Ureidoglycollate lyase, a new metalloenzyme of peroxisomal urate degradation in marine fish liver

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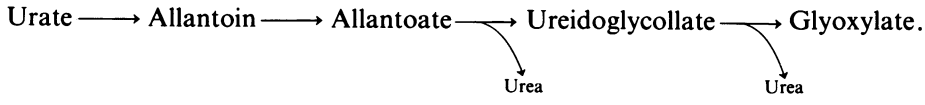
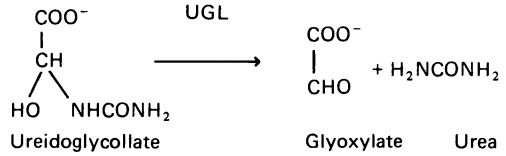
Ureidoglycollate lyase (UGL, EC 4.3.2.3), which catalyses the degradation of *S*(−)-ureidoglycollate to urea and glyoxylate, was found in the peroxisomes of marine fish (sardine and mackerel) liver. The enzyme highly purified from sardine liver had an M_r of about 121000, with two identical subunits. When UGL was purified in the presence of 1 mM-EDTA, a much less active form was obtained. It was markedly activated by bivalent metal ions, particularly by Mn^{2+}. The Mn^{2+}-activated enzyme remained active when free Mn^{2+} was removed by gel filtration on Sephadex G-50, suggesting that UGL may be a metalloenzyme and the activation resulted from the binding of Mn^{2+} to the apoenzyme. UGL was found to be essential in peroxisomal urate degradation, since allantoate, the intermediate of urate catabolism, was found to be degraded to urea and glyoxylate in a two-step reaction catalysed by allantoicase (EC 3.5.1.5) and UGL via *S*(−)-ureidoglycollate as an intermediate in fish liver peroxisomes, but not in a one-step reaction as previously believed.

INTRODUCTION

In micro-organisms and frog liver, urate is degraded to allantoin by uricase (EC 1.7.3.3); allantoin is then degraded to allantoate by allantoinase (EC 3.5.2.5). Allantoate formed is degraded to ureidoglycollate and urea by allantoicase (EC 3.5.1.5), and ureidoglycollate is further degraded to glyoxylate and another molecule of urea by ureidocollate lyase (UGL, EC 4.3.2.3) (Trijbels & Vogels, 1969; Vogels & Van der Drift, 1976):



In fish liver, allantoate has been believed to be degraded to urea and glyoxylate in one-step reaction catalysed by 'allantoicase' (Scott *et al.*, 1969; Smellie, 1955; Hanks *et al.*, 1982). We have reported that uricase, allantoinase and allantoicase are located in the peroxisomes in mackerel (*Pneumatophorus japonicus*) liver (Noguchi *et al.*, 1979). In the present paper, the peroxisomal localization and some properties of UGL in sardine (*Sardinopus melanosticta*) and mackerel liver are described. UGL was found to be essential in peroxisomal urate degradation, since allantoate was found to be degraded to glyoxylate and urea in a two-step reaction via *S*(−)-ureidoglycollate as an intermediate (Scheme 1). Evidence that UGL may be a metalloenzyme was obtained.



Scheme 1.

EXPERIMENTAL

Materials

Sodium ureidoglycollate was obtained from Sigma. Other chemicals were of the highest grade available. All apparatus (HLC 803D, GE4 gradient maker) and columns (TSK gel 3000SW, TSK gel DEAE-5PW) for h.p.l.c. were from Toyo Soda Manufacturing Co., Tokyo, Japan.

Sucrose-density-gradient centrifugation of the homogenate from sardine and mackerel liver

All procedures were carried out at about 4 °C. Fresh sardine or mackerel liver was cut into very small pieces with scissors and homogenized by a once-only processing in 4 vol. of 0.25 M-sucrose/20 mM-glycylglycine, pH 7.4, in a Potter–Elvehjem homogenizer with a Teflon pestle at 800 rev./min. The homogenate was filtered through coarse cheesecloth and centrifuged at 300 *g* for 5 min to sediment nuclei and any whole cells. A portion (4 ml) of the homogenate was layered on a 30 ml linear sucrose gradient (24–54%, w/w) in 20 mM-glycylglycine, pH 7.4, and centrifuged at 132400 *g* for 70 min in a Hitachi 55P-72 ultracentrifuge with a RPV-50T vertical rotor. Fractions (2.5 ml) were collected from the bottom of the tube.

Enzyme assays

Catalase (EC 1.11.1.6) (Aebi, 1974) and cytochrome *c* oxidase (EC 1.9.3.1) (Wharton & Tzagoloff, 1967) were assayed as described in the cited references. Allantoicase (EC 3.5.3.4) was assayed by the 'Procedure 1' of the 'Analysis of the product of the allantoicase reaction'

Abbreviations used: UGL, ureidoglycollate lyase; LDH, lactate dehydrogenase.

subsection below, except that ultrafiltration of the reaction mixture was omitted. The assay of UGL was modified from those previously described (Choi *et al.*, 1968; Trijbels & Vogels, 1969). The reaction mixture contained 100 mM-triethanolamine/HCl, pH 7.6, 3 mM-phenylhydrazine hydrochloride, 0.4 mM-sodium ureidoglycollate, 0.1% Triton X-100 and the enzyme solution in 1 ml. The increase in A_{324} due to the reaction of the liberated glyoxylate with phenylhydrazine was monitored at room temperature. The reaction was linear after a lag period of about 3 min. A unit of enzyme activity is defined as the amount of enzyme that catalyses the formation of product or a decrease in substrate of 1 μmol/min.

Preparation of allantoicases from mackerel, sardine and frog liver

Mackerel liver allantoicase has been reported to be located in the peroxisomal membrane (Noguchi *et al.*, 1979). All procedures were carried out at about 4 °C. Mackerel liver was homogenized in 4 vol. of 10 mM-Tris/HCl (pH 7.5)/1 mM-EDTA in a Potter–Elvehjem homogenizer with a Teflon pestle. The homogenate was centrifuged at 300 *g* for 5 min to sediment cell debris. The supernatant was centrifuged at 105000 *g* for 40 min. The precipitate was washed twice by re-homogenization and centrifugation as described above. The final precipitate suspended in the buffer (9.0 mg/ml) contained most of the allantoicase activity and was used for analysis of the reaction product. The supernatant and washings contained most of the UGL activity. UGL activity was less than 1% of the allantoicase activity in the suspension of the precipitate. Sardine liver allantoicase was purified as described by Noguchi *et al.* (1986). Frog (*Rana catesbeiana*) liver allantoicase was prepared as previously described (Takada & Noguchi, 1983).

Analysis of the product of the allantoicase reaction

A portion of each allantoicase preparation was incubated with 50 mM-potassium allantoate in 0.1 M-sodium citrate, pH 6.5, in total volume of 1 ml for 1 or 3 h at 37 °C. After incubation, the reaction mixture was centrifuged at 15000 *g* for 10 min at 4 °C (only with mackerel enzyme), and the supernatant was deproteinized by ultrafiltration with Centricon-10 (Amicon) at 4 °C. The filtrate was subjected to differential glyoxylate analysis (Valentine & Wolfe, 1961; Trijbels & Vogels, 1966; Choi *et al.*, 1968; Vogels & Van der Drift, 1970) or to treatment with sardine liver UGL.

Procedure 1. A portion of the filtrate was adjusted to 100 μl with 0.1 M-sodium citrate, pH 6.5, and 50 μl of 0.1 M-KOH was added to the filtrate. After 10 min at room temperature, 50 μl of 0.1 M-HCl and 0.75 ml of 0.1 M-triethanolamine/HCl, pH 8.2, were added. Then 50 μl of 5 mM-NADH and 2 μl (3 units) of pig liver LDH (Toyobo, Tokyo, Japan) were added and the decrease in A_{340} was measured after 60 min against the blank without the filtrate.

Procedure 2. A portion of the filtrate was adjusted to 100 μl with 0.1 M-sodium citrate, pH 6.5, and heated in a boiling-water bath for 7 min and cooled rapidly to room temperature. Then 0.85 ml of 0.1 M-triethanolamine/HCl, pH 8.2, was added and glyoxylate formed was determined as described above.

Procedure 3. Except that boiling was omitted and 5 μl of sardine liver UGL (5 munits) was finally added, this was the same as Procedure 2.

Procedure 4. This was the same as Procedure 2, except that boiling was omitted.

Sodium ureidoglycollate was degraded to glyoxylate and urea completely by alkali treatment and less effectively by boiling (about 60% in 7 min), but sodium glyoxylate and potassium allantoate were stable when subjected to these treatments (Valentine & Wolfe, 1961; Trijbels & Vogels, 1966; Choi *et al.*, 1968).

Purification of UGL from peroxisomal and soluble fraction of sardine liver

All purification procedures were carried out at 0–4 °C, unless specified otherwise. Fresh sardine liver (about 100 g) was homogenized in 2 vol. of 0.25 M-sucrose in 10 mM-Tris/HCl (pH 7.5)/0.1% 2-mercaptoethanol in a Waring blender (two 10 s bursts with a 1 min interval between each burst) and centrifuged at 12000 *g* for 30 min. The supernatant (soluble fraction) was pooled. The particulate fraction was re-homogenized in 2 vol. of 10 mM-Tris/HCl (pH 7.5)/0.1% 2-mercaptoethanol and centrifuged at 12000 *g* for 30 min. The supernatant (peroxisomal extract) was pooled. UGL was prepared from the soluble fraction and the peroxisomal extract by the same procedures. $(NH_4)_2SO_4$ was added to the enzyme solution to 30% saturation. After 30 min, the precipitate was removed by centrifugation. Additional $(NH_4)_2SO_4$ was added to the supernatant to 60% saturation. After 30 min, the precipitate was collected by centrifugation, dissolved in a minimal volume of 10 mM-Tris/HCl (pH 7.5)/10% (v/v) glycerol/0.1% 2-mercaptoethanol (buffer A) and desalted by dialysis against buffer A. $MnCl_2$ (2 mM) was added to the non-diffusable solution (the dialysis residue) and the solution was rapidly warmed to 50 °C and quickly chilled in an ice-cold bath. Precipitate inactive with respect to UGL activity was removed by centrifugation. The supernatant was applied to a column (3.8 cm × 17 cm) of DEAE-cellulofine (Seikagaku Kogyo Co., Tokyo, Japan) equilibrated with buffer A containing 2 mM-$MnCl_2$. After washing the column with 500 ml of buffer A, the enzyme was eluted by a 2-litre linear NaCl gradient (0–0.25 M) in buffer A. The enzyme was eluted as a single activity peak at 0.1 M-NaCl. $(NH_4)_2SO_4$ was added to the active fractions to 60% saturation. The precipitate was collected by centrifugation, dissolved in 5 ml of buffer A and applied to a column of Sephacryl S-200 (Pharmacia, 2.5 cm × 90 cm) equilibrated with buffer A containing 0.2 M-NaCl and 2 mM-$MnCl_2$. The column was eluted with the same buffer at a flow rate of 20 ml/h. The enzyme was eluted as a single activity peak with an M_r of about 120000. Active fractions were pooled and applied to a column (2.2 cm × 13 cm) of hydroxyapatite equilibrated with 5 mM-potassium phosphate buffer (pH 7.5)/10% glycerol/0.1% 2-mercaptoethanol. After washing the column with 225 ml of the same buffer, the enzyme was eluted with a 600 ml linear gradient of potassium phosphate (5–250 mM). The enzyme was eluted at 90 mM-phosphate. Active fractions were pooled and concentrated to 0.5 ml by ultrafiltration with PM-10 and Centricon-10 (Amicon). Portions were subjected to h.p.l.c. on a column of TSK gel 3000 SW (a matrix for gel permeation; 0.75 cm × 60 cm) equilibrated with

50 mM-potassium phosphate (pH 7.5)/0.2 M-NaCl at 25 °C. The column was eluted with the same buffer at a flow rate of 0.7 ml/min. Active fractions were pooled and concentrated as described above and the buffer was changed to 10 mM-Tris/HCl, pH 8.0. Portions were subjected to h.p.l.c. on a column of TSK gel DEAE-5PW (an anion-exchange resin; 0.75 cm × 7.5 cm) equilibrated with 10 mM-Tris/HCl, pH 8.0. After washing the column with the same buffer (10 ml), the enzyme was eluted by a 40 ml linear NaCl gradient (0–0.5 M) in the same buffer. The enzyme was eluted at 110 mM-NaCl. Active fractions were pooled and concentrated by ultrafiltration.

Other methods

An antibody against peroxisomal UGL was prepared by the procedures previously described (Takada & Noguchi, 1985). The serum was stored at −70 °C. Protein determination, immunodiffusion analysis, polyacrylamide-disc-gel electrophoresis, SDS/polyacrylamide-gel electrophoresis and sucrose-density-gradient centrifugation for the determination of approximate M_r were carried out as described previously (Takada & Noguchi, 1985).

RESULTS AND DISCUSSION

UGL activity was detected in the liver of sardine and mackerel. A representative sedimentation in a sucrose density gradient for the homogenate from sardine liver is present in Fig. 1. The peroxisomes and mitochondria were separated; the peroxisomes, marked by catalase, were at a density of about 1.25 g·ml^{-1} and the mitochondria, marked by cytochrome *c* oxidase, at a density of about 1.19 g·ml^{-1}. UGL activity showed a distribution profile similar to that of catalase. Some 30% of the UGL activity was recovered in the peroxisomes and the remainder in the soluble fraction. When the same experiment was carried out with mackerel liver, nearly identical results were obtained; UGL activity was present both in the peroxisomes and in the soluble fraction.

The peroxisomal and soluble UGLs were purified and characterized from sardine liver as described in the Experimental section. Results of the purification are shown in Table 1. The purified peroxisomal and soluble enzymes [in 10 mM-Tris/HCl (pH 8.0)/0.1 M-NaCl/10% glycerol] may be stored at −20 °C for at least 3 months without loss of activity.

The purified peroxisomal and soluble UGLs were

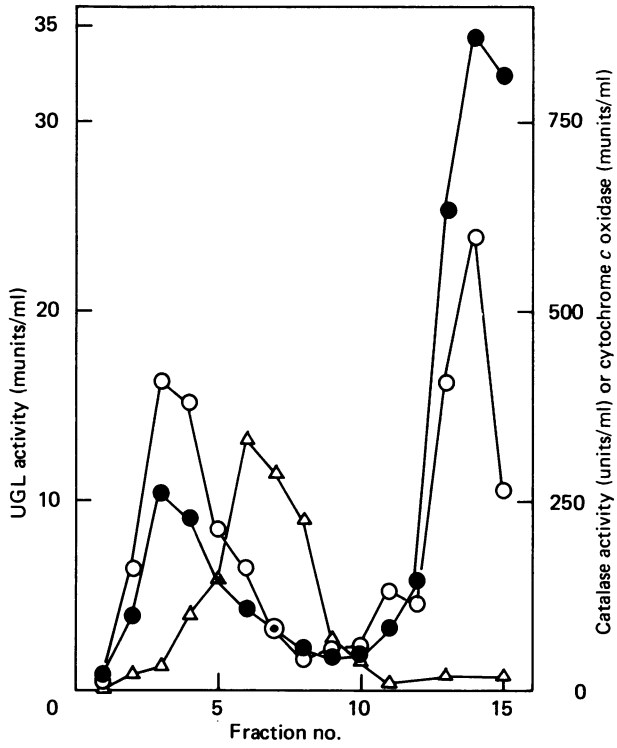


Fig. 1. Subcellular distribution of UGL in sardine liver

Postnuclear homogenate from sardine liver (4 ml, equivalent to about 1 g of original tissue) was layered on to a 30 ml linear sucrose gradient (24–54%, w/w) in 20 mM-glycylglycine, pH 7.4, and centrifuged at 132400 *g* for 70 min with a RPV-50T vertical rotor. Fractions (2.5 ml) were collected from the bottom of the tube. ○, Catalase; △, cytochrome *c* oxidase; ●, UGL. Mackerel liver showed distribution profiles similar to those shown by sardine liver.

Table 1. Purification of ureidoglycollate lyase (UGL) from sardine liver peroxisomal extract and soluble fraction

Details of the purification procedures are described in the text. Crude peroxisomal extract, soluble fraction and each $(NH_4)_2SO_4$ fraction were assayed after incubation with 2 mM-$MnCl_2$ for 5 min at room temperature. Abbreviations used: P, peroxisomal extract; S, soluble fraction.

	Total protein (mg)		Total activity (munits)		Specific activity (munits/mg)	
	P	S	P	S	P	S
Peroxisomal extract	2500	–	21500	–	8.6	–
Soluble fraction	–	11750	–	46300	–	3.94
$(NH_4)_2SO_4$	538	2350	17900	36000	33.3	15.3
Heat treatment	260	950	17000	30000	65.4	31.5
DEAE-cellulofine	73.4	78.3	12100	17550	165	224
Sephacryl S-200	9.6	3.86	7720	4950	807	1282
Hydroxyapatite	2.8	1.56	4240	3040	1510	1940
TSK gel 3000SW	1.12*	1.12*	2150	2200	1920	1960
TSK gel DEAE 5PW	0.38*	0.29*	1670	1220	4400	4200

* Protein concentration was estimated from A_{280}.

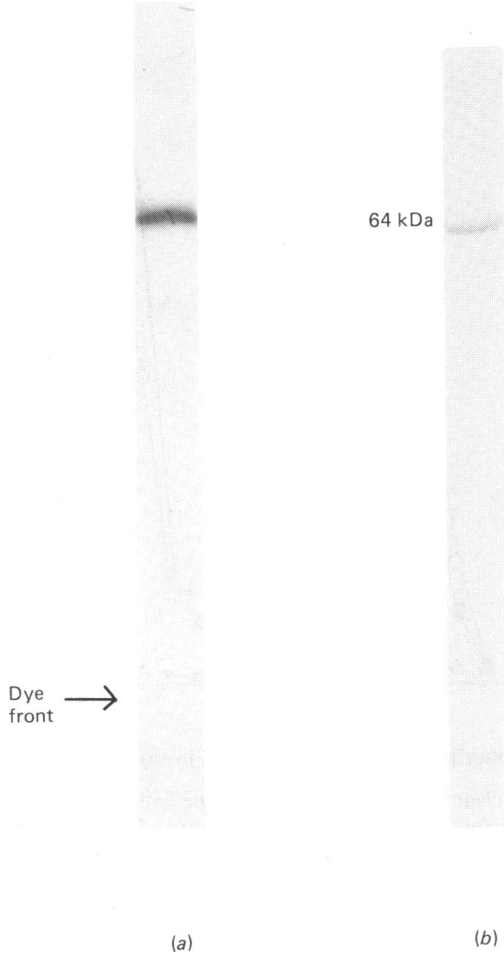


Fig. 2. Polyacrylamide-gel electrophoresis of the purified peroxisomal UGL from sardine liver

Electrophoresis was carried out in Tris/glycine buffer, pH 8.7 (*a*), or in Tris/glycine buffer, pH 8.3, in the presence of SDS (*b*). The migration was from top to bottom. Nearly identical results were obtained with UGL from soluble fraction. M_r markers used in (*b*) were ribonuclease A (13700), chymotrypsinogen A (25000), ovalbumin (43000) and bovine serum albumin (67000). Gels were stained for protein in 0.25% Coomassie Blue in methanol/acetic acid/water (5:1:5, by vol.) and destained by diffusion in 7% (v/v) acetic acid.

nearly identical with respect to physical, catalytic and immunological properties. On polyacrylamide-disc-gel electrophoresis at pH 8.7 in 7%-(w/v)-acrylamide gel, each enzyme preparation migrated to the anode as a single protein band (mobility 0.30). On SDS/polyacrylamide-disc-gel electrophoresis in 12.5%-acrylamide gel, each enzyme preparation migrated as a single band with an M_r of about 64000 (Fig. 2). The M_r of each enzyme was estimated as about 121000 by sucrose-density-gradient centrifugation, suggesting that UGL is composed of two identical subunits. The catalytic properties of each enzyme preparation were examined. The pH optimum was near 7.5 with 0.4 mM-ureidoglycollate as substrate. The apparent K_m value (with ureidoglycollate as substrate) was estimated to be about 330 μM. Purified peroxisomal and soluble UGL produced a single fused precipitin band on Ouchterlony double-diffusion analysis with antibody against peroxisomal UGL.

On the basis of the previous reports (Lazarow & de Duve, 1973; Leighton *et al.*, 1975) that catalase is present only in the peroxisomes, the distribution of UGL and catalase in the peroxisomes and in the soluble fraction, and the near-identity in the properties between the peroxisomal and soluble UGLs, it is suggested that most of UGL in the soluble fraction is from broken peroxisomes.

It has been reported that allantoicase from frog liver produced *S*(−)-ureidoglycollate and urea from allantoate (Trijbels & Vogels, 1969). Sardine liver UGL degraded most (89%) of the ureidoglycollate produced by frog liver allantoicase, but only 39% of synthetic sodium ureidoglycollate under the same conditions (Table 2), suggesting that the UGL used only one of the two optical isomers of ureidoglycollate [*S*(−)-ureidoglycollate] as substrate.

When the crude peroxisomal extract was prepared in the presence of 1 mM-EDTA and the $(NH_4)_2SO_4$ fraction (30–60%) was extensively dialysed against buffer A supplemented with 1 mM-EDTA, and when the non-

Table 2. Degradation of chemically or enzymically (by frog liver allantoicase) synthesized ureidoglycollate by sardine liver UGL

Highly purified frog liver allantoicase was incubated in 50 mM-potassium allantoate/0.1 M-sodium citrate, pH 6.5, at 37 °C for 3 h. After incubation, the reaction mixture was deproteinized by ultrafiltration with Centricon-10 at 4 °C. A 5 μl portion of the filtrate [containing 36 μmol/of *S*(−)-ureidoglycollate/ml, determined as glyoxylate formed by alkali treatment] was adjusted to 100 μl with 0.1 M-sodium citrate, pH 6.5, and used for each treatment. Glyoxylate formed was determined with NADH (50 μl of a 5 mM solution) and LDH (2 μl, 3 units) after addition of 0.85 ml (0.75 ml for alkali treatment) of 0.1 M-triethanolamine/HCl, pH 8.2. Each value represents the mean of the two determinations. For alkali treatment, 50 μl of 0.1 M-KOH was added to the filtrate and, after 10 min at room temperature, the mixture was neutralized with 50 μl of 0.1 M-HCl. For UGL treatment, 5 munits of sardine liver UGL after hydroxyapatite chromatography was added with LDH and NADH, and the time course of the decrease in A_{340} was monitored; the reaction was almost complete by 30–40 min and a further decrease in A_{340} was not observed after 60 min.

Treatment	Substrate...	Glyoxylate formed (nmol)	
		Synthetic sodium *S*,*R*(±)-ureidoglycollate	*S*(−)-Ureidoglycollate produced by frog liver allantoicase
Alkali		203 (100%)	180 (100%)
Sardine UGL		79.4 (39.1%)	160 (89.5%)
None		16.0 (7.9%)	25.4 (14.1%)

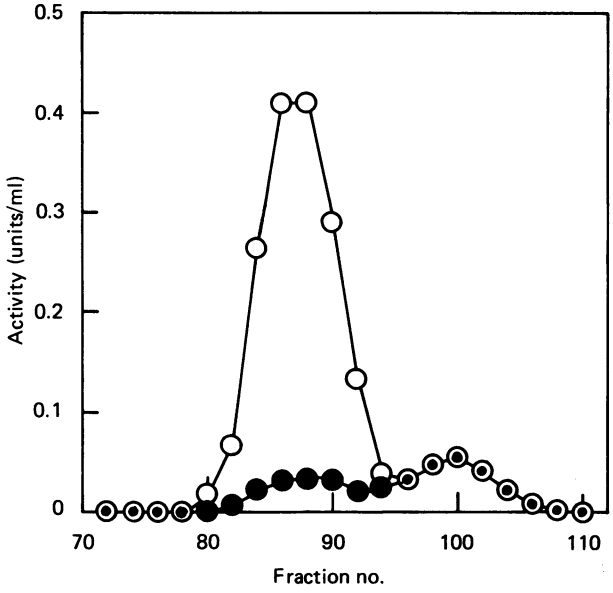


Fig. 3. Sephacryl S-200 chromatography of UGL from the peroxisomal extract in the presence of EDTA

Preparation of the peroxisomal extract from fresh sardine liver (50 g) and $(NH_4)_2SO_4$ fractionation were carried out as described in the Experimental section, except that all buffers used were supplemented with 1 mM-EDTA. The $(NH_4)_2SO_4$ fraction (30–60% satd.) was applied to a column (2.5 cm × 90 cm) of Sephacryl S-200 equilibrated with 10 mM-Tris/HCl (pH 7.5)/0.2 M-NaCl/10% glycerol/0.1% 2-mercaptoethanol/1 mM-EDTA. Fractions (3 ml) were collected. UGL was assayed with 10–25 μl of the fraction, with (○) or without (●) incubation of the enzyme with 3 mM-$MnCl_2$ for 5 min at room temperature before assay. Similar results were obtained with the soluble fraction.

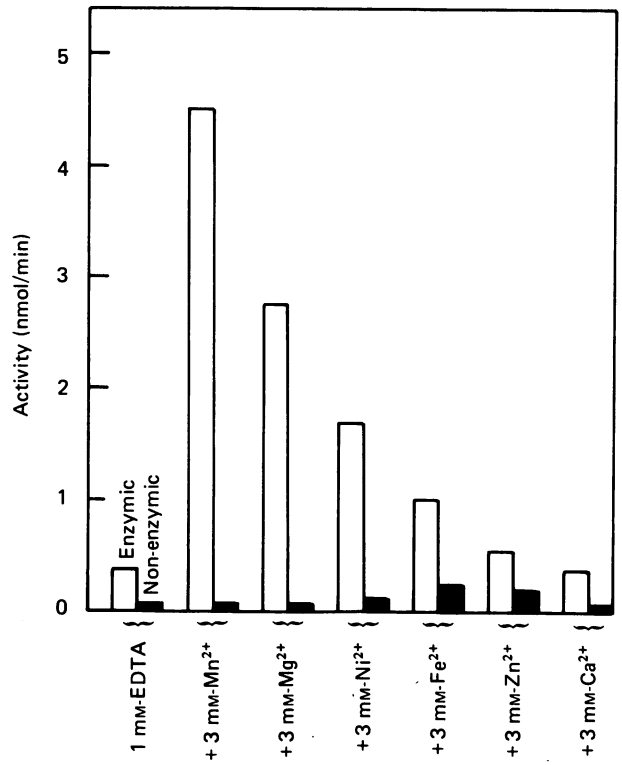


Fig. 4. Effects of bivalent metal ions on much less active UGL (M_r 120000)

A 3 μl portion of each 100 mM bivalent metal salt solution ($MnCl_2$, $MgCl_2$, $FeSO_4$, $NiCl_2$, zinc acetate or $CaCl_2$) was added to 100 μl of much less active UGL fraction (M_r 120000) after gel filtration on Sephacryl S-200 (Fig. 3). After incubation at room temperature for 5 min, 25 μl of the mixture was used for assay. Buffer solution containing only 3 mM bivalent metal ion was used to determine non-enzymic reaction. □, Enzymic; ■, non-enyzmic.

diffusable portion was subjected to gel filtration on a column (90 cm × 2.5 cm) of Sephacryl S-200 equilibrated with buffer A supplemented with 0.2 M-NaCl and 1 mM-EDTA, two peaks of UGL activity were obtained. The faster-migrating activity, with an M_r of about 120000, was markedly activated by Mn^{2+}, but the slower-migrating activity, with an M_r of about 60000, was not (Fig. 3). On the basis of M_r, the faster-migrating enzyme may be the much less active form of UGL highly purified in the present study. Similar results were obtained with the soluble fraction, except that the slower-migrating activity was higher (about 30% of the total activity) than that in the peroxisomal extract. The slower-migrating enzyme was not further characterized in the present study.

The effects of different bivalent metal ions on peroxisomal much less active UGL were examined (Fig. 4). The enzyme was markedly activated by bivalent metal ions, particularly by Mn^{2+}. Non-enzymic degradation of ureidoglycollate was not affected by bivalent metal ions. The activation by Mn^{2+} was dependent on the Mn^{2+} concentration, and reached saturation level at 1 mM-Mn^{2+}. The Mn^{2+}-enhanced activity was not lost by the removal of free Mn^{2+} by gel filtration [centrifuge column procedure of Penefsky (1977)] on Sephadex G-50, suggesting that the activation resulted from the binding of Mn^{2+} to the apoenzyme (Fig. 5). Similar results were obtained with much less active UGL from the soluble fraction. These findings show that UGL may be a metalloenzyme.

When subcellular fractions of sardine or mackerel liver prepared by sucrose-density-gradient centrifugation (Fig. 1) were incubated with 2 mM-$MnCl_2$ for 5 min at room temperature before assay, UGL activities in the peroxisomal and soluble fractions were activated 1.9–2.0-fold and 1.6–1.7-fold respectively, suggesting that UGL was not fully saturated with bivalent metal ion *in vivo*. The purified enzyme preparations were not activated by Mn^{2+}, probably because of the addition of $MnCl_2$ during purification.

The product of fish liver allantoicase reaction was examined to discover whether it is glyoxylate or ureidoglycollate and whether UGL is indeed involved in peroxisomal urate degradation, since allantoate has been believed to be degraded to urea and glyoxylate in one-step reaction in fish liver (Scott *et al.*, 1969; Smellie 1955; Hanks *et al.*, 1982). Allantoicase from mackerel and sardine liver produced an alkali- and heat-labile glyoxylate derivative, but only a small amount of free glyoxylate in a manner dependent on the enzyme concentration (Table 3). In addition, most (over 83%) of the glyoxylate derivative was degraded by sardine liver UGL, yielding glyoxylate (Table 3). On the basis of both the differential glyoxylate analysis (Valentine & Wolfe, 1961;

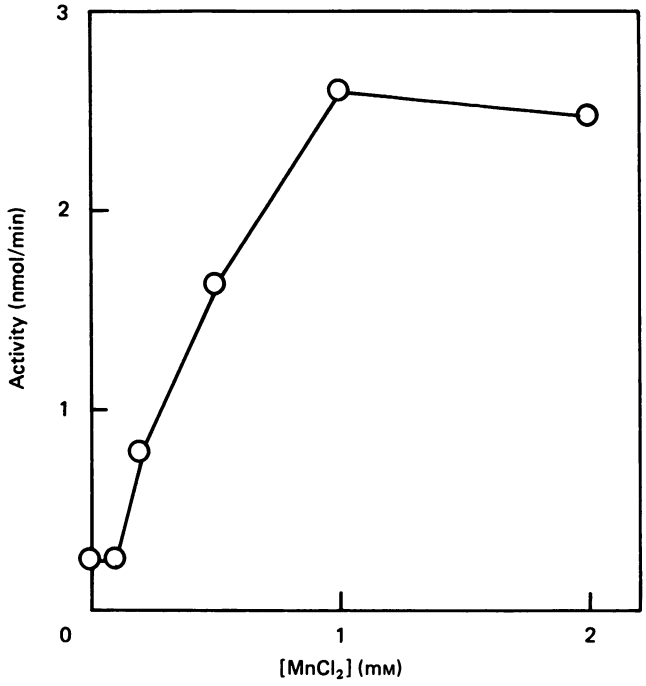


Fig. 5. Continuation of Mn^{2+}-concentration-dependent activation of much less active UGL after removal of free Mn^{2+}

Much less active UGL fraction (M_r 120000) after gel filtration on Sephacryl S-200 (Fig. 5) was used. After the buffer was changed to 10 mM-Tris/HCl (pH 7.5)/10% glycerol by gel filtration on Sephadex G-25 equilibrated with this buffer, 20 μl of $MnCl_2$ solution was added to 80 μl of the enzyme solution to give a final $MnCl_2$ concentration of 0–2 mM and incubated for 5 min at room temperature. Free Mn^{2+} was then removed from the mixture by the centrifuge column procedure of Penefsky (1977). A small column of Sephadex G-50 (superfine grade) in a 1 ml syringe was equilibrated with 10 mM-Tris/HCl (pH 7.5)/10% glycerol and centrifuged at 100 *g* for 3 min. The mixture (90 μl) was transferred to the top of the column and centrifuged at 100 *g* for 5 min. A 25 μl aliquot of the effluent was used for assay. The recovery of the activity during the column centrifugation was 85±4%.

Trijbels & Vogels, 1966; Choi *et al.*, 1968; Vogels & Van der Drift, 1970) and the enzymic degradation by sardine UGL, the product of the allantoicase reaction was suggested to be *S*(−)-ureidoglycollate. (With the mackerel enzyme the reaction was not fully proportional to the protein added, presumably because over 20% of the substrate was consumed during incubation.) It is not clear whether the small amount of free glyoxylate found was from non-enzymic or enyzmic (due to contaminating UGL) degradation of ureidoglycollate. Hydrolysis of ureidoglycollate at a significant rate in buffer solutions has been reported (Choi *et al.*, 1968; Trijbels & Vogels, 1966). These findings suggested that allantoate is degraded in a two-step reaction to urea and glyoxylate with *S*(−)-ureidoglycollate as an intermediate and UGL is essential in the peroxisomal urate degradation.

UGL has been reported to be present in various micro-organisms and frog liver (Vogels & Van der Drift, 1976; Trijbels & Vogels, 1969). There are, however, few reports on the characterization of the highly purified enzyme. These enzymes are slightly stimulated by Mn^{2+}. The present fish liver enzyme required bivalent metal ions, particularly Mn^{2+}, for its activity. Preparation of much less active enzyme, its marked activation by bivalent metal ions and binding of Mn^{2+} to the apoenzyme suggested that it is a metalloenzyme. It is, however, not clear whether Mn^{2+} is bound to it *in vivo*. The present study suggested that UGL may be the fourth component of the urate-degradation pathway in fish liver peroxisomes. To elucidate the further function of peroxisomes in urate metabolism, it will be necessary to examine whether other enzymes involved in microbial urate metabolism are also present in fish liver peroxisomes.

Table 3. Degradation of the products of mackerel and sardine liver allantoicase reactions by alkali treatment, boiling and sardine liver UGL

Mackerel and sardine liver allantoicase were incubated in 50 mM-potassium allantoate/0.1 M-sodium citrate, pH 6.5, at 37 °C for 1 and 3 h respectively. After incubation, the reaction mixtures were deproteinized by centrifugation at 15000 *g* for 10 min (only with mackerel enzyme) and ultrafiltration with Centricon-10 at 4 °C. A 10 and a 20 μl portion of each filtrate were adjusted to 100 μl with 0.1 M-sodium citrate, pH 6.5, and used for each treatment. Glyoxylate formed was linearly proportional to the amount of the filtrate used. For boiling treatment, the filtrate was heated in a boiling-water bath for 7 min and rapidly cooled to room temperature. Glyoxylate formed was determined after addition of 0.85 ml of 0.1 M-triethanolamine/HCl, pH 8.2. Alkali and UGL treatments and determination of glyoxylate were as described in the text and legend of Table 2. Each value represents the mean of two determinations. Abbreviation: N.D., not detected.

		Glyoxylate formed (μmol)			
		Mackerel allantoicase		Sardine allantoicase	
Treatment	Enzyme... None	2.25 mg	4.5 mg	0.43 μg	0.86 μg
Alkali	N.D.	7.67	12.3 (100%)	5.75	10.4 (100%)
Sardine UGL	N.D.	7.87	12.9 (105%)	4.42	8.66 (83.4%)
Boiling*	–	2.98	6.42 (52.1%)	3.36	7.69 (64.7%)
None	N.D.	0.62	0.88 (7.1%)	0.51	1.02 (9.8%)

* Glyoxylate, formed presumably by non-enzymic degradation of allantoate (0.97 μmol) during boiling, was subtracted from each value.

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