Structure and Properties of Arginase from the Polychaete Annelid *Pista pacifica* Berkeley

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(Received 7 June 1974)

An oligomeric arginase with a molecular weight of 205 000 is present in the intestinal tissues of the polychaete annelid *Pista pacifica*. The presence of an active subunit with a molecular weight of 34 000 was demonstrated. The enzyme specificity, effect of thiol reagents on activity, kinetic properties of the intact enzyme and the active subunit were investigated. Treatment of the arginase with EDTA results in its dissociation into an inactive subunit; the active oligomeric structure can be regenerated by addition of Mn^{2+} . The correlation of characteristics of arginase from a certain species and that species, mode of nitro-telism is discussed.

Arginase (EC 3.5.3.1, L-arginine ureohydrolase), once thought to be present only in ureotelic species (Clementi, 1937), is widely distributed throughout the animal kingdom. Significant differences in arginases from ureotelic, uricotelic and ammonotelic organisms have been observed with respect to molecular weights, K_m values, inhibition by *p*-chloromercuribenzoate and stability during dialysis (Mora *et al.*, 1965, 1966; Soru, 1965; Brown, 1966; Soberón *et al.*, 1967; Reddy & Campbell, 1968; Rossi & Grazi, 1969; Middlehoven, 1969; Hirsch-Kolb *et al.*, 1971). Recent studies, however, indicate that there is no known correlation between the characteristics of arginase and a species' excretory nitrogen metabolism (Reddy & Campbell, 1970; Hartenstein, 1971).

The only annelid arginase characterized so far is that of the terrestrial oligochaete, *Lumbricus terrestris* (Reddy & Campbell, 1968). This arginase is unique in that it has a molecular weight of 27 000 whereas the molecular weights of all other arginases so far investigated are about 130 000 or 260 000 (Reddy & Campbell, 1968, 1970). The marine polychaete *Pista pacifica* contains an arginase in its tentacles, body wall and gut tissues (K. L. O'Malley, unpublished work). Unlike the monomeric enzyme of *L. terrestris*, the arginase of *P. pacifica* is oligomeric. Properties of the enzyme presented in this paper include: molecular weights of enzyme and subunits, enzyme specificity, effect of thiol reagents on enzyme activity, the presence and kinetic properties of an active subunit, and the reversible Mn^{2+} -dependent dissociation of the enzyme into subunits.

Experimental

Materials

All chemicals and reagents used in this study were purchased from Sigma Chemical Co., St. Louis,

Mo., U.S.A., except for the phenol reagent (Fisher Chemical Co., Pittsburgh, Pa., U.S.A.) and Blue Dextran 2000 (Pharmacia Fine Chemicals AB, Uppsala, Sweden).

Methods

Enzyme source and assay. *P. pacifica* Berkeley was collected from mudflats near Charleston, Oreg., U.S.A. Only recently collected animals were used for tissue preparations.

Preliminary tissue homogenates were prepared in 50 mM-Tris-HCl (pH 7.5) containing 100 mM-KCl and 50 mM-MnCl₂. Unless otherwise stated the standard assay medium used at 25°C contained 0.85 M-arginine adjusted to pH 9.5 with 1 M-HCl, 5 mM-MnCl₂, 0.5 M-sodium glycinate buffer adjusted to pH 9.5 with 1 M-NaOH, and a sample of the enzyme in a final volume of 1 ml. The assay was stopped after 10 min with 5 ml of 0.5 M-HClO₄ and urea determined as described by Archibald (1945). One enzyme unit is defined as the amount of enzyme that produces 1 μ mol of urea/min. Specific activity is expressed in enzyme units/mg of protein. Protein was measured by the procedure of Lowry *et al.* (1951) as modified by Linn & Lehman (1965) with bovine serum albumin as a standard.

Disc gel electrophoresis. *P. pacifica* arginase gives a sharp band after electrophoresis in a 3% (w/v) polyacrylamide gel and therefore running gels of 3% polyacrylamide were prepared as described by Swaney & Klotz (1971). Stacking gels were prepared as described by Davis (1964) with the Tris buffer adjusted to pH 7.5. Samples (100 μ g) were dissolved in large-pore solution and photochemically polymerized on top of the stacking gel. Electrophoresis was performed in Tris-glycine buffer (6 g of Tris, 28.8 g of

glycine, water to 1 litre, pH 9.0) for about 30 min with a current of 5 mA/tube. After removal from the tubes, gels were stained with 1% (w/v) Amido Schwartz in 7% acetic acid (20°C) and destained with frequent changes of 7% (v/v) acetic acid.

Electrophoresis in sodium dodecyl sulphate. Electrophoresis was carried out by the method of Shapiro *et al.* (1967) modified for a 3% (w/v) polyacrylamide gel. Samples and standards (1 mg/ml) were dissolved in a 1% (w/v) sodium dodecyl sulphate solution and 0.01 M-sodium phosphate buffer, heated to 100°C for 3 min, and then incubated for 2 h at 37°C. Electrophoresis was performed at a constant current of 8 mA/gel for 4 h. The gels were removed from their tubes and stained with Coomassie Brilliant Blue [1.25 g in 454 ml of 50% (v/v) methanol and 46 ml of acetic acid] for 10 h at 20°C. Gels were then destained by frequent changes of destaining solution [7.5% (v/v) acetic acid and 5% (v/v) methanol].

Molecular weight. The molecular weight of *P. pacifica* arginase was estimated by gel filtration on Sephadex G-200-120 at 15°C as described by Andrews (1965). The column (1.8 cm × 90 cm) was equilibrated with 0.01 M-Tris-HCl buffer (pH 7.5), and eluted with the same buffer at a flow rate of 20 ml/h. Pyruvate kinase (mol.wt. 237 000), γ -globulin (mol.wt. 150 000), catalase (mol.wt. 195 000), rabbit muscle aldolase (mol.wt. 160 000), bovine serum albumin (mol.wt. 71 000), ovalbumin (mol.wt. 41 000), and α -chymotrypsinogen A (mol.wt. 25 700) were used as molecular-weight standards. Molecular weights of standards were adjusted according to their behaviour on Sephadex G-200 as described by Andrews (1965). The calibrating proteins were detected in 3 ml fractions by their extinction at 280 nm. Arginase activity was determined by urea formation.

Results

Purification of *P. pacifica* arginase

The scheme outlined below represents several steps described in the purification of rat liver arginase

(Schimke, 1962) and rabbit liver arginase (Vielle-Breitburd & Orth, 1972) adapted for the purification of *P. pacifica* arginase. The purification procedure is summarized in Table 1. Unless otherwise stated all procedures were carried out at 10°C.

Extraction. Several worms were cut open in cold sea-water and the intestines quickly removed, weighed and homogenized in a ground-glass homogenizer with 3 vol. of 0.05 M-Tris-HCl buffer (pH 7.5) containing 0.1 M-KCl and 0.05 M-MnCl₂. The homogenate was centrifuged for 15 min at 13 000g in a Sorvall RC2-B refrigerated centrifuge and the pellet was discarded.

Heat treatment. The supernatant containing the enzyme was incubated at 60°C for 20 min, cooled and centrifuged for 10 min at 13 000g. The pellet was discarded.

Ethanol fractionation. Ethanol (3 vol.) containing 0.05 M-MnCl₂ at -10°C was added to the supernatant. The resulting precipitate was removed by centrifugation for 10 min at 15 000g at -10°C. The pellet was then completely redissolved by stirring gently in a small amount of 0.01 M-Tris-HCl buffer (pH 9.0) prepared by the method of Long (1961) with all solutions at 10°C, and dialysed overnight against the same buffer.

DEAE-cellulose chromatography. The dialysed solution was placed on a DEAE-cellulose column (1.8 cm × 10 cm) equilibrated with 0.01 M-Tris-HCl (pH 9.0). The column was washed with 2 column volumes of the equilibration buffer. Enzyme elution was performed with a linear gradient of NaCl (0.0–0.4 M) in the same buffer. The activity was eluted over a narrow range of molarity (at approx. 0.2 M) in a single peak. No additional arginase activity was eluted by washing the column with the same buffer containing 1 M-NaCl. The active fractions were pooled and freeze-dried.

Chromatography on Sephadex G-200. Freeze-dried enzyme was redissolved in approx. 2 ml of 0.01 M-Tris-HCl (pH 9.0) and chromatographed on a column (1.8 cm × 90 cm) of Sephadex G-200 equilibrated with the same buffer. The enzyme was eluted as a single

Table 1. Purification of *P. pacifica* arginase

Increase in specific activity of *P. pacifica* arginase due to purification procedures is listed below. Arginase activity and total protein were measured as described in the text.

Procedure	Vol. (ml)	Total activity (units)	Total protein (mg)	Yield (%)	Specific activity (units/mg)
Extract	10.0	25.0	310.2	100	0.08
Extract supernatant	9.0	23.6	21.9	94	1.1
Heat	8.5	17.3	7.7	69	2.2
Ethanol	2.0	16.5	1.6	66	10.3
DEAE-cellulose*	5.0	16	0.5	64	32.0
Sephadex G-200	20.0	15.8	0.2	63	79.0

* After freeze-drying and redissolution.

symmetrical peak. The active fractions were pooled and used as the enzyme source in subsequent experiments.

The specific activity of the enzyme after chromatography on Sephadex G-200 was about 1000-fold greater than the specific activity of the crude extract. When active fractions were freeze-dried and electrophoresed on 3% (w/v) polyacrylamide gels at pH 9.0, a single band is obtained.

pH optimum

A pH optimum of 10.5 was observed in four different experiments. The enzyme was assayed in 0.5M-Tris-HCl buffer between pH values 7 and 9; 0.5M-sodium glycinate was used as buffer between pH values 9 and 12. Although the optimum pH was 10.5, assays were conducted at pH 9.5 in this study so that results would be comparable with other reported data.

Specificity

Agmatine sulphate, D-arginine, L-arginine, arginine acid, L-canavanine, γ -guanidinobutyric acid, and β -guanidinopropionic acid were tested as potential substrates. Only two, L-arginine and L-canavanine, were hydrolysed to urea at a measurable rate. The ratio of arginase/canavanase activity was about 22 at pH 9.5.

Substrate kinetics

The apparent K_m value for L-arginine was 155–160mM in several experiments with both purified and crude extracts. As shown in Fig. 1 there was no indication of inhibition by high substrate concentrations.

On the same graph L-ornithine (100mM) appears to be a competitive inhibitor. Hill plots for both enzyme and enzyme plus inhibitor at the concentrations shown yield h values of 1.034 and 1.040 respectively where h equals the slope of $\log[V_0/(V_{max} - V_0)]$ versus $\log[S]$.

Effect of temperature

The effect of temperature on K_m values was examined at 10°, 15°, 25°, 37° and 45°C. The Michaelis constants were essentially unaltered over this temperature range (Table 2).

The effect of temperature on arginase activity was determined between 0° and 70°C. The temperature optimum is approx. 60°C. Fig. 2 shows a plot of $\log v$ versus $1/T$ for temperatures between 0° and 60°C. The substrate concentration used for these assays was 0.85M. At this concentration 95–100% of the calculated maximum velocity was obtained. From Fig. 2 the energy of activation (E) was calculated

Fig. 1. Kinetic behaviour of *P. pacifica* arginase

A double-reciprocal plot of $1/V_0$ versus $1/[S]$ for both enzyme and enzyme plus inhibitor. The reaction mixture contained the concentration of arginine indicated plus 0.1 ml of purified enzyme. ●, Enzyme kinetics without ornithine; ▲, enzyme activity in the presence of 0.1 ml of 100mM-ornithine.

Table 2. K_m values for L-arginine between 10° and 45°C

K_m values were average values of four experiments. The apparent K_m values for L-arginine were determined from a Lineweaver-Burk plot of $1/V_0$ versus $1/[S]$. K_m values were calculated from the relationship, $K_m = \text{slope} \times V_{max}$.

Temperature (°C)	K_m (mM)
10	160
15	153
25	155
37	162
45	150

from slope = $-E/4.576$. Energy of activation was found to be 34 kJ/mol (8.09 kcal/mol).

Effect of thiol compounds

Either cysteine, dithiothreitol, GSH or 2-mercaptoethanol (5 μ mol in each case) was added to the assay medium and the effect noted. The reaction was initiated with 0.1 ml of purified enzyme (1 mg/ml) and incubated for 1h. Standards were prepared in the presence of the same amount of thiol compound and used for colorimetric correction. All thiol compounds tested had a stimulatory effect on *P. pacifica* arginase.

Fig. 2. Plot of $\log v$ versus $1/T$ ($^{\circ}\text{K}^{-1}$) from which the energy of activation for *P. pacifica* arginase was obtained. Purified enzyme (0.2 ml; 1 mg/ml) was used in the assay medium equilibrated to the appropriate temperatures. Arginase activity was determined colorimetrically by measuring E_{540} .

2-Mercaptoethanol showed the greatest stimulatory effect, the arginase activity being 3.5 times greater than with no thiol compound.

The effect of *p*-hydroxymercuribenzoate on this arginase depends on experimental conditions. When the enzyme was preincubated for 30 min at 25°C with 5 mM-*p*-hydroxymercuribenzoate and then added to the assay medium, the same amount of urea was produced as when the enzyme was preincubated with an equivalent amount of buffer. However, when the enzyme plus *p*-hydroxymercuribenzoate were incubated for 30 min in the principle assay medium, a slight increase in enzyme activity was noted.

Presence of an active subunit

The native enzyme dissociated under the following conditions: acetone fractionation at -10°C ; chromatography on CM-cellulose in 0.01 M-Tris-HCl (pH 7.5); freeze-drying followed by rechromatography on a Sephadex G-200 column in Tris-HCl buffer (pH 9.0). Fractions in the latter case included two activity peaks, one corresponding to the high-molecular-weight unit and the other to a molecular weight of about 34000. When the peak fraction of the lower molecular weight was rechromatographed on the same column there was no evidence of aggregation (Fig. 3). Active subunit fractions were pooled and freeze-dried. Freeze-dried subunit (2 mg/ml) migrated as a single electrophoretic component on a 3% (w/v) polyacrylamide gel at pH 9.0.

Fig. 3. Appearance of subunit on Sephadex G-200

(a) Breakdown of the enzyme after redissolution in buffer after freeze-drying; (b) rechromatography of the subunit from (a) showing the absence of aggregation. ●, E_{280} ; ▲, arginase activity. V_e is the elution volume.

K_m value of subunit

Values of 114–118 mM were obtained for the K_m value of the arginase subunit from a double-reciprocal plot of $1/V_o$ versus $1/[S]$. A Hill plot indicated an h value of 1.07.

Effects of manganese

P. pacifica arginase is active in the absence of Mn^{2+} in the assay buffer (Table 3); preincubation at 37° or 55°C with 5 mM- MnCl_2 increased enzyme activity by less than 3%.

The purified enzyme, however, can be inactivated by incubation with 30 mM-EDTA in 0.01 M-Tris-HCl buffer (pH 9.0) for 1 h at 37°C followed by dialysis against the same buffer containing 5 mM-EDTA at 10°C . Under these conditions no enzymic activity can be detected in a Mn^{2+} -free assay buffer. Activity can be restored by incubation of the enzyme with 5 mM- MnCl_2 for 30 min at 37°C . The amount of activity which can be regenerated depends on the length of dialysis time after inactivation, as well as enzyme concentration. After 4 days of dialysis against EDTA, dilute samples of enzyme (0.1 mg/ml) show a 40% loss of their activity despite being assayed in

Table 3. Effect of exogenous Mn^{2+} on *P. pacifica* arginase activity

Purified enzyme (0.1 ml; 1 mg/ml) and 0.1 ml of subunit from dissociated Sephadex G-200 enzyme were incubated for 30 min in the principle assay system except that the Mn^{2+} concentration was varied as indicated.

[Mn^{2+}] (mM)	Urea (μ mol/30 min)	
	Native enzyme	Subunit
0	0.31	0.37
0.025	0.31	0.37
0.050	0.31	0.39
0.100	0.32	0.37
0.500	0.30	0.36
1.000	0.31	0.39
2.500	0.31	0.38
5.000	0.31	0.37

 Fig. 4. EDTA inactivation of *P. pacifica* arginase

Purified enzyme (0.1 mg/ml) was incubated for 1 h with 30 mM-EDTA and then dialysed for 1–4 days against 0.01 M-Tris-HCl buffer (pH 9.0) containing 5 mM-EDTA, with daily changes of the dialysis buffer. Portions were taken each day and tested for activity in the standard assay medium containing either 5 mM-EDTA (■), or 5 mM- $MnCl_2$ (▲). ●, Arginase dialysed against 0.01 M-Tris-HCl buffer not containing EDTA. The fully Mn^{2+} -activated original sample was taken as 100%.

the presence of Mn^{2+} (Fig. 4). Concentrated samples (1 mg/ml), on the other hand, seem to lose no activity after dialysis in the same manner.

When the enzyme was inactivated by incubation with 30 mM-EDTA in 0.01 M-Tris-HCl buffer (pH 9.0) for 1 h at 37°C and dialysed for 10 h against the same buffer containing 5 mM-EDTA at 10°C, a molecular weight of 93 000 was obtained on Sephadex G-200. After 4 days of dialysis against EDTA the molecular weight was 34 000. Concentrated samples of EDTA-treated enzyme (1 mg/ml) did not dissociate even after 4 days of dialysis.

The reversible Mn^{2+} -dependent dissociation of *P. pacifica* arginase was also investigated. EDTA-treated enzyme (0.1 mg/ml, after 4 days of dialysis as described above) which had a molecular weight of 34 000 on Sephadex G-200, was incubated with 5 mM- $MnCl_2$ in 0.01 M-Tris-HCl (pH 7.5) for 30 min at 37°C. Rechromatography of this sample on Sephadex showed a single active enzyme fraction corresponding to the molecular weight of the untreated molecule (205 000). This indicates that the observed reactivation was accompanied by reassociation of subunits.

Sodium dodecyl sulphate-polyacrylamide-gel electrophoresis

P. pacifica arginase migrated as a single band during gel electrophoresis in sodium dodecyl sulphate. When the logarithm of the molecular weight is plotted versus the relative mobility a molecular weight of 36 000 is obtained.

Discussion

A highly purified arginase from rat liver has a molecular weight of 118 000 (Schimke, 1962; Hirsch-Kolb & Greenberg, 1968). In 8 M-urea rat liver arginase dissociates into subunits of 30 800 molecular weight (Hirsch-Kolb & Greenberg, 1968). This value is similar to the subunit molecular weight of bovine liver arginase (30 000) (Hirsch-Kolb *et al.*, 1970) and human liver arginase (30 000) (Carvajal *et al.*, 1971). Molecular-weight data are consistent with a tetrameric arrangement for these enzymes. Arginases with molecular weights between 118 000 and 160 000 have been found primarily among ureotelic species. Molecular weights ranging from 228 000 to 276 000, corresponding to reputed octameric arrangements, have been found for arginases from uricotelic species (Reddy & Campbell, 1970). However, a ureotelic land planarian shows an arginase with a molecular weight of 238 000 (Reddy & Campbell, 1970; Campbell, 1966) and a uricotelic seagull has a liver arginase with a molecular weight of 134 000 (Reddy & Campbell, 1970). The earthworm is unique in possessing a monomeric arginase with a molecular weight (27 000) slightly lower than the molecular weight of mammalian enzyme subunits (Reddy & Campbell, 1968).

The molecular weight of *P. pacifica* arginase (205000) does not closely resemble the molecular weights of other arginases. The subunit has a molecular weight of about 34000–36000 as determined by gel chromatography and electrophoresis in sodium dodecyl sulphate. This value is slightly higher than the native molecular weight of earthworm arginase as well as the subunit values of rat, bovine and human liver arginases. However, rabbit liver arginase has been shown to have a molecular weight of 110000 with a subunit molecular weight of 36500 (Vielle-Breitbart & Orth, 1972; Rogers & Moore, 1963), a value which more closely resembles *P. pacifica* arginase. It is apparent that the molecular-weight values obtained for this enzyme indicate an oligomeric structure. However, the data do not fit the tetrameric or octameric arrangements proposed for other species; perhaps a hexameric assemblage would be more consistent. Obviously the definitive quaternary structure will require more precise molecular-weight data.

Rat liver arginase is reported to be homogeneous and composed of a single type of subunit (Schimke, 1962; Carvajal *et al.*, 1971). Rabbit liver arginase, on the other hand, appears to be composed of several isoenzymes and two different subunits (Vielle-Breitbart & Orth, 1972). Two isoenzymes were described for human liver (Bascur *et al.*, 1966) and other mammalian arginases (Poremska *et al.*, 1971). *P. pacifica* arginase appears to be homogeneous and composed of a single subunit.

The high K_m value obtained for *P. pacifica* is in the range of the values (>100mM) found for chicken liver and lizard liver arginases (Mora *et al.*, 1965, 1966). As with chicken liver arginase, high concentrations of arginine did not inhibit *P. pacifica* arginase. Competitive inhibition by L-ornithine is characteristic of arginase from *P. pacifica* as well as chicken liver, *Neurospora crassa* (Mora *et al.*, 1966) and silkworm (Reddy & Campbell, 1969).

Thiol compounds have variable effects on arginases from different sources although experimental conditions may partially account for the differing results. As illustrated by *P. pacifica* arginase, the effect of thiol groups on enzyme activity depends on whether the enzyme is incubated with the thiol-group inhibitor *p*-hydroxymercuribenzoate before the addition of arginine or whether the enzyme is added directly to a solution of arginine plus *p*-hydroxymercuribenzoate. When bullfrog and bovine arginases (Carlisky & Sadnik, 1972) are preincubated with GSH, dithiothreitol, 2-mercaptoethanol or cysteine, these thiol-containing reagents have a stimulatory effect whereas *p*-chloromercuribenzoate has an inhibitory effect. *P. pacifica* arginase is unaffected by preincubation with *p*-hydroxymercuribenzoate. When the enzyme is not preincubated with GSH, dithiothreitol, 2-mercaptoethanol or cysteine, these reagents stimulate

P. pacifica arginase activity. These same compounds were slightly inhibitory in the case of insect arginase (Reddy & Campbell, 1969). The activity toward L-arginine is inhibited by *p*-chlorohydroxymercuribenzoate in the crayfish (Hartenstein, 1971), chicken liver (Mora *et al.*, 1966) and cockroach fat-body (Reddy & Campbell, 1969). Rat liver (Mora *et al.*, 1966) and silkworm (Reddy & Campbell, 1969) are unaffected. Under the same conditions, *P. pacifica* arginase was slightly stimulated by *p*-hydroxymercuribenzoate.

The arginases present in the livers of uricotelic and ureotelic animals have a similar substrate specificity. All hydrolyse L-arginine and to some extent L-canavanine (Mora *et al.*, 1965). *Helix pomatia* and *Helix aspersa* also hydrolyse homoarginine (Baret *et al.*, 1972). Arginase/canavanase ratios at pH9–10 for reported species have no apparent pattern among ureoteles and uricoteles. High ratios (>10) as obtained for *P. pacifica* arginase have been reported for rat liver (Mora *et al.*, 1966), bovine liver (Brown, 1966) and silkworm (Reddy & Campbell, 1969).

Few temperature optima have been reported. The arginase of the sockeye salmon has a temperature optimum of 45°C (Cvancara, 1971), *H. pomatia* and *H. aspersa* between 60° and 65°C (Baret *et al.*, 1972), and horse liver arginase has a temperature optimum of 45–50°C (Greenberg & Mohamed, 1945). The temperature optimum of *P. pacifica* is about 60°C.

The requirement for Mn^{2+} as a metallic cofactor seems to be common for most reported arginases although other bivalent cations, Co^{2+} , Fe^{2+} or Ni^{2+} , may also be activators (Greenberg & Mohamed, 1945; Middlehoven, 1969; Hirsch-Kolb & Greenberg, 1968). Some investigators have found that binding of Mn^{2+} to the enzyme seems to vary for arginases isolated from different species. Hirsch-Kolb & Greenberg (1968) reported that 4 molecules of manganese were bound per molecule of activated rat liver arginase although the binding of the metal ions was not identical; two ions were loosely bound and their removal caused a 50% loss in activity. The remaining ions were tightly bound and removal caused irreversible inactivation. However, rabbit liver arginase (Vielle-Breitbart & Orth, 1972) and human liver arginase (Carvajal *et al.*, 1971) apparently bind each Mn^{2+} with an equal affinity such that removal results in the dissociation of the enzyme. In the case of human liver arginase the addition of Mn^{2+} causes a reassociation of the enzyme (Carvajal *et al.*, 1971). Similarly in *P. pacifica* arginase removal of Mn^{2+} ions by EDTA resulted in the dissociation of the enzyme into subunits. That Mn^{2+} or some other bivalent cation is required for quaternary structure is indicated by the aggregation of the EDTA-inactivated subunit in the presence of $MnCl_2$ to re-form the oligomeric protein. Presumably the lack of aggregation indicated in Fig. 3 is due

to the lack of Mn^{2+} ions in the eluting buffer. Mn^{2+} -deficient assay buffer did not seem to effect the enzymic activity of either the native enzyme or the subunit (Table 3); the presence of a chelator was necessary before an effect was noticeable. It may be that Mn^{2+} ions are tightly bound to *P. pacifica* arginase or that other bivalent ions would be more effective. This was not investigated.

In a search to categorize arginases with respect to modes of nitrogen excretion, reports on arginases of ureo-, ammono-, and urico-teles served more to point out the disparities rather than the similarities among arginase properties. The information presented in this paper on an arginase from a polychaete worm, together with other data (Reddy & Campbell, 1970; Hartenstein, 1971), further emphasizes the fact that none of the characteristics of arginase observed to date is consistent with a particular mode of nitrotelism.

We greatly appreciate the advice and assistance of Dr. Leonard Simpson. This work was supported by N.S.F. Research Grant No. GB-34292 to R. C. T.

References

- Andrews, P. (1965) *Biochem. J.* **96**, 595-606
- Archibald, R. M. (1945) *J. Biol. Chem.* **157**, 507-518
- Baret, R., Girard, C. & Riou, J. (1972) *Biochimie* **54**, 421-430
- Bascur, V., Cabello, J., Véliz, M. & González, A. (1966) *Biochim. Biophys. Acta* **128**, 149-154
- Brown, G. W., Jr. (1966) *Arch. Biochem. Biophys.* **114**, 184-194
- Campbell, J. W. (1966) *Comp. Biochem. Physiol.* **18**, 179-189
- Carlisky, N. J. & Sadnik, I. L. (1972) *Comp. Biochem. Physiol.* **41B**, 785-792
- Carvajal, N., Venegas, A., Oestreicher, G. & Plaza, M. (1971) *Biochim. Biophys. Acta* **250**, 437-442
- Clementi, A. (1937) *Enzymologia* **4**, 205-216
- Cvancara, V. A. (1971) *Comp. Biochem. Physiol.* **40B**, 819-822
- Davis, B. (1964) *Ann. N.Y. Acad. Sci.* **121**, 404-427
- Greenberg, D. M. & Mohamed, M. S. (1945) *Arch. Biochem.* **8**, 365-375
- Hartenstein, R. (1971) *Comp. Biochem. Physiol.* **40B**, 781-795
- Hirsch-Kolb, H. & Greenberg, D. M. (1968) *J. Biol. Chem.* **243**, 6123-6129
- Hirsch-Kolb, H., Heine, J. P., Kolb, H. J. & Greenberg, D. M. (1970) *Comp. Biochem. Physiol.* **37**, 345-359
- Hirsch-Kolb, H., Kolb, H. & Greenberg, D. M. (1971) *J. Biol. Chem.* **246**, 395-401
- Linn, S. & Lehman, I. R. (1965) *J. Biol. Chem.* **240**, 1287-1293
- Long, C. (1961) *Biochemists' Handbook*, p. 33, Van Nostrand, Princeton
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265-275
- Middlehoven, W. J. (1969) *Biochim. Biophys. Acta* **119**, 110-121
- Mora, J., Tarrab, R., Martuscelli, J. & Soberón, G. (1965) *Biochem. J.* **96**, 588-594
- Mora, J., Tarrab, R. & Bojalil, L. F. (1966) *Biochim. Biophys. Acta* **118**, 206-209
- Poremska, Z., Baranczyk, A. & Jachimowicz, J. (1971) *Acta Biochim. Pol.* **18**, 77-85
- Reddy, S. R. R. & Campbell, J. W. (1968) *Biochim. Biophys. Acta* **159**, 557-560
- Reddy, S. R. R. & Campbell, J. W. (1969) *Comp. Biochem. Physiol.* **28**, 515-534
- Reddy, S. R. R. & Campbell, J. W. (1970) *Comp. Biochem. Physiol.* **32**, 499-509
- Rogers, S. & Moore, M. (1963) *J. Exp. Med.* **117**, 521-545
- Rossi, N. & Grazi, G. (1969) *Eur. J. Biochem.* **7**, 348-352
- Schimke, R. T. (1962) *J. Biol. Chem.* **237**, 459-468
- Shapiro, A. L., Vinuela, E. & Maizel, J. V. (1967) *Biochem. Biophys. Res. Commun.* **28**, 815-824
- Soberón, G., Ortiz-Pineda, J. & Tarrab, R. (1967) *Nat. Cancer Inst. Monogr.* **27**, 823-295
- Soru, E. (1965) *J. Chromatogr.* **20**, 325-333
- Swaney, J. B. & Klotz, I. M. (1971) *Arch. Biochem. Biophys.* **147**, 475-486
- Vielle-Breitbart, F. & Orth, G. (1972) *J. Biol. Chem.* **247**, 1227-1235