine between 8.0 and 9.5. The pH optimum for both enzymes was 7.25. There was little difference between the activities in different buffers although phosphate was somewhat better and was used in the routine assay.

TABLE II

THE EFFECT OF VARIOUS INHIBITORS ON LIVER AND THYROID TRANSAMINASE

Inhibitors	*Concentration (M) necessary for 50% inhibition*	
	Thyroid	*Liver*
p-Chloromercuriphenylsulfonic acid	$3 \cdot 10^{-4}$	$3 \cdot 10^{-4}$
Iodoacetamide	No inhibition*	No inhibition
KCN	$5 \cdot 10^{-5}$	$5 \cdot 10^{-5}$
NaN_3	No inhibition	No inhibition
Isonicotinic acid hydrazide	$2 \cdot 10^{-3}$	$2 \cdot 10^{-3}$
Diiodotyrosine	No inhibition	$2.5 \cdot 10^{-3}$
Thyroxine	$5 \cdot 10^{-4}$	$5 \cdot 10^{-4}$
3,5,3'-Triiodothyronine	$1 \cdot 10^{-4}$	$1 \cdot 10^{-4}$
KSCN	No inhibition	No inhibition
KI	No inhibition	No inhibition
NaF	No inhibition	No inhibition
$CoCl_2$	No inhibition	No inhibition
Propylthiouracil	No inhibition	No inhibition

* At $2 \cdot 10^{-3}$ M concentrations.

Table II shows the concentration of a variety of inhibitors necessary to cause 50% inhibition of liver and thyroidal transaminase. Neither enzyme was inhibited by the classical thyroid inhibitors, thiocyanate, iodide, cobalt, fluoride or the thiourylene compounds. Both enzymes were inhibited by the sulfhydryl reagent, PCMPS, but neither was inhibited by iodoacetamide in the concentration used. The enzymes were sensitive to the carbonyl reagent cyanide, but only slightly inhibited by isonicotinic acid hydrazide. In the concentrations tested, up to 2.0 mM, azide was not inhibitory. As is the case with rat-liver tyrosine–α-ketoglutarate transaminase, each of these enzymes was inhibited by the thyroid hormones, thyroxine and triiodothyronine. Triiodothyronine affected 50% inhibition at one fifth the concentration necessary for thyroxine to inhibit to the same degree. The only difference between the two enzymes in their response to inhibitors was that seen with diiodo-

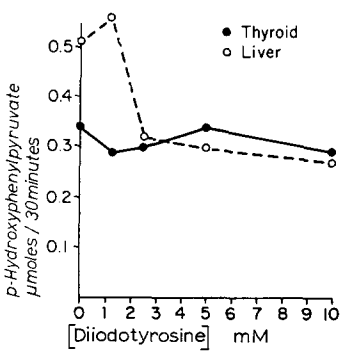


Fig. 6. The effect of increasing concentrations of diiodotyrosine on catalysis by liver and thyroid transaminase. The assay described under METHODS was used except that the tyrosine concentration was 2.5 mM instead of 10 mM. Diiodotyrosine 1.0 to 10 mM was added to the reaction mixture just prior to tyrosine. 0.1 mg of enzyme protein was used in each assay.

tyrosine. Fig. 6 shows the effect of increasing concentrations of diiodotyrosine upon production of *p*-hydroxyphenylpyruvate by liver and thyroid transaminase. In these studies tyrosine concentration was reduced to 2.5 mM because diiodotyrosine has been shown by LITWACK to be a competitive inhibitor of rat liver tyrosine-α-ketoglutarate transaminase[10]. Thyroid transaminase was not inhibited significantly by diiodotyrosine in concentrations up to the limits of solubility of the compound at pH 7.25 (10 mM). Liver transaminase, on the other hand, was inhibited 45% by diiodotyrosine at 2.5 mM. Increasing the concentration of diiodotyrosine to 10 mM did not increase the inhibition significantly.

TABLE III

THE EFFECT OF PREINCUBATION WITH SUBSTRATE ON PCMPS INHIBITION OF THYROID AND LIVER TRANSAMINASE

	% Inhibition	
	Thyroid	*Liver*
1. Regular assay	0	0
2. Regular assay + PCMPS, $3 \cdot 10^{-5}$ M	0	5
3. Preincubated enzyme with PCMPS, $1.5 \cdot 10^{-4}$ M (PCMPS, $3 \cdot 10^{-5}$ M in reaction tube)	62	66
As in (3) but enzyme previously incubated with:		
4. Pyridoxal phosphate, $1 \cdot 10^{-3}$ M	54	64
5. α-Ketoglutarate, $6 \cdot 10^{-4}$ M	59	67
6. Tyrosine, $1 \cdot 10^{-3}$ M	34	26
7. Pyridoxal phosphate + α-ketoglutarate	35	40
8. Pyridoxal phosphate + tyrosine	32	29
9. Pyridoxal phosphate + α-ketoglutarate + tyrosine	20	10
10. α-Ketoglutarate + tyrosine	35	24
11. Pyridoxal phosphate + diiodotyrosine	30	58

Table III shows the effect of preincubation of liver and thyroid transaminase with their substrates upon inhibition by PCMPS. In these experiments the enzymes were preincubated with pyridoxal phosphate, α-ketoglutarate, tyrosine or combinations of these compounds in the concentrations indicated in the Table III. After 5 min PCMPS was added to a concentration of $1.5 \cdot 10^{-4}$. The mixture was again incubated for 5 min at which time sufficient reaction rate mixture was added to dilute the PCMPS to $3 \cdot 10^{-5}$ M in the final reaction. As can be seen, this concentration of inhibitor in the final reaction causes little inhibition. However, when this amount of inhibitor is preincubated with the enzyme at $1.5 \cdot 10^{-4}$ M then diluted to $3 \cdot 10^{-5}$ M, more than 60% inhibition occurs. Pretreating the enzyme with pyridoxal phosphate or α-ketoglutarate alone afforded little or no protection from PCMPS. On the other hand, pretreating the enzyme with tyrosine alone or combinations of pyridoxal phosphate, tyrosine, and/or α-ketoglutarate reduced the inhibition by PCMPS to 35%. The only difference between the two enzymes in these studies was that the combination of diiodotyrosine and pyridoxal phosphate protected the thyroid transaminase, but did not protect the liver enzyme.

Table IV shows the relative activities of liver and thyroid transaminase with tyrosine, monoiodotyrosine, and diiodotyrosine as amino acid substrates. Both en-

TABLE IV

RELATIVE ACTIVITIES OF LIVER AND THYROID TRANSAMINASE WITH VARIOUS SUBSTRATES

Amino acid substrate	*μmoles of keto acid formed per mg protein per h*	
	Liver	*Thyroid*
Tyrosine	5.0	6.2
Monoiodotyrosine	4.9	6.4
Diiodotyrosine (at pH 6.5)	0.25	0.35

zymes utilize tyrosine and monoiodotyrosine equally well. The activity of both enzymes with diiodotyrosine as substrate was about 5% of the activity seen with the other tyrosines. When diiodotyrosine was used as the amino acid the pH optimum for the reaction was 6.5 instead of 7.25. Because the keto acid analogue of diiodotyrosine is extremely unstable, the production of glutamic acid was tested enzymatically and confirmed the low rate of diiodotyrosine transamination and the lower pH optimum.

During the purification of bovine liver transaminase it was necessary to add diethyldithiocarbamate to the assay mixture to inhibit the oxidation of *p*-hydroxyphenylpyruvate. In both the supernatant fluid after high-speed centrifugation (90 000 × *g*) and the fraction precipitated between 70% and 90% saturation with $(NH_4)_2SO_4$ a considerable amount of *p*-hydroxyphenylpyruvate oxidation occurred which could be inhibited by diethyldithiocarbamate. The liver transaminase eluted from hydroxylapatite did not contain significant diethyldithiocarbamate-sensitive *p*-hydroxyphenylpyruvate oxidase. Table V compares the amount of removal of *p*-hydroxyphenylpyruvate from solution by fractions of liver and thyroid nomogenates. In these studies 0.3 μmole of *p*-hydroxyphenylpyruvate were incubated at 38° with potassium phosphate buffer 100 μmoles (pH 7.25) and sufficient tissue protein to cause a significant removal of *p*-hydroxyphenylpyruvate. 10–20 times as much thyroid protein was used as liver protein. The table shows that the supernatant fraction from liver has about 50% as much diethyldithiocarbamate-sensitive

TABLE V

DISAPPEARANCE OF *p*-HYDROXYPHENYLPYRUVATE IN THE PRESENCE OF LIVER AND THYROID FRACTIONS

Fraction	*Reduction in p-hydroxyphenylpyruvate (μmoles/h)*			
	Supernatant, 90 000 × g		*Residue, 90 000 × g*	
	Liver (2.4 mg protein)	*Thyroid (40 mg protein)*	*Liver (1.6 mg protein)*	*Thyroid (24 mg protein)*
Without diethyldithiocarbamate	0.113	0.092	0.037	0.083
With diethyldithiocarbamate, 5 mM	0.048	0.096	0.034	0.086
Diethyldithiocarbamate-sensitive	0.065	0	0	0
(Transaminase activity μmoles/h per amount of protein indicated above)	(0.126)	(0.08)	(0.085)	(0.36)

TABLE VI

THE EFFECT OF RABBIT ANTISERUM AGAINST THYROID TRANSAMINASE ON ACTIVITY OF THYROID AND LIVER ENZYME

Procedure		*Activity (μmoles p-hydroxyphenylpyruvate formed/mg protein/h)*	
30-min incubation at 38° with	*Centrifugation 1000 × g for 10 min*	*Supernatant*	
		Liver	*Thyroid*
0.15 M NaCl	+	5.0	7.0
Preinjection rabbit serum	+	4.8	6.2
Antiserum, undiluted	−	4.6	6.4
Antiserum, undiluted	+	0.9	1.4
Antiserum diluted 1:4	+	4.4	6.0
Antiserum diluted 1:40	+	5.1	6.8

p-hydroxyphenylpyruvate oxidase activity when assayed under the conditions described. There is no appreciable diethyldithiocarbamate-sensitive activity in the thyroid fractions although sufficient protein waa used to demonstrate it if present at a level of 10% of the transaminase activity. Under conditions more suited to the assay of the enzyme, *p*-hydroxyphenylpyruvate oxidase, some activity may have been demonstrable. Incubation of either liver or thyroid protein with *p*-hydroxyphenyl-

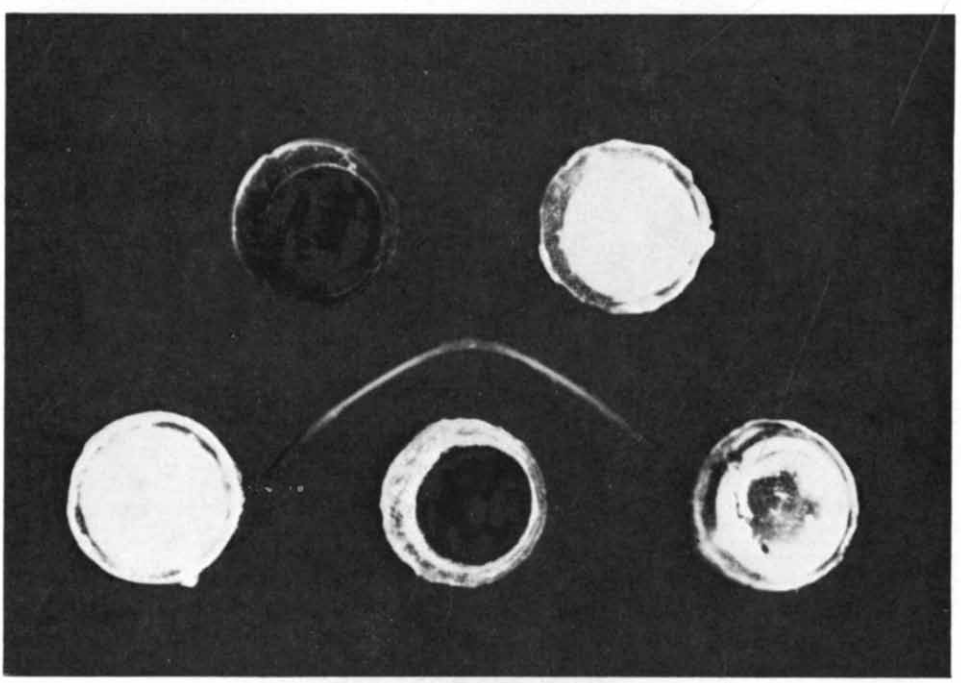


Fig. 7. Immunodiffusion study of bovine liver and thyroid transaminase. Rabbit thyroid transaminase antiserum was placed in the lower center well. Equal amounts of transaminase activity from the thyroid (upper left well) and the liver (upper right well) were placed in the upper wells.

pyruvate caused the disappearance of some substrate. This disappearance occurred even in the absence of oxygen and the product of the reaction is not known.

Antiserum prepared by injecting the most purified preparation of thyroid transaminase with Freund's adjuvants into a rabbit showed a single precipitation line on immunoelectrophoresis against the antigen enzyme. This antiserum when mixed with the enzyme for 30 min did not inactivate it. However, as shown in Table VI, when the enzyme and antiserum were mixed, incubated for 30 min at 38° and centrifuged at 1000 × g for 10 min, almost all of the activity was precipitated. Table VI shows that liver transaminase when handled in the same manner is also precipitated by rabbit antithyroid transaminase. Preinjection rabbit serum has no effect upon either enzyme after 30 min incubation. If enzyme and rabbit serum, removed either before or after transaminase injection, are incubated together for more than 1 h, the enzyme is inactivated.

Fig. 7 is a photograph of an Ouchterlony double immunodiffusion plate. Rabbit antiserum against bovine thyroid transaminase was placed in the center well (lower well) and an equal number of units of thyroid (upper left well) and liver transaminase (upper right well) were placed in the other wells. The two enzymes meet in a single line without spur formation.

DISCUSSION

These studies were undertaken to find an enzyme in the thyroid that would produce the α-keto analogues of monoiodotyrosine and diiodotyrosine, compounds which represent possible intermediates in the production of thyroid hormones. Tyrosine-α-ketoglutarate transaminase, known to be in thyroid tissue, would catalyse the production of these compounds. Bovine thyroid tyrosine transaminase was purified and compared with bovine liver transaminase in an attempt to distinguish differences that might suggest the enzymes had different biological significance.

Thyroid and liver tyrosine transaminases were prepared in the same manner so that isozymes with different characteristics might have been excluded during preparation. Biochemically the two enzymes were very similar: the K_m values for keto and amino acid substrates were of the same order of magnitude; the pH optimum was the same; the sensitivity to heat was the same; and the response to most inhibitors was comparable. None of the classical thyroid gland inhibitors affected either enzyme. Both enzymes utilized tyrosine and monoiodotyrosine as amino acid substrate equally well, and diiodotyrosine as amino acid substrate poorly. The rate of transamination of diiodotyrosine was 5% of the rate when the other tyrosines were used. Both enzymes were precipitated by rabbit antiserum produced against the thyroid enzyme. In double immunodiffusion studies against the rabbit antiserum the precipitin lines formed by the two enzymes met in a single line without spur formation.

The only difference between the two enzymes was the effect of diiodotyrosine as an inhibitor. The liver transaminase was inhibited 50% by diiodotyrosine at 2.5 mM concentration. Thyroid transaminase was not inhibited by diiodotyrosine in concentrations up to 10 mM. Tyrosine when preincubated with either transaminase, partially protected it from the inhibitory effects of PCMPS. Diiodotyrosine was equally effective in protecting thyroid transaminase, but protected liver trans-

aminase very little if at all. These differences with respect to diiodotyrosine may represent biological differences between the liver and thyroid transaminases or may be artifacts resulting from the preparation of the enzymes.

In the liver tyrosine transaminase catalyses the first step in a series of reactions which convert tyrosine to acetoacetate. The second step in this sequence is the oxidation of *p*-hydroxyphenylpyruvate by the copper dependent enzyme, *p*-hydroxyphenylpyruvate oxidase. No copper dependent *p*-hydroxyphenylpyruvate oxidase could be demonstrated in the thyroid. This may suggest that tyrosine transaminase serves a different function in the thyroid than in the liver.

If tyrosine transaminase represents a step in the biosynthesis of thyroid hormones, the substrate may be monoiodotyrosine. FISCHER, SCHULZ AND OLIVER[11] have shown that thyroid microsomes when incubated with monoiodotyrosine, pyridoxal phosphate, manganese, and a peroxide source will produce 3,5,3'-triiodothyronine. FEUER[12] found that early after the injection of radioiodine in rabbits and rats, the specific activity of triiodothyronine was higher than that of thyroxine. Whether this triiodothyronine was iodinated to form thyroxine or thyroxine was formed without a triiodothyronine intermediate could not be determined.

When the thyroid transaminase described here was incubated with diiodotyrosine and monoiodotyrosine, pyridoxal phosphate, α-ketoglutarate, and thyroid microsomes, thyroxine and triiodothyronine are formed. A relationship between this *in vitro* reaction and thyroid hormone synthesis *in vivo* has not been established. Further studies designed to determine the role of tyrosine transaminase in thyroxine synthesis are in progress.

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