The first committed step in the degradation of L-arginine in *Saccharomyces cerevisiae* is catalyzed by arginase (EC 3.5.3.1). Arginase catalyzes the hydrolysis of L-arginine to L-ornithine and urea. In yeast, arginase and ornithine transcarbamoylase (EC 2.1.3.3), which catalyzes the first committed step in the biosynthesis of arginine, are both present in the cytoplasm. Thus, in the presence of the active site ligands for ornithine transcarbamoylase and arginase, there is the potential for the coupling of the two pathways into a futile urea cycle resulting in the uncontrolled hydrolysis of ATP. To avoid this, arginase and ornithine transcarbamoylase form a multienzyme complex in which ornithine transcarbamoylase activity is inhibited while arginase remains active. Formation of the multienzyme complex results in the uncoupling of the two pathways and the elimination of the wasteful hydrolysis of ATP. This system was first characterized by Wiame and co-workers (Bechet and Wiame, 1965; Messenguy and Wiame, 1969; Wiame, 1971; Penninckx et al., 1974; Penninckx, 1975; Penningkx and Wiame, 1976) and has subsequently been studied in our laboratory (Eisenstein et al., 1984; Eisenstein, 1985; Duong et al., 1986; Eisenstein et al., 1986; Hensley, 1988).

An analysis of the three-dimensional structure of ornithine transcarbamoylase and arginase by electron microscopy demonstrated that both enzymes are trimers (Duong et al., 1986). These studies also showed that in the multienzyme complex, the two enzymes associate face-to-face with a one-to-one stoichiometry. Furthermore, equilibrium sedimentation studies demonstrated complex formation in solution, yielding a $K_0$ of 23 nM, indicating that ornithine transcarbamoylase and arginase are tightly associated in the complex (Eisenstein et al., 1986). Although much is known about the structure and function of yeast ornithine transcarbamoylase and ligand-binding-promoted conformational changes in this enzyme which are required for complex formation (Eisenstein et al., 1984; Eisenstein, 1985; Eisenstein and Hensley, 1986; Eisenstein et al., 1986), less is known about the structural and enzymatic properties of yeast arginase.

The enzymatic activity of arginase from a number of sources has been shown to be manganese-dependent (Hirsch-Kolb and Greenberg, 1968; Hirsch-Kolb et al., 1970, 1971; Carvajal et al., 1977; Munakata et al., 1976; Fujimoto et al., 1976; Stewart and Caron, 1977; Garganta and Bond, 1986). Preliminary evidence from our laboratory, using sedimentation equilibrium, suggested that release of weakly associated manganese simply by dialysis resulted in the reversible loss of enzymatic activity without a change in the quaternary structure of arginase. However, dialysis of arginase against chelating agents appeared to cause complete dissociation of the trimeric quaternary structure into monomers. It was of interest, therefore, to investigate the influence of metal ions on
the catalytic activity of arginase, as well as assess their impact on the tertiary and quaternary structure of the enzyme and determine their role, if any, in multienzyme complex formation.

To establish the role of metals in the structure and catalytic function of arginase, a rapid and reproducible protocol was developed that enabled the purification of milligram quantities of enzyme. Results presented in this study suggest that there may be two types of metals associated with yeast arginase. It is proposed that one type, presumably manganese, which is characterized by its relative ease of removal, is essential for catalysis and may play a small but significant role in stabilizing the quaternary structure of arginase. The second type of metal, whose identity has yet to be established, may be involved in stabilizing the tertiary and quaternary structure of arginase, since upon its release, large changes in circular dichroism and sedimentation coefficient relative to the native enzyme are observed.

**EXPERIMENTAL PROCEDURES**

**RESULTS**

Purification of Yeast Arginase—Arginase was purified approximately 100-fold from the protease-deficient, arginase-overproducing strain of *S. cerevisiae* (22321). Fig. 1 shows the elution profile of arginase from the Green A-Sepharose column. Following a large breakthrough, arginase is eluted with a salt gradient at approximately 0.10 M NaCl. The specific activity is represented by the filled squares and runs with the first major peak following the breakthrough. The pooled fractions are denoted by the closed bar. Fig. 2 shows the elution profile of arginase from the Spherogel TSK-G3000 HPLC gel filtration column. Arginase elutes as a single, narrow peak. The pooled fractions are again denoted by the closed bar. The summary of the purification protocol is presented in Table I. From 50 grams of cells, 10-20 mg of highly purified arginase are obtained. The specific activity ranges from 885 ± 60 units mg⁻¹. Homogeneity of arginase was determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Fig. 3 shows Coomassie Brilliant Blue R 250 stained gels run as described under "Experimental Procedures." Arginase is represented by a single band.

**Spectroscopic Characterization**—The UV absorption spectrum of arginase shown in Fig. 4 is given as specific absorbance versus wavelength based on the extinction coefficient of ε₂₉₇ nm = 0.71 cm⁻¹ mol⁻¹ (Eisenstein, 1985). The spectrum is dominated by the absorbance of the 3 tryptophans/polypeptide chain (Sumrada and Cooper, 1984). Consequently, there is little fine structure observed from the 6 phenylalanines or 11 tyrosines in the amino acid sequence (cf. Wetlaufer, 1962). The fluorescence excitation and emission spectra for arginase are shown in Fig. 5. For the excitation spectrum, the emission was monitored at 337 nm. For the emission spectrum, the excitation was monitored at 295 nm. The emission spectrum has a wavelength maximum of 337 nm. The excitation spectrum has a wavelength maximum of 284 nm.

**Steady-state Kinetics Studies**—Fig. 6 shows the data from steady-state kinetics experiments represented as specific activity versus arginine concentration. The data were analyzed in terms of the Michaelis-Menten equation yielding a *Kₘ* for arginine of 15.7 ± 1.3 mM and a *Vₘₐₓ* of 887.5 ± 30.4 units mg⁻¹. The inset to Fig. 6 shows the specific activity plotted as a function of the log of the arginine concentration. This demonstrates that the Michaelian kinetics are observed over all arginine concentrations.

**Effect of Manganese on the Activity of Arginase**—To determine the role that manganese plays on the activation of arginase, manganese concentration was varied during the heat activation step ("Experimental Procedures"). Fig. 7 is a plot of the specific activity of arginase as a function of the manganese concentration used in the heat activation step. The data are described by a rectangular hyperbola. The activation is half-maximal at a manganese concentration of 12.1 μM, and the enzyme achieves maximum specific activity (845 units mg⁻¹) above 0.6 mM. The inset to Fig. 7 shows the specific activity plotted as a function of the log of the manganese concentration, demonstrating that the hyperbolic function maintains over a wide concentration range.

**Effect of Chelating Agents on the Structure of Arginase**—Velocity sedimentation experiments were undertaken to determine the role of metals on the structure of arginase. The sedimentation studies were done at different rotor speeds, 55,000 rpm for the two samples dialyzed against buffers without chelating agents and 60,000 rpm for arginase incubated with EDTA. The sedimentation coefficients were calculated using nonlinear least-squares analysis with multiple

**TABLE I**

<table>
<thead>
<tr>
<th>Step</th>
<th>Volume</th>
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<th>Amount</th>
<th>Specific activity</th>
<th>Yield</th>
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<tr>
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<td>2,410</td>
<td>16,870</td>
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<td>100</td>
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<td>pH 7.0, heat treatment</td>
<td>170</td>
<td>423</td>
<td>10,025</td>
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<td>7,379</td>
<td>47.0</td>
<td>44</td>
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<tr>
<td>Green A-Sepharose chromatography</td>
<td>67</td>
<td>33</td>
<td>16,170</td>
<td>490</td>
<td>90</td>
</tr>
<tr>
<td>TSK-G3000 gel filtration</td>
<td>8</td>
<td>14.7</td>
<td>9,629</td>
<td>665*</td>
<td>57</td>
</tr>
</tbody>
</table>

* Protein concentration was determined by the method of Kalcik (1947).

*1 unit = 1 μmol of urea formed per mg arginase min⁻¹.

* Specific activity was determined at 25 mM arginine at pH 9.5 in 0.1 M glycine buffer at 30 °C. This assay was used subsequent to a 5-min heat activation of arginase at 45 °C (in the presence of 10 mM MnCl₂).

* Protein concentration was determined from the extinction coefficient for purified arginase, ε = 0.71 cm⁻¹ mol⁻¹ at 279 nm (Eisenstein, 1985).

* As shown in Fig. 6, if the arginine concentration used in the assay were extrapolated to infinite arginine concentration, the specific activity would be approximately 885 units mg⁻¹.
that the spectrum for the fully unfolded enzyme in 6 M guanidinium chloride (open squares) is strikingly different than that observed for arginase in the presence of chelating agents (Fig. 8).

**DISCUSSION**

Previously, we have reported the purification and characterization of ornithine transcarbamoylase (Eisenstein et al., 1984) as well as an architectural study of ornithine transcarbamoylase, arginase, and complex formation (Eisenstein, 1985; Eisenstein and Hensley, 1986; Eisenstein et al., 1986; Duong et al., 1986; Hensley, 1988). For further studies, however, it was important to first investigate the biochemical attributes of arginase and develop a purification protocol that would routinely yield homogeneous material.

The summary of the newly developed purification procedure for arginase from *S. cerevisiae* strain 02232i is given in Table I. The procedure involves two chromatography steps which include ion-exchange and gel filtration columns. The ion-exchange column, Green A-Sepharose, which has been modified from the procedure used by Garganta and Bond (1986) for the purification of rat liver arginase, results in a large increase in specific activity of the enzyme. After a concentration step, an HPLC-gel filtration step is used which enables both high resolution and rapid separation of arginase from the remaining contaminants. Our protocol also takes advantage of the heat stability of arginase at 55 °C as well as the solubility of the enzyme under slightly acidic conditions. Using this protocol, up to 40 mg of highly purified arginase can be obtained from 100 g of plasmid-containing, arginase-overproducing, protease-deficient yeast cells. The high purity of the enzyme isolated from this protocol is demonstrated by polyacrylamide gel electrophoresis under denaturing conditions as shown in Fig. 3. Arginase yields a single band when stained with Coomassie Blue R-250. The band represents 5 μg of purified arginase and is located at approximately 37,000 daltons.

The protease-deficient background is instrumental in yielding an undegraded, purified protein. It was noticed that if manganese was omitted at all during the purification or if EDTA was added at any point in the purification, arginase activity was lost and could not be regained. Thus, two modes of protease inhibition are unavailable: the use of EDTA, a strong inhibitor of metal-requiring lysosomal enzymes and the use of higher pH buffers, because MnCl₂ is oxidized at a higher pH. Therefore, although the strain is protease-deficient, all steps must be carried out as quickly as possible and at 4 °C to ensure an undegraded, purified enzyme.

Yeast arginase has been purified previously by Penninckx et al. (1974) and Eisenstein (1985). However, these protocols would not routinely yield large amounts of homogeneous enzyme required for the studies to determine the roles metal ions play in the maintenance of the catalytic function and the stabilization of the quaternary structure of arginase. Eisenstein (1985) previously established a purification protocol from a protease-deficient, arginase-overproducing strain of *S. cerevisiae*, 1C1931a. This protocol entailed three chromatography steps: an ion-exchange column, an affinity column, and a low pressure gel filtration step. Using this procedure, 2-10 mg of arginase could be purified from 140 g of 1C1931a cells in 1 week. Because strain 02232i expresses larger quantities of active enzyme than strain 1C1931a, we hoped to use previously developed methodology for the purification. Unfortunately, chromatography steps employed previously did not routinely yield homogeneous protein when using 02232i.

The maximal specific activity of arginase purified by this
Purification and Characterization of Yeast Arginase

**FIG. 6. Initial velocity versus arginine concentration.** Initial velocity as a function of arginine concentration was measured in 0.1 M glycine, pH 9.5 and 1 mM MnCl<sub>2</sub> at 30 °C. Arginase concentration was 300 ng ml<sup>-1</sup>. Inset shows the initial velocity versus the log of the arginine concentration.

Protocol is 885 ± 60 units mg<sup>-1</sup>. This is demonstrated in Fig. 6 by the hyperbolic function described by the data of specific activity versus arginine concentration. The value of 885 units mg<sup>-1</sup> is higher than the value of 530 units mg<sup>-1</sup> reported by Eisenstein (1985) for strain 1C1931a and is similar to the value of 865 units mg<sup>-1</sup> determined from the data for yeast arginase by Penninckx <em>et al.</em> (1974).<sup>5</sup> While this latter method produced an enzyme of high specific activity, it yielded quantities of arginase that were too low for a biophysical characterization of both the enzyme and the role of ligands and metals in the maintenance of arginase structure and function as well as in complex formation.

The effect of arginine concentration on the activity of arginase is shown in Fig. 6. The steady-state kinetics for arginine are hyperbolic and yield an apparent $K_m$ for arginine of 15.7 ± 1.3 mM. The apparent Michaelis constants reported for mammalian liver arginases are in the range of 6–20 mM (Hirsch-Kolb <em>et al.</em>, 1970) thus, similar to yeast arginase.

Since there are 3 tryptophans/polypeptide chain in yeast arginase (Sumrada and Cooper, 1984), spectroscopy could be utilized to monitor both ligand- and metal-binding-promoted structural changes in arginase. The ultraviolet absorption spectrum of arginase, as shown in Fig. 4, is dominated by the absorbance of the 3 tryptophans in the polypeptide chain.

**FIG. 7. Urea formation versus manganese concentration.** Urea formation as a function of manganese concentration in the heat activation of arginase was measured in 0.1 M glycine, pH 9.5 at 30 °C in the presence of 25 mM arginine. Inset shows urea formation versus the log of the manganese concentration. Arginase concentration was 300 ng ml<sup>-1</sup>.

---

<sup>5</sup> Penninckx <em>et al.</em> (1974) actually report their value as 53,087 units mg<sup>-1</sup>. However, as discussed by Eisenstein (1985), they report their specific activity in terms of umol urea formed h<sup>-1</sup> rather than micromoles of urea formed min<sup>-1</sup>. Also, the assay of Penninckx <em>et al.</em> (1974) employed a heat activation step of 10 min at 50 °C rather than 5 min at 45 °C and is carried out at pH 9.8 rather than pH 9.5. Furthermore, they determined the concentration of purified arginase by the method of Kalckar (1947), rather than by the molar extinction coefficient at 279 nm, which underestimates the concentration of the enzyme.
The spectrum is characterized by a maximum in absorbance at 279 nm and a shoulder at 290 nm, both typical of tryptophan absorbance. The fluorescence spectra of arginase, shown in Fig. 5, is characterized by an emission maximum of 337 nm, suggesting that the tryptophans in arginase are in a moderately hydrophobic environment. Preliminary results from fluorescence studies suggest that as the metals are released, the environments of the three tryptophans become more polar, and there is a shift to longer lifetimes, consistent with a more polar environment.4

To determine the effect of manganese on the activation of yeast arginase, manganese concentration was varied during the heat activation step of the assay. The half-maximal concentration for the heat activation of arginase by manganese is 12.1 μM with a maximal specific arginase activity of 844.8 ± 32.5 units mg⁻¹. The $V_{max}$ for yeast arginase in terms of both manganese and arginine are similar. Hirsch-Kolb et al. (1970) report that the optimal manganese concentration for mammalian liver arginase activity is 40 mM. We find that such high concentrations of manganese actually inhibit yeast arginase activity by up to 80% depending on the concentration of arginase present during the heat activation step (data not shown). Furthermore, the concentration of manganese normally present in our heat activation (10 mM) also has a inhibitory effect on arginase activity.5

In order to determine the role metal ions play in the stabilization of the tertiary and quaternary structure of yeast arginase, velocity sedimentation experiments were undertaken. Preliminary studies of Eisenstein (1985), using sedimentation equilibrium, suggested that arginase, incubated with EDTA, dissociated fully into monomers similar to the results of Carvajal et al. (1971, 1977). Velocity sedimentation studies of yeast arginase in the presence of exogenous manganese obtained a sedimentation coefficient of 5.95 S, whereas a sedimentation coefficient of 5.92 S is obtained when exogenous metal is removed by dialysis. This excellent agreement indicates no gross structural change ensues upon removal of the weakly associated metal ion. When the enzyme was incubated with EDTA, however, the sedimentation coefficient decreases to 4.65 S. A decrease in the sedimentation coefficient for arginase in the presence of EDTA may result from an increase in effective molecular volume of the enzyme, reflecting an increased frictional coefficient. Alternatively, this decrease in $v$-value could be attributed to a dissociation of the trimeric quaternary structure into constituent monomers. For proteins with similar shape and size to the folded arginase monomer, a sedimentation coefficient of approximately 3.5 S would be expected (Creighton, 1983). This analysis assumes that dissociated monomers maintain their folded conformation. It is more likely that if the monomers are present, they are partially or significantly unfolded. An unfolded polypeptide chain of 37,000 Da would sediment at a rate less than 3.5 S. Since the observed sedimentation coefficient of 4.65 S in the presence of EDTA is intermediate between that derived for the trimer and that expected for either a folded or unfolded monomer, the chelating agent may remove a structural metal that results in the destabilization of the trimer and promotes the dissociation of the trimers into monomers. Preliminary equilibrium sedimentation experiments indicate that in the presence of EDTA, arginase may exist in a monomer-trimer equilibrium.6

Circular dichroism experiments were undertaken to determine the effect of removal of the weakly associated metal as well as the effect of chelating agents on the secondary structure of arginase. Fig. 8 demonstrates that there is an overall decrease in ellipticity as metal is removed. Analysis of the data by the method of Provencher and Glockner (1981) reveals, however, that arginase treated with exogenous manganese and arginase dialyzed against a buffer containing no metals retain similar amounts of each type of secondary structure. Incubation with EDTA results in an increase in both β-sheet and β-turn structures. The CD spectrum of the sample treated with EDTA, though, does not resemble that for the enzyme that has been treated with guanidinium chloride (Fig. 8).

Summary—A large scale purification protocol for arginase from S. cerevisiae has been developed and the physical characterization begun. This protocol yields a preparation of arginase which is highly purified (> 99%) and has a higher specific activity than that obtained previously in our laboratory (Eisenstein, 1985). Previous results suggested that treatment of arginase with EDTA caused the trimeric enzyme to dissociate fully into monomers. However, results presented here on an enzyme purified by a new purification protocol yielding a significantly higher specific activity show that treatment with EDTA results in only partial dissociation of arginase into monomers. In the absence of the weakly and strongly bound metals, the data suggest that arginase may exist in a monomer-trimer equilibrium. Thus, metals play an important role in maintaining both the catalytic activity of the enzyme as well as contributing to the stability of the tertiary and quaternary structure. Only the trimeric form of the enzyme has been shown to form the tight complex with ornithine transcarbamoylase (Eisenstein, 1985; Eisenstein et al., 1986; Duong et al., 1986). It remains to be determined what roles metals ultimately play in promoting multienzyme complex formation.

Acknowledgments—We thank Drs. Francine Messenguy and Jean-Marie Wiame for generously providing S. cerevisiae strain No. 022321 and Dr. Marc S. Lewis for the use of his analytical ultracentrifuge. We also wish to thank Dr. Patrick J. Fleming for his comments on the manuscript and his many helpful discussions.

S. M. Green, A. Ginsburg, M. S. Lewis, and P. Hensley, manuscript in preparation.

S. M. Green, J. R. Knutson, and P. Hensley, manuscript in preparation.

4 S. M. Green, J. R. Knutson, and P. Hensley, manuscript in preparation.

5 10 mM manganese can actually inhibit arginase activity by up to 50%. If the optimal manganese concentration were used in the heat activation step during the steady-state kinetics experiments for arginase, the specific activity would be in the vicinity of 1200 units mg⁻¹.

6 S. M. Green, A. Ginsburg, M. S. Lewis, and P. Hensley, manuscript in preparation.
REFERENCES


Sedimentation Studies: Velocity and equilibrium measurements were carried out in a Beckman Model T ultracentrifuge equipped with a refrigerated water-bath system. Samples with chroomatographed type anticomplementary and aortic buffer were used. The data were calculated from the equation:

\[ k = \frac{1}{m} \left( \frac{1 + m + n}{n} + \frac{1}{m} \right) \]

where \( m \) is the residue of the sample, \( n \) is the residue of the coefficient, \( k \) is the molar weight of the sample, and \( m \) is the molar weight of the coefficient.

The sedimentation coefficient was corrected for both sample and temperature effects by normalizing the value obtained at the density of water using the equation:

\[ k_{0} = k_{s} \frac{1}{1 + \frac{1}{1 + \frac{1}{2}}} \left( \frac{1 + \frac{1}{1 + \frac{1}{2}}} - \frac{1}{1 + \frac{1}{2}} \right) \]

where \( k_{s} \) is the sedimentation coefficient in water, \( k_{0} \) is the sedimentation coefficient in buffer or temperature \( m \), \( n \) is the relative viscosity of the solution, \( m \), and \( n \) is the molar weight of the sample, \( m \) and \( n \) are the molar weight of the buffer, and \( m \) is the temperature of the sample.

Fluorometry: All fluorimetric assays were carried out on an FE./C-200 200-fluorimeter at 25°C in the single photon mode using 2 mm slit which corresponds to an 8 mm bandwidth. The excitation spectrum was measured at an emission wavelength of 351 nm. The emission spectrum was measured at the excitation wavelength of 305 nm. Anisotropy was 0.21 nm and 0.5 nm.

Circular Dichroism: CD spectra were measured on a JASCO J-700 spectropolarimeter interfaced with a JASCO J-700 100-200 A scanning system. The mean residue ellipticity (deg cm² dmól⁻¹) was calculated using the following equation:

\[ [\theta]_220 = \frac{1}{c} \frac{m}{l} \frac{1}{n} \frac{1}{1 + \frac{1}{1 + \frac{1}{2}}} \left( \frac{1 + \frac{1}{1 + \frac{1}{2}}} - \frac{1}{1 + \frac{1}{2}} \right) \]

where \( [\theta]_220 \) is the ellipticity at 220 nm, \( m \) is the molar residue molecular weight for the sample, and \( n \) is the molar residue molecular weight for the solvent. The mean residue ellipticity at 220 nm was calculated using the following equation:

\[ [\theta]_220 = \frac{1}{c} \frac{m}{l} \frac{1}{n} \frac{1}{1 + \frac{1}{1 + \frac{1}{2}}} \left( \frac{1 + \frac{1}{1 + \frac{1}{2}}} - \frac{1}{1 + \frac{1}{2}} \right) \]

where \( [\theta]_220 \) is the ellipticity at 220 nm, \( m \) is the molar residue molecular weight for the sample, and \( n \) is the molar residue molecular weight for the solvent. The mean residue ellipticity at 220 nm was calculated using the following equation:

\[ [\theta]_220 = \frac{1}{c} \frac{m}{l} \frac{1}{n} \frac{1}{1 + \frac{1}{1 + \frac{1}{2}}} \left( \frac{1 + \frac{1}{1 + \frac{1}{2}}} - \frac{1}{1 + \frac{1}{2}} \right) \]

where \( [\theta]_220 \) is the ellipticity at 220 nm, \( m \) is the molar residue molecular weight for the sample, and \( n \) is the molar residue molecular weight for the solvent. The mean residue ellipticity at 220 nm was calculated using the following equation:

\[ [\theta]_220 = \frac{1}{c} \frac{m}{l} \frac{1}{n} \frac{1}{1 + \frac{1}{1 + \frac{1}{2}}} \left( \frac{1 + \frac{1}{1 + \frac{1}{2}}} - \frac{1}{1 + \frac{1}{2}} \right) \]

where \( [\theta]_220 \) is the ellipticity at 220 nm, \( m \) is the molar residue molecular weight for the sample, and \( n \) is the molar residue molecular weight for the solvent. The mean residue ellipticity at 220 nm was calculated using the following equation:

\[ [\theta]_220 = \frac{1}{c} \frac{m}{l} \frac{1}{n} \frac{1}{1 + \frac{1}{1 + \frac{1}{2}}} \left( \frac{1 + \frac{1}{1 + \frac{1}{2}}} - \frac{1}{1 + \frac{1}{2}} \right) \]
Purification and Characterization of Yeast Arginase

1. Elution Volume, ml

<table>
<thead>
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<th>Volume, ml</th>
<th>Absorbance, cm²</th>
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</thead>
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</tr>
<tr>
<td>31,000</td>
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<td>0.00</td>
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<td>92,500</td>
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</table>

Figure 2. Elution of arginase from Sphero-Gel TSK-G3000, pH 7.6. Fractions of 1 ml were collected and SDS polyacrylamide gel run to assess purity. Fractions containing one band (denoted by bar) were collected, concentrated, and dialyzed against 20 mm Hepes, pH 7.5, 0.5 mm L-lysine, 0.5 mm NaCl, 0.5 mm MgCl₂, 0.5 mm MnCl₂, and 1 mm ATP.

Figure 3. SDS polyacrylamide gel electrophoresis of purified arginase. Lane 1 contains 5 µg of purified arginase, lane 2 and 3 contain 10 and 15 µg, respectively. Lanes 4-6 contain: 62,200 Da, bovine serum albumin; 68,300 Da, ovalbumin; 240,000 Da, yeast glycosylated plasmid. 31,000 Da, yeast arginase inhibited; 21,500 Da and 23,000 Da, samples in 1% SDS and 1% (w/v) Na₂EDTA were heated at 100°C for 5 min prior to electrophoresis.
The purification and characterization of arginase from Saccharomyces cerevisiae.
S M Green, E Eisenstein, P McPhie and P Hensley


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