Characterization of argininosuccinate lyase (EC 4.3.2.1) from *Chlamydomonas reinhardtii*

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We have isolated and characterized argininosuccinate lyase (ASL; EC 4.3.2.1) from the photosynthetic green alga, *Chlamydomonas reinhardtii*. The general properties of *Chlamydomonas* ASL are very similar to those described previously for ASLs from phylogenetically diverse organisms. The algal ASL has a native M_r , determined by gel-filtration chromatography, of $218\,000 \pm 25\,000$, and a pI of 5.4–5.6. The K_m for argininosuccinate at 37 °C and pH 7.5 is 0.26 mM. The subunit M_r of *Chlamydomonas* ASL is approx. 50000, determined by SDS/polyacrylamide-gel electrophoresis, in contrast with a previously reported value of 39000. Rabbit antisera prepared against the M_r -50000 protein completely abolished ASL activity *in vitro*. In contrast, serum prepared against the M_r -39000 protein was ineffective in inhibiting ASL activity. Despite the general similarity of the physical properties of *Chlamydomonas* ASL and those of other ASLs, antiserum raised against the algal ASL did not cross-react with ASL preparations from *Escherichia coli*, *Saccharomyces cerevisiae* or bovine liver.

INTRODUCTION

Argininosuccinate lyase (ASL; EC 4.3.2.1) catalyses the terminal reaction in arginine biosynthesis: argininosuccinate $\rightleftharpoons$ arginine + fumarate. The enzyme has been studied from a wide range of sources, including bovine liver and kidney (Havir *et al.*, 1965; Bray & Ratner, 1971), human liver (O'Brien & Barr, 1981), *Neurospora* (Cohen & Bishop, 1966), jack bean (Rosenthal & Naylor, 1969) and *Chlamydomonas* (Matagne & Schlosser, 1977). Over this wide phylogenetic range the gross physical and chemical properties of ASL appear to have been conserved. The general consensus is that the active enzyme is a tetramer of M_r approx. 200000 and is composed of four identical M_r -50000 subunits. The K_m for ASL from these diverse sources similarly shows little variation and is in the range 0.13–0.43 mM-argininosuccinate.

In *Chlamydomonas* ASL is coded for by the *arg-7* locus on linkage group I (Loppes *et al.*, 1972), for which many mutants have been isolated and characterized (see, e.g., Loppes & Matagne, 1972; Loppes *et al.*, 1972; Matagne, 1978). ASL assumes special importance in *Chlamydomonas* in an attempt to develop a molecular transformation system for this alga, since *arg-7* is one of the few loci in *Chlamydomonas* for which auxotrophic mutations are available as selective markers for transformation.

With the development of the λ gt11 expression vector (Young & Davis, 1983a,b), it is potentially possible to isolate the *arg-7* gene by screening a *Chlamydomonas* library in this vector with antibody raised against *Chlamydomonas* ASL and to use the cloned *arg-7* gene as a selective marker for transformation of *arg-7* auxotrophs. As a first step towards his goal we have isolated and partially characterized ASL from *Chlamydomonas reinhardtii* and have raised an antibody to this enzyme which completely abolished ASL activity *in vitro*.

MATERIALS AND METHODS

Materials

Arginine and argininosuccinate (barium salt) were obtained from Sigma Chemical Co. Before use, 50 mg of barium argininosuccinate in 9.85 ml of water was converted into the potassium salt by addition of 0.15 ml of 1 M- K_2SO_4 . The $BaSO_4$ precipitate was removed by centrifugation at 12000 g for 5 min at 4 °C. Sephadex IEF and Pharmalyte Ampholines were purchased from Pharmacia. Bio-Gel A 0.5 m and A 1.5 m were obtained from Bio-Rad, and DE-52 DEAE-cellulose was from Whatman.

Definition of enzyme unit

One unit of ASL activity is defined throughout as the amount of ASL that produces 1 μ mol of arginine/h from argininosuccinate at 37 °C.

Assay for ASL

Method I. ASL activity in crude enzyme preparations was assayed as follows. Arginine, produced by ASL action on argininosuccinate, was converted into urea by an excess of arginase. The urea was then assayed colorimetrically (Spandrio & Ceriotti, 1963). This procedure obviated potential underestimations of arginine production by ASL, owing to the presence of arginase in the crude extracts. Briefly, the enzyme preparation was incubated at 37 °C for 20 min, with 30 mM-argininosuccinate, 225 units of arginase and 75 mM-potassium phosphate buffer, pH 7.5, in a final reaction volume of 200 μ l. The reaction was terminated by the addition of 0.4 ml of 7.5% (w/v) trichloroacetic acid, and the mixture left on ice for 10 min and centrifuged for 10 min at 4 °C in an Eppendorf Microfuge. Duplicate 200 μ l samples were taken from

Abbreviations used: ASL, argininosuccinate lyase; PAGE, polyacrylamide-gel electrophoresis.

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the supernatant and added to 2 ml of acid mixture (90 ml of conc. H_2SO_4 , 270 ml of conc. H_3PO_4 , 1 ml of 0.1 M- FeCl_3 , made up to a final volume of 1 litre with water) and 0.1 ml of 1-0-1,2-propanedione-2-oxime [3% (v/v) in 95% (v/v) ethanol]. The mixtures were incubated at 100 °C in the dark for 1 h, cooled in the dark for 10 min, and the A_{540} was read against a blank containing no argininosuccinate.

Method II. ASL activity in more highly purified enzyme preparations was assayed with argininosuccinate as substrate, by measuring arginine production colorimetrically by the Sakaguchi reaction. The enzyme preparation was incubated at 37 °C for 20 min with 10 μl of 12 mM-argininosuccinate (potassium salt) and 10 μl of 1 M-potassium phosphate buffer, pH 7.5, in a final volume of 200 μl . The reaction was terminated by the addition of 400 μl of 7.5% trichloroacetic acid, and the mixture left on ice for 10 min and centrifuged for 10 min in an Eppendorf Microfuge. To each duplicate 200 μl supernatant sample was added 1 ml of distilled water and 250 μl of α -naphthol (0.04% in ethanol)/thymine [20 mg/ml in 10% (w/v) NaOH] mixture (1:1, v/v). Colour development in the reaction mixture was initiated by the addition of 100 μl of commercial bleach (diluted 1:7 in water), and the reaction was allowed to proceed for exactly 1 min. The reaction was terminated by the addition of 100 μl of 2% (w/v) $\text{Na}_2\text{S}_2\text{O}_3$, and the A_{515} of the samples was read against a blank lacking substrate.

Protein assay

Protein was assayed by the method of Lowry *et al.* (1951), with bovine albumin as standard.

Growth of *Chlamydomonas reinhardtii*

Wild-type *Chlamydomonas reinhardtii* strain 125+ was grown in 1.5% agar plates containing medium I of Sager & Granick (1953). For each enzyme preparation 150 8 cm plates were inoculated with 0.1–0.5 ml of a pilot culture which had been grown in liquid medium with bubbling and continuous illumination (7000 lx) to a cell density of 1×10^6 – 3×10^6 ml⁻¹. The plates were then incubated at 23 °C with continuous illumination (5000 lx) for 4–6 days or until growth became confluent. The plates were harvested by scraping the cells off with razor blades, from which we routinely obtained 65–80 g wet wt. of cells.

The protein yields in the crude extracts obtained by this procedure were routinely lower (by approx. 50%) than those reported by Matagne & Schlosser (1977). Differences in enzyme recovery during the initial purification steps are also apparent between the data in the present work and those of Matagne & Schlosser (1977). The reason for these discrepancies is unclear, but may be related to the fact that different strains of *Chlamydomonas* were employed in the two studies and were grown under different conditions [strain 125+ on agar plates containing Sager & Granick (1953) medium I in the present work; strain 137C in liquid medium 40N of Loppes *et al.* (1972) by Matagne & Schlosser (1977)].

Preparation of cell extracts

Cell extracts were prepared essentially by the method of Matagne & Schlosser (1977). Harvested cells were resuspended in 160 ml of 20 mM-potassium phosphate

buffer (pH 7.5)/0.1 mM-phenylmethanesulphonyl fluoride. The cell suspension was then sonicated in a melting-ice/water bath with three bursts each at 200 W for 1.5 min, separated by 3 min cooling periods. Cell disruption was verified by light-microscopy. The sonicated cells were then centrifuged at 12000 *g* for 15 min at 4 °C. The resulting supernatants constituted the crude cell extracts from which ASL was purified.

Electrophoretic methods

Denaturing SDS/PAGE. This was performed in the discontinuous system of Laemmli (1970). Slab gels were used with a 5%-acrylamide stacking gel and a 12.5%-acrylamide resolving gel. Analytical gels were 0.15 cm thick and preparative gels 0.6 cm thick. In both cases a 12 cm resolving gel was used. Analytical gels were run at 15 mA constant current, and preparative gels at 25 mA. For protein detection, both analytical and preparative gels were stained in 0.1% (w/v) Coomassie Blue R250 in 7.5% (v/v) acetic acid/5% (v/v) methanol. Gels were destained for 1–2 h in 7.5% acetic acid/50% methanol, after which the methanol concentration was lowered to 5%.

Electroelution protocol. Enzyme preparation (10–15 mg), partially purified by ion-exchange chromatography (e.g. Fig. 1) was fractionated by preparative SDS/PAGE as described above. After detection of the protein bands, the bands of interest were excised and the gel slices placed in dialysis membranes filled with Laemmli electrode buffer, pH 8.9. The sealed dialysis membranes were then placed in a Bio-Rad SubCell horizontal electrophoresis apparatus and the proteins were eluted from the gel slices by electrophoresis in Laemmli electrode buffer, pH 8.9, at 50 mA constant current for 1–2 h. After electroelution, the buffer inside the dialysis membranes containing the electroeluted protein was dialysed exhaustively against distilled water and freeze-dried to a powder. The protein was stored in this form at –20 °C until use. From 10 mg of enzyme preparation (Fig. 1) approx. 0.1–0.3 mg of ASL was recovered.

Flat-bed isoelectric focusing. To prepare enzyme samples for isoelectric focusing, 5–12 mg of enzyme preparation purified by ion-exchange chromatography (Fig. 1) was dialysed for at least 4 h at 4 °C against 1% (w/v) glycine, pH 7.0. After dialysis, the sample was added to a slurry containing 4.4 g of Sephadex IEF and 4.7 ml of Pharmalyte Ampholines and made up to final volume of 70 ml with distilled water. The slurry was poured on to a 115 mm × 230 mm plate and isoelectric focusing carried out at 10–20 W constant power for 17 h with cooling. The anode buffer was 0.1 M- H_3PO_4 , and the cathode buffer 0.1 M-NaOH. For broad-pH-range isoelectric focusing, 3.8 ml of pH 3.5–10 Ampholines was mixed with 0.9 ml of pH 4.0–6.5 Ampholines. For narrow-pH-range isoelectric focusing, 4.7 ml of pH 4.0–6.5 Ampholine alone was used.

After isoelectric focusing, the gel was divided into 3 mm sections and small samples of gel were removed and resuspended in 1.0 ml of distilled water for pH determination. The remainder of each gel slice was resuspended in 1.0 ml of 20 mM-potassium phosphate buffer, pH 7.5, left overnight at 4 °C, and samples were removed for enzyme (Method II) and protein assays.

Table 1. Partial purification of ASL from *Chlamydomonas*

ASL activity was assayed by Method I* or Method II† (see the Materials and methods section).

Purification step	Volume (ml)	Total protein (mg)	Total activity (units)	Yield (%)	Specific activity (units/mg)
Crude extract*	178	571	1155	100	2.02
(NH ₄) ₂ SO ₄ pellet*	32	150	645	56	4.30
Ion-exchange chromatography†	81	17	255	22	15.00

Gel-filtration protocol

Ion-exchange-purified enzyme preparations, containing 20 mg of protein in 2 ml of 20 mM-potassium phosphate buffer, pH 7.5, were loaded on to 1.6 cm × 80 cm Bio-Gel A 1.5 m columns, pre-equilibrated with 20 mM-potassium phosphate buffer, pH 7.5, at 4 °C. The columns were run at a flow rate of 8.2–8.5 ml/h, and 4.3 ml fractions were collected. Protein and enzyme assays (Method II) on the samples were performed as described above. Columns were calibrated with the following M_r standards: Dextran 2000 (2×10^6), thyroglobulin (6.69×10^5), ferritin (4.40×10^5), catalase (2.32×10^5) and aldolase (1.58×10^5).

Antibody production

The following protocol was used to raise rabbit antibodies against *Chlamydomonas* proteins which had been purified by excising the appropriate stained band from SDS/polyacrylamide gels, followed by electroelution to recover the protein (see above). An initial injection of 300 µg of freeze-dried protein, resuspended in 1.0 ml of phosphate-buffered saline, pH 7.0, and mixed with an equal volume of Freund's complete adjuvant, was administered intraperitoneally. Subsequent injections, also of 100–300 µg of protein, were administered 4 weeks after the initial injection and then at 2-week intervals thereafter until antibody was raised. Freund's incomplete adjuvant was used for all subsequent injections.

Partial purification of the immunoglobulin fraction from crude serum was obtained by precipitation with 33% -satd. (NH₄)₂SO₄ for 1 h at 4 °C. The precipitate was pelleted by centrifugation (20 min at 12000 g, in a Sorvall SS34 rotor, at 4 °C), resuspended in phosphate-buffered saline (one-fifth the original volume of crude serum) and dialysed against phosphate-buffered saline for 24 h at 4 °C with three changes of buffer. The partially purified serum was stored in 1 ml batches at –80 °C until use.

RESULTS

Initial purification of ASL

The initial steps used to purify ASL were essentially those described by Matagne & Schlosser (1977). Crude cell extracts (see the Materials and methods section) were mixed with solid (NH₄)₂SO₄ (21 g/100 ml of extract) at room temperature and stirred for 30 min. The mixture was then centrifuged at 30000 g (Sorvall SS34 rotor) for 15 min at 4 °C and the supernatant recovered. Additional

Fig. 1. SDS/PAGE analysis of ASL preparations

Lane 1, ASL preparation purified by ion-exchange chromatography (Table 1; see the Materials and methods section). Lane 2, *Chlamydomonas* ASL isolated by preparative electrophoresis (see the Materials and methods section). Lane 3, M_r standards: α -tubulin (55000), β -tubulin (53000). Lane 4, M_r standards: bovine albumin (67000), ovalbumin (43000), α -chymotrypsinogen A (25000), myoglobin (17000). Notice the prominent presence of M_r -39000 protein in the ASL preparation purified by ion-exchange chromatography (lane 1).

(NH₄)₂SO₄ (16 g/100 ml of supernatant) was added to the supernatant and the solution was stirred for 1 h at 4 °C, followed by centrifugation at 30000 g as above. The resulting precipitate was resuspended in 20–30 ml of 20 mM-potassium phosphate buffer, pH 7.0, and dialysed overnight at 4 °C against a 1000-fold excess of the same buffer.

The resulting solution (30–40 ml) was applied to a 15 cm × 2.5 cm column of DEAE-cellulose, pre-equil-

Fig. 2. Flat-bed isoelectric focusing of *Chlamydomonas* ASL

A 10 mg sample of enzyme preparation purified by ion-exchange chromatography (Table 1) was dialysed against 1% (w/v) glycine, pH 7.0, and focused at 20 W for 17 h with pH 4.0–6.5-range Ampholines. After completion of the run, the gel was divided into fractions and both the ASL activity (●) and pH (○) in each fraction were determined.

brated with 20 mM-potassium phosphate buffer, pH 7.5, and washed until the A_{280} of the eluate was 0.05. Proteins adsorbed to the column were eluted with a 400 ml linear gradient of NaCl (0–0.3 M) in 20 mM-potassium phosphate buffer at a flow rate of 25 ml/h. Fractions containing ASL activity were eluted between 0.09 M- and 0.19 M-NaCl, and were pooled and dialysed against a 1000-fold excess of 20 mM-potassium phosphate buffer, pH 7.5.

A summary of the purification protocol is given in Table 1. This initial procedure resulted in only slight (routinely 4–7-fold) purification of enzyme activity. Furthermore, little, if any, enrichment of ASL activity was obtained by the $(\text{NH}_4)_2\text{SO}_4$ precipitation step.

The pooled fractions from the ion-exchange column which contained enzyme activity were composed of a heterogeneous mixture of proteins, as shown by SDS/PAGE, including a major protein migrating with an apparent M_r of 39000 (Fig. 1). This protein was also a major component after the enzyme activity of the pooled ion-exchange fractions was additionally purified by non-denaturing gel electrophoresis (results not shown). This is consistent with the observations by Matagne & Schlosser (1977), who identified their M_r -39000 protein as the ASL subunit.

Attempts to purify ASL further from the pooled ion-exchange fractions, by either Bio-Gel A 0.5 m or Sephadex G-200 column chromatography, were unsuccessful, routinely yielding ASL preparations which contained many proteins, as shown by SDS/PAGE, and with specific activities of 20–25 units/mg (results not shown; cf. Table 1).

Isoelectric focusing

In an attempt to obtain ASL of a purity suitable for antibody production, ASL fractions partially purified by ion-exchange chromatography (Fig. 1; Table 1) were further fractionated by flat-bed isoelectric focusing (see the Materials and methods section). An initial determination of the pI of ASL by using wide-pH-range Ampholines (pH 3.5–10) indicated the pI to lie between pH 5 and 6. Subsequent use of narrow-pH-range Ampholines demonstrated the peak enzyme activity to

Fig. 3. SDS/PAGE analysis of M_r -39000 protein isolated by preparative electrophoresis (see the Materials and methods section)

Lane 1, M_r standards: phosphorylase *b* (94000), bovine albumin (67000), ovalbumin (43000), carbonic anhydrase (30000), trypsin inhibitor (20100) and α -lactalbumin (14400). Lane 2, M_r -39000 protein.

focus between pH 5.4 and 5.6 (Fig. 2). However, although the specific activities of the peak enzyme fractions were enhanced (40–45 units/mg), SDS/PAGE analysis of the enzyme fractions showed the presence of at least ten protein bands, and they were therefore unsuitable for antibody production (results not shown).

SDS/PAGE analysis of the protein distribution through the pH gradient also yielded the unexpected result that the profile of ASL activity did not correspond to the distribution of an M_r -39000 protein band, the subunit M_r of ASL reported by Matagne & Schlosser (1977). The M_r -39000 protein focused in the pH range 4.7–5.0, in contrast with the ASL activity, which focused at pH 5.4–5.6. Instead, a protein with an apparent M_r of about 50000 coincided with ASL activity.

Immunological studies

To investigate which of these proteins represented ASL, rabbit antibodies were raised against both (see the Materials and methods section) and tested for their ability to inhibit enzyme activity in ion-exchange-purified enzyme preparations. Since none of the protein-fractionation techniques employed above gave enzyme preparations sufficiently pure for specific anti-ASL antibody production, ion-exchange-purified enzyme preparations were fractionated by preparative SDS/PAGE, the bands corresponding to the M_r -39000 and -50000

Fig. 4. Effects of anti-39K and anti-50K antisera on *Chlamydomonas* ASL activity

A 50 μ l portion of an ASL preparation (0.4 mg/ml) purified by ion-exchange chromatography (Table 1; Fig. 1) was incubated with increasing volumes of immune or pre-immune sera (see the Materials and methods section) for 2 h at room temperature in a final reaction volume of 150 μ l. ASL activities in the samples were then determined by Method II (see the Materials and methods section). Inhibition of ASL activity is expressed as a percentage of the activity in 50 μ l of the ASL preparation to which buffer alone had been added. Δ , Anti-39K pre-immune serum; $\square$, Anti-39K immune serum; $\bullet$, anti-50K pre-immune serum; $\circ$, anti-50K immune serum.

proteins excised, and the proteins recovered by electroelution (see the Materials and methods section). As shown by analytical SDS/PAGE, each protein preparation contained a single protein band migrating with the appropriate apparent M_r (Figs. 1 and 3). These proteins were then used for antibody production (see the Materials and methods section).

The results demonstrated that rabbit antibodies prepared against the M_r -39000 protein (anti-39K) failed to inhibit enzyme activity (Fig. 4), even though 125 I-labelled anti-39K IgG fraction 'recognized' the M_r -39000 protein band in Western blots of ion-exchange-purified enzyme preparations (results not shown). In contrast, the IgG fraction prepared from rabbits immunized with the M_r -50000 protein was active against ASL and completely inhibited enzyme activity (Fig. 4). No immunological cross-reactivity was seen between the M_r -39000 and -50000 proteins on Western blots probed with either anti-39K or anti-50K serum, indicating that the M_r -39000 protein was not a proteolytic fragment of the M_r -50000 protein (results not shown).

The species-specificity of the rabbit serum raised against *Chlamydomonas* ASL (anti-ASL) was examined by Western blot analysis. No cross-reactivity was found between anti-ASL and crude enzyme preparations from *Escherichia coli* and *Saccharomyces cerevisiae* or purified bovine liver ASL (results not shown).

Fig. 5. Determination of the native M_r of ASL

(a) A 20 mg sample of an ASL preparation, purified by ion-exchange chromatography (Fig. 1), was applied to a column (80 cm $\times$ 1.6 cm) of Bio-Gel A 0.5 m pre-equilibrated with 20 mM-potassium phosphate, pH 7.5, and fractionated at 4 $^{\circ}$ C (flow rate 8.5 ml/h). The protein content of each fraction was monitored continuously at 280 nm (—). The ASL activity ($\circ$) in each fraction was determined by Method II. (b) The apparent M_r of ASL was determined from the relative mobility of the peak of ASL activity in (a). The column was calibrated with Blue Dextran 2000 (M_r , 2×10^6 ; $\bullet$), thyroglobulin (6.69×10^5 ; ∇), ferritin (4.40×10^5 ; Δ), catalase (2.32×10^5 ; $\circ$) and aldolase (1.58×10^5 ; $\square$). Arrow indicates relative mobility of ASL.

Fig. 6. Determination of the K_m for ASL

Samples (50 μ l) of an ASL preparation (0.32 mg/ml), partially purified by $(\text{NH}_4)_2\text{SO}_4$ precipitation (specific activity 3.9 units/mg), were incubated for 20 min at 37 $^{\circ}$ C with increasing concentrations of argininosuccinate (0–8 mM) in a final reaction volume of 200 μ l. Arginine production was determined by Method II (see the Materials and methods section). A double-reciprocal plot of the initial rates of arginine production (v) as a function of argininosuccinate concentration gave $K_m = 0.26$ mM.

Determination of ASL native M_r

To determine the native M_r of ASL, 20 mg of an enzyme preparation, purified by the steps outlined in Table 1, was fractionated on a column (80 cm $\times$ 1.5 cm) of Bio-Gel A 1.5 m, pre-equilibrated with 20 mM-potassium phosphate, pH 7.5 (see the Materials and methods section). The column was calibrated with reference proteins as described in the Materials and methods section. In this method ASL migrated with an apparent M_r of 218000 ± 25000 (Fig. 5).

K_m determination

The K_m for *Chlamydomonas* ASL was determined over an argininosuccinate concentration range of 0–8 mM, in which arginine production was linear with time for at least 20 min. The initial rates of arginine production were obtained from these data by least-squares linear-regression analysis and are plotted as a function of substrate concentration in the form of a double-reciprocal plot, from which the K_m was obtained (Fig. 6). *Chlamydomonas* ASL exhibited normal Michaelis–Menten kinetics in this concentration range, and no evidence of co-operative behaviour was observed. The K_m was determined to be 0.26 mM for argininosuccinate at pH 7.5 and 37 °C.

DISCUSSION

The gross physical properties of *Chlamydomonas* ASL resemble those described for other ASLs from phylogenetically widely separated organisms. On gel filtration, *Chlamydomonas* ASL migrated with an apparent native M_r of 218000 ± 25000 (Fig. 5). Comparable native M_r values have been reported for ASL isolated from such distinct sources as human and bovine tissues (Havir *et al.*, 1965; Bray & Ratner, 1971; Ratner, 1972; O'Brien & Barr, 1981), jack bean (Rosenthal & Naylor, 1969) and *Neurospora* (Cohen & Bishop, 1966). Similarly, *Chlamydomonas* ASL has a pI of 5.4–5.6 (Fig. 2) and a K_m of 0.26 mM for argininosuccinate (Fig. 6). Again, these values are comparable with those reported for ASL isolated from the above sources.

A discrepancy exists between the subunit M_r determined for *Chlamydomonas* ASL in this study (50000) and that reported previously (Matagne & Schlosser, 1977; 39000). Purification of ASL by the procedure described by those authors (Table 1) did indeed yield enzyme preparations a major component of which had an M_r of 39000 (Fig. 1). Similarly, the composition of enzyme preparations was enriched for this protein by fractionation on non-denaturing polyacrylamide gels (data not shown), as reported by Matagne & Schlosser (1977). However, in our hands, in every enzyme preparation isolated by these methods many other proteins were additionally present. One such protein, invariably present, migrated with an apparent M_r of 50000 on SDS/PAGE (Fig. 1).

The available evidence strongly suggests that the M_r -50000 protein is in fact the ASL subunit. On isoelectric focusing the profile of the M_r -50000 protein closely correlated with the ASL activity profile, in contrast with

the distribution of the M_r -39000 protein, which showed little correlation. More importantly, anti-39K antiserum failed to inhibit ASL activity, whereas anti-50K antiserum completely abolished such activity (Fig. 4). Evidence that the M_r -39000 protein was the ASL subunit was, in contrast, based solely on the fact of its prominence in enzyme preparations exhibiting ASL activity (Matagne & Schlosser, 1977). From these data it is reasonable to conclude that the apparent subunit M_r of *Chlamydomonas* ASL is 50000 and that the M_r -39000 protein is a contaminant in the enzyme preparations. This conclusion is underscored by the fact that the subunit M_r of ASL has been reported as approx. 50000 in all other cases examined, and an M_r value of 52010 has been calculated from the nucleotide sequence of the cloned yeast *arg4* gene (Beacham *et al.*, 1984). A subunit M_r of 50000 suggests that the native form of *Chlamydomonas* ASL is most probably a tetramer.

Although ASLs from a wide range of sources show similarities in their gross physical properties, antibodies prepared against purified ASLs in contrast show marked species specificity. No species cross-reactivity was seen between *Chlamydomonas* anti-ASL and ASL preparations from *E. coli*, *S. cerevisiae* or bovine liver. Similarly, rabbit anti-human ASL failed to recognize rat liver ASL and only weakly cross-reacted with bovine liver ASL (O'Brien & Barr, 1981). This lack of species cross-reactivity suggests that detection of ASL sequences by immunological screening of gene libraries in expression vectors such as λ gt11 will require homologous antibody probes.

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