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# Purification and Characterization of Arginine Kinase From the American Cockroach (*Periplaneta americana*)

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The isolation and characterization of homogeneous arginine kinase from the cockroach is reported. The purification protocol produces 6.6 mg of pure enzyme from 6.8 g of whole cockroach. The purified enzyme cross-reacts with a heterologous antibody and monoclonal antibody against arginine kinase from the shrimp. Both antibody preparations also cross-react with extracts from several species known to contain monomeric arginine kinase, but fail to react with extracts from organisms containing dimeric arginine kinase. Cockroach arginine kinase has a molecular mass of approximately 43,000 determined from measurements by gel filtration and gel electrophoresis. Compared with other arginine kinases, the enzyme from the cockroach is relatively thermostable (50% activity retained at 50°C for 10 min) and has a pH optima of 8.5 and 6.5–7.5, for the forward and reverse reactions, respectively. Treatment with 5,5′dithiobis[2-nitrobenzoic acid] indicates that arginine kinase has a single reactive sulfhydryl group and, interestingly, the reaction is biphasic. The Michaelis constants for the phosphagen substrates, arginine: 0.49 mM, phosphoarginine: 0.94 mM, and nucleotide substrates MgATP: 0.14 mM, MgADP: 0.09 mM, are in the range reported for other arginine kinases. A 1% solution of pure enzyme has an absorbance of 7.0 at 280 nm. Calculations based on circular dichroic spectra indicate that arginine kinase from the cockroach has 12% $\alpha$-helical structure. The intrinsic protein fluorescence emission maximum at 340 nm suggests that tryptophan residues are below the surface of the protein and not exposed to solvent. Arginine kinase from the cockroach and shrimp are known to be deleterious immunogens towards humans. The availability of pure protein, its characterization and potential regulation of activity, will be useful in developing agents to control the cockroach population and its destructive role in agriculture and human health. Arch. Insect Biochem. Physiol. 56:51–60, 2004. © 2004 Wiley-Liss, Inc.

KEYWORDS: cockroach; shrimp; arginine kinase; creatine kinase; enzyme purification; physical properties; invertebrates

## INTRODUCTION

Arginine kinase catalyzes the reversible phosphorylation of arginine using ATP:

$$\text{Mg ATP + L-arginine} \rightleftharpoons \text{MgADP + L-arginine phosphate + } H^+$$

The enzyme is widely distributed among invertebrates (Moreland et al., 1967) and is functionally analogous to the more thoroughly studied creatine kinase found in vertebrates (Watts, 1973). These phosphagen kinases may directly (Bessman and Geiger, 1981) or indirectly (Ouellet and Shoubridge, 1992) regulate availability of ATP. In insects with generalized high metabolic turnover (Rosenthal et al., 1977), AK[1] is important for the onset of rapid motility. In a preliminary survey of numerous invertebrates, it was found that the domestic cockroach (*Periplaneta americana*) is a rich source of AK activity. The purification and characterization of AK in the cockroach is particularly sig-

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Abbreviations used: AK = arginine kinase (EC 2.7.3.3) arginine:ATP phosphotransferase; Buffer A = 0.1 M Tris/HCl, 10 mM mercaptoethanol, 1 mM EDTA, 50 $\mu$M $NaN_3$ and 25 $\mu$M phenylmethylsulfonylfluoride, pH 8.0; Buffer B = 10 mM Tris/HCl, 10 mM mercaptoethanol, and 0.1 mM EDTA, pH 8.0; CK = creatine kinase (EC 2.7.3.2) creatine:ATP phosphotransferase.

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nificant because of the role of this organism as a domestic pest, as a destructive agent in agriculture, and as a carrier of antigens evoking strong responses among humans (Crespo et al., 1995). One of those antigens has now been identified as AK protein, which elicits allergenic asthma among atepic individuals (Binder et al., 2001).

Arginine kinase has been purified and characterized from numerous species and can be generally categorized as either a monomeric or dimeric protein (Morrison, 1973; Seals and Grossman, 1987). Studies have shown that AK is structurally homologous to the dimeric CK isozymes (Anosike and Watts, 1975; Suzuki et al., 2000). Indeed, a hybrid dimer of CK and AK can be prepared containing a single subunit from each of the parent homodimers (Seals and Grossman, 1987).

In the present study, we report the isolation of pure AK from the cockroach. Several catalytic and physical properties are characterized and compared with those of the enzyme from other sources, including AK previously isolated from shrimp (France et al., 1997).

## MATERIALS AND METHODS

### Materials

All reagents used were obtained from Sigma Chemical Co. (St. Louis, MO) unless noted otherwise. Live organisms were collected locally and species designation confirmed in the Department of Biology (University of South Florida).

### Assay of Activity and Determination of Protein Concentration Spectrophotometric Assay

Catalytic activity was routinely measured in the reverse direction (formation of arginine and ATP) by the enzyme-linked spectrophotometric assay with the production of NADPH + $H^+$ (Rosalki, 1967). The final mixture (0.5 mL) contained 10 mM arginine phosphate, 1.125 mM ADP, 0.4 mM NADP, 0.5 U/mL hexokinase, 1 U/mL glucose 6-phosphate dehydrogenase, 10 mM dithiothreitol, 3 mM magnesium acetate, and 20 mM glucose in 0.1 M Tris/HCl buffer, pH 8.0. The increase in absorbance at 340 nm was measured using a Beckman Coulter DU 640 Spectrophotometer (Fullerton, CA).

A microtiter assay with activity corresponding to formation of thiolated NADH + $H^+$ was also used to assay AK activity. The assay measured the increase in the reduced pyridine nucleotide of a thiolated NAD quantitated by the absorbance at 405 nm (Florini, 1989). The final composition of this assay mixture was 10 mM arginine phosphate, 20 mM glucose, 10 mM magnesium acetate, 1 mM ADP, 0.4 mM thio-NAD, 10 mM dithiothreitol, 0.5 U/mL hexokinase, and 1 U/mL glucose 6-phosphate dehydrogenase, in 0.1 M glycylglycine buffer at pH 6.75. Each well contained a total assay volume of 200 µL. The change in absorbance was measured using a Benchmark plate reader (BioRad, Richmond, CA).

### pH Stat Assay

The forward reaction (formation if arginine phosphate and ADP) catalyzed by AK was measured by a direct pH stat assay (Grossman, 1983) using a Radiometer Analytical Autoburette ABU 901 (Chicago, IL). The assay mixture (2.0 mL) contained 5 mM L-arginine, 2 mM ATP, 3 mM magnesium acetate, and 2 mM dithiothreitol, pH 8.0. The reaction vessel was continuously purged with dry nitrogen and the temperature was held constant at 25°C with a circulating water bath. A solution of 0.01 M NaOH, used as the titrant, had been calibrated against a standard solution of HCl. Prior to assay, the AK was dialyzed against 2.0 mM Tris/HCl buffer containing 1 mM EDTA and 10 mM dithiothreitol, pH 8.0.

### Protein Determination

Protein concentrations were determined using the assay described by Bradford (1976) with bovine serum albumin as the calibration standard.

### AK and CK in Local Invertebrates

Local invertebrate species were collected and immediately frozen. Species included *Pagurus longicarpus* (Hermit Crab), *Uca pugilator* (Fiddler

Crab), *Talorchestia megalophthalma* (Beach Flea), *Romalea microptera* (Lubber Grasshopper), *Orthomorpha coeactata* (Millipede), *Phyllophoga smithi* (White Grub), *Trichoplasia ni* (Cabbage Looper), *Periplaneta americana* (American Cockroach), *Tetramonum caespitum* (Ant), *Littorina obtusata* (Marsh Snail), *Melongena corona* (Florida Crown Conch), *Crassostrea virginica* (Eastern Oyster), *Ophiothrix oerstedii* (Brittle Star), *Mellita quinqueisperforata* (Sand Dollar), *Meloidogyne hapla* (Root Knot Nematode), *Pectinaria gouldii* (Ice Cream Cone Worm), *Arenicola marina* (Bristle Worm), and *Tubulanus sexlineatus* (Ribbon Worm).

Cockroaches used were determined to all be *Periplaneta americana*. Extracts were prepared by mincing thawed samples in Buffer A, followed by centrifugation at 7,000 rpm for 1 min in a microcentrifuge. The supernatants were diluted with buffer to a final protein concentration of 1.0 mg/mL, as determined by dye-binding assay (Bradford, 1976). The phosphagen kinase activity was determined using the colorimetric assay for the forward reaction (France and Grossman, 1996), with either arginine or creatine as substrate.

## Purification

The purification of AK from the American cockroach (*Periplaneta americana*) is a modification of the method developed by France et al. (1997) for the purification of AK from the Gulf shrimp (*Penaeus aztecus*). A total of 6.8 g of frozen cockroach bodies was prepared by removing the head and gut and minced in 0.1 M Tris/HCl, 10 mM mercaptoethanol, 1 mM EDTA, 50 µM $NaN_3$, and 25 µM phenylmethylsulfonylfluoride, pH 8.0 (Buffer A) in a total volume of 27 mL. The suspension was centrifuged at 4,500*g* for 25 min and the pellet discarded. The supernatant was heated at 50°C for 1 min and placed on ice for 1 min. This suspension was then centrifuged at 4,500*g* for 25 min. The resulting supernatant was added to CM-cellulose previously equilibrated with 10 mM Tris/HCl, 10 mM mercaptoethanol, and 0.1 mM EDTA, pH 8.0 (Buffer B), in a 1:1 volume ratio of CM-cellulose to supernatant. The cellulose suspension was gently stirred on ice for 15 min, centrifuged for 25 min at 4,500*g*, and filtered through glass wool to remove the cellulose. The filtrate was then applied to a Sephadex G-100 column (4 × 70 cm) (Amersham Pharmacia Biotech, Piscataway, NJ) and developed with Buffer B. Ten-milliliter fractions were collected and assayed for activity. Those fractions exhibiting AK activity were combined and applied to a Sephadex DEAE-50 column (2.5 × 14.5 cm) previously equilibrated with Buffer B. Arginine kinase was eluted from the DEAE column in a single step with Buffer A containing 0.3 M NaCl.

## Preparation of Polyclonal Antibody

Shrimp AK was purified as described previously (France et al., 1997). Polyclonal antibodies were generated in a female New Zealand white rabbit by subcutaneous injections with 500 µg of purified *P. aztecus* AK in phosphate buffered saline (0.8% NaCl, 0.02% KCl, 10 mM sodium phosphate, pH 7.5) and Freund's complete adjuvant. The rabbit was boosted twice at 10-day intervals with 500 µg purified AK and Freund's incomplete adjuvant. After the detection of anti-AK using the ELISA procedure, whole blood was collected and the polyclonal antibodies were purified from the serum by repeated precipitation with 33% ammonium sulfate. The precipitate was then solubilized in and dialyzed extensively against 0.02 M sodium phosphate, pH 7.4. The IgG fraction was isolated using DEAE-Sephadex as described by Dunbar and Schwoebel (1990) and quantified using the value of 1.38 mL/mg-cm for the extinction coefficient (Robyt and White, 1987).

## Preparation of Monoclonal Antibodies

Monoclonal antibodies against purified shrimp AK (1 mg in 1 mL 0.01 M Tris/HCl, pH 8.0), were developed by the Hybridoma Core Lab, University of Florida (Gainsville, FL). Three antibody preparations, designated 12D5, 12F1, and 11F1 exhibited reactivity with AK using the Western blot assay. The 11F1 type was found to be most inhibitory towards AK activity according to the microtiter plate assay for production of reduced thiolated NAD.

### Western Blot Assay

Western blotting detection (Dunbar, 1994) of AK was performed using polyacrylamide electrophoresis gels (12% running, 4% stacking) in the absence and presence of SDS.

### Sulfhydryl Determination

Reactive sulfhydryls were determined by the method of Ellman (1959). Purified AK was dialyzed against a 0.1 M Tris/HCl, pH 8.0 to remove dithiothreitol. The sample was filtered through a syringe with a 0.45-μm cellulose acetate membrane (Corning, NY) to remove any aggregated protein. A solution of 5,5′dithiobis[2-nitrobenzoic acid] (0.1 mL of 1 mM stock) was added to a 0.90-mL sample containing 0.09 mg/mL of AK. The reaction was allowed to proceed for 15 min at room temperature. Identical protocols were repeated with either 5,5′dithiobis[2-nitrobenzoic acid] or protein replaced by an equivalent volume of buffer. After correction for the controls, the number of sulfhydryl groups per molecule of protein was calculated from the concentrations of protein and sulfhydryl groups determined by the measurement of the absorbance of 3-carboxylate-4 nitrothiophenolate at 412 nm and application of its molar extinction coefficient, $1.14 \times 10^4$ L/mol · cm.

### Circular Dichroism and Intrinsic Fluorescence

Circular dichroic spectra in the far ultraviolet were acquired with an Aviv Model 215 spectropolarimeter (Rheometric Scientific, Inc., Lakewood, NJ).

Intrinsic fluorescence was measured with an SLM 48000 spectrofluorometer (Spex, Edison, NJ), with excitation at 295 nm from a Xenon arc source.

## RESULTS

### Distribution

Extracts from several locally collected invertebrate species exhibiting AK activity were analyzed using a heterologous antibody raised against shrimp AK. Positive responses were exhibited on Western blotting with several arthropods (hermit crab, fiddler crab, beach flea, lubber grasshopper, white grub, ant, cockroach) and mullosks (marsh snail, Florida crown conch, eastern oyster). However, while exhibiting AK activity, extracts from the millipede and cabbage looper failed to react with anti-shrimp AK antibodies. Among the chordata (lizard, frog, grouper, chicken, and rabbit), only CK activity was detected and none cross-reacted with anti-AK antibodies. Interestingly, species within those classes of organisms that are known to contain dimeric AK did not react with antibodies against shrimp (monomeric) AK. All organisms within the classes known to contain monomeric AK protein did react with antibodies against shrimp AK. Equal activities of AK in the cockroach and shrimp exhibited considerably more intense reaction with Coomassie blue for the sample from shrimp after resolution by SDS-PAGE and subsequent identification of AK using Western blots and anti-shrimp AK.

### Purification

The isolation of AK from the cockroach involves a general procedure used previously for purification of several phosphagen kinases (Grossman and Mollo, 1979; Seals and Grossman, 1987; France and Grossman, 1996). Table 1 summarizes the isolation, beginning with about 6.8 g of decapitated roach carcass. The protocol results in isolation of

TABLE 1. Purification of Arginine Kinase from the Cockroach

| Procedure | Total volume (mL) | Total protein (mg) | Total activity (μmole $min^{-1}$) | Specific activity (μmole $min^{-1}mg^{-1}$) | Fold enrichment | % Yield |
|---|---|---|---|---|---|---|
| Homogenization/centrifugation | 26 | 114 | 228 | 2.0 | — | 100 |
| Heat (50°C/1min) | 26 | 109 | 230 | 2.1 | 1.0 | 101 |
| CM-Cellulose | 47 | 48 | 184 | 3.8 | 1.9 | 81 |
| Sephadex G-100 | 188 | 15 | 194 | 12.9 | 6.5 | 85 |
| DEAE-Sephadex | 40 | 6.6 | 143 | 21.7 | 10.8 | 63 |

6.6 mg enzyme, which is shown to be homogenous by the criteria of SDS-PAGE. The enrichment factor is about 11-fold, with a yield of 63%.

## Molecular Mass and Subunits

Arginine kinase most often occurs as a monomeric or dimeric protein. In order to determine the molecular size and number of subunits, we determined the molecular mass for the native and denatured protein. Based on results from the elution profile obtained using a calibrated Sephadex G-100 column, the molecular mass of AK is 43,000 (Fig. 1A). The molecular mass obtained from an analysis of mobility in SDS-PAGE is 43,000 (Fig. 1B and C). The results indicate that AK from the cockroach is a monomeric protein.

## Sulfhydryl Groups

Titration of AK with 5'dithiobis[2-nitrobenzoic acid] was biphasic. A rapid initial reaction, complete within 2 sec of mixing, constituted 60% of the total dinitrothiobenzoate produced. After 10 min, the reaction was essentially complete. The total change in absorbance of dinitrothiobenzoate, corrected for both protein and reagent blanks, corresponded to 0.82 mol of sulfhydryl for 1.0 mol of AK.

## Heat Inactivation

Arginine kinase activity was unaffected by heating for one minute at 65°C, but lost all activity when heated at 70°C for the same period (Fig. 2A).

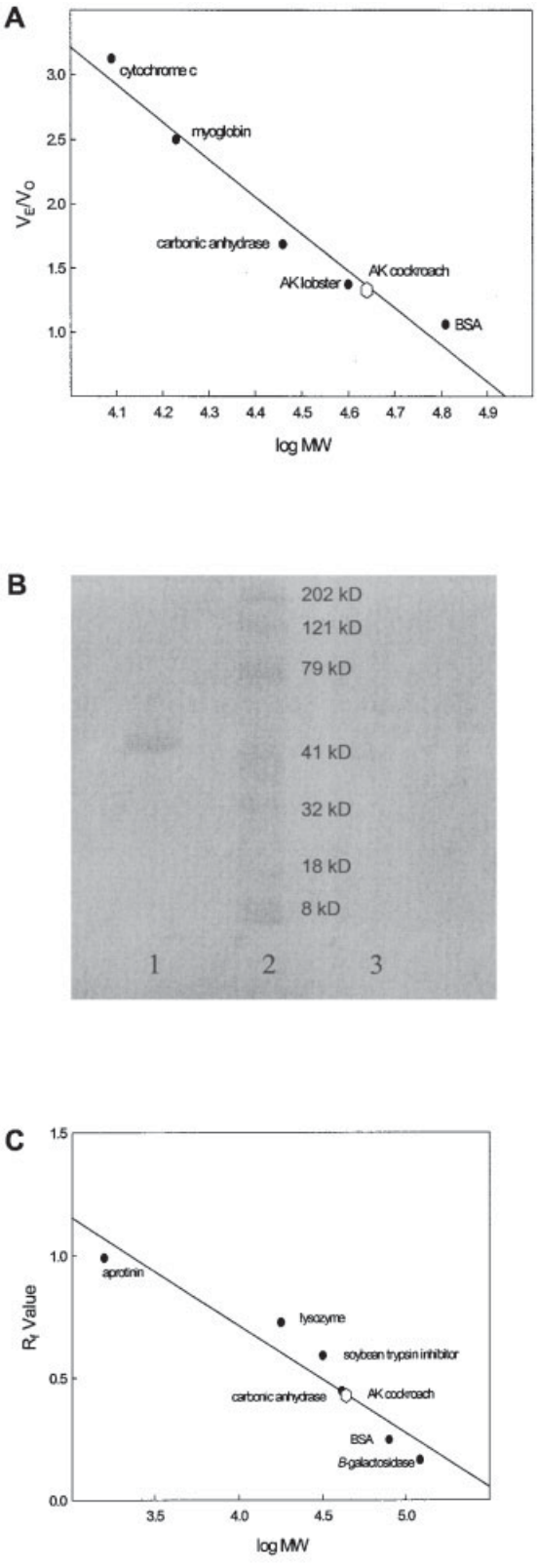


Fig. 1. Determination of molecular mass of AK. Gel filtration chromatography and SDS polyacrylamide gel electrophoresis: **A:** Native AK. A sample (0.3 mL) of AK (0.5 mg/ml) was placed on a column of Sephadex G-100 (2 × 53 cm) previously equilibrated and developed with 0.01 M Tris/HCl, pH 8.0, 10 mM mercaptoethanol, 1 mM EDTA, 5 µM $NaN_3$, and 25 µM PMSF. Fractions of 1.0 mL were collected. **B:** SDS/PAGE of purified AK. Samples of AK (**Lane 1**), molecular mass standards (**Lane 2**), and ovalbumin (**Lane 3**) were subjected to electrophoresis according to the method of Laemmli (1970). **C:** Calibration curve for results of SDS/PAGE illustrated in B.

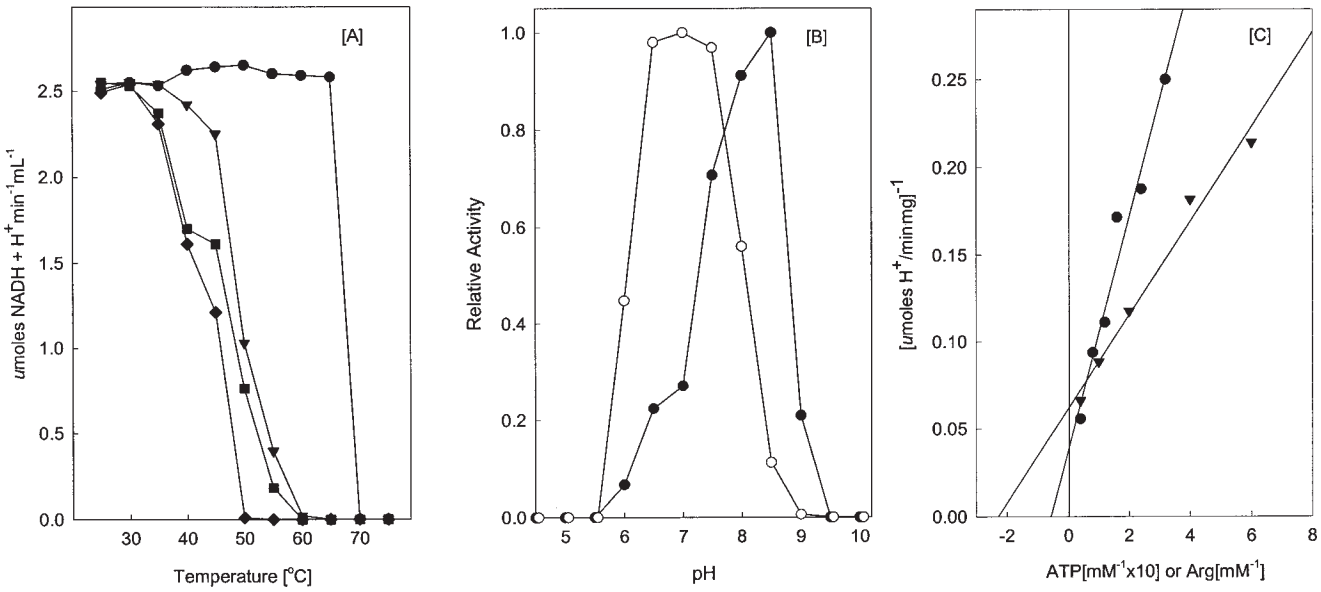


Fig. 2. **A:** Thermal inactivation. Crude extracts (25% tissue homogenate in buffer A) were centrifuged (4,900*g*), heated for various times, and rapidly cooled in ice. Arginine kinase activity was determined spectrophotometrically as described previously: circles, 1 min; triangles, 5 min; squares, 10 min; diamonds, 15 min. **B:** Dependence of AK activity on pH. Activity in the reverse direction (open circles) was determined using the colorimetric thio-NAD assay. A sample of AK (72 µg) was added to assay reagents prepared with buffers over the following ranges: 0.1 M sodium acetate buffer, pH 4.5–6.0, 0.1 M sodium phosphate buffer, pH 6.5–7.5, and 0.1 M Tris/HCl buffer, pH 8.0–10.0. The pH stat assay was used for activity measurements in the forward direction (closed circles). **C:** Determination of $K_m$ values for the forward reaction. The enzymatic activity was measured for the forward reaction using the pH stat assay. The reaction mixture contained variable concentrations of arginine, ATP, with magnesium acetate in 1.0 mM excess above nucleotide. ATP (closed circles); arginine (triangles). Analysis of the reverse reaction (not shown) used the enzyme-linked assay that contained variable concentrations of phosphoarginine, ADP, and magnesium acetate. The magnesium acetate concentration was held in 1.125 mM excess above the ADP to account for magnesium ADP binding (4,150 $M^{-1}$) (Morrison et al., 1961).

Longer exposure times to temperatures over the range of 37° to 60°C were required for complete inactivation. Approximately 50% of the activity was lost when AK is heated at 50°C for 10 min.

## pH optima

Activity as a function of pH was determined in the forward direction using the pH stat assay and in the reverse direction using the enzyme-linked spectrophotometric assay (Fig. 2B). The dependence of activity on pH for the forward reaction (phosphoarginine synthesis) exhibits a maximum at pH 8.5. The pH optimum for the reverse reaction (ATP synthesis) is somewhat broader, occurring between pH 6.5 and 7.5.

## $K_m$ Values

The Michaelis constants were determined for the AK reaction in the forward and reverse directions. Figure 2C illustrates a Lineweaver Burk plot for typical rate data obtained for the reverse reaction. The values derived are: $K_m^{MgATP}$ = 0.14 mM; $K_m^{arginine}$ = 0.49 mM; $K_m^{MgADP}$ = 0.09 mM; $K_m^{phosphoarginine}$ = 0.94 mM.

## Absorption, Circular Dichroic, and Fluorescence Spectra

The absorption spectra of purified AK exhibits the transition bands characteristic of a protein without absorbing prosthetic groups or cofactors (Fig.

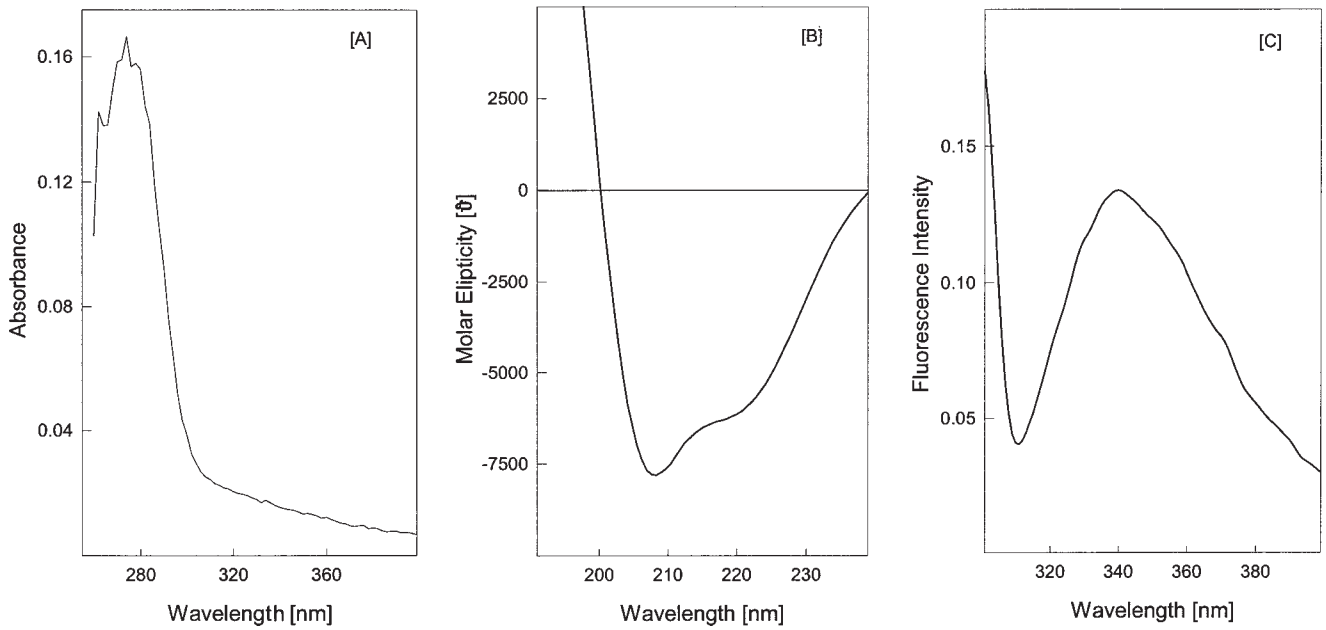


Fig. 3. **A:** Absorption spectrum. Sample of AK (0.22 mg/mL) in 0.1 M Tris/HCl, 10 mM mercaptoethanol, pH 8.0, was scanned from 250 to 600 nm. **B:** Circular dichroism spectrum. Sample of 0.25 mg/mL in 2 mM potassium phosphate, 1 mM mercaptoethanol, pH 8.0. **C:** Intrinsic fluorescence spectrum: excitation at 295 nm, sample of AK (0.1 mg/mL in 0.1M Tris/HCl, 2 mM DTT, pH 8.0).

3A). A 1% solution of AK is equivalent to an absorbance of 7.0 based on the protein concentration and absorbance at 280 nm. The circular dichroic spectra displays minima at 222 and 208 nm (Fig. 3B). From calculations using equation $f_n = \theta_{222} + 2{,}340/30{,}300$ (Chen et al., 1972), a value of 12% α-helical content is derived. The relative fluorescence, determined at an excitation wavelength of 295 nm, shows an emission maximum at 340 nm (Fig. 3C), characteristic of tryptophan residue fluorescence.

## DISCUSSION

Arginine kinase occurs in monomeric and dimeric forms in a wide range of invertebrate organisms (Moreland et al., 1967). Many invertebrates contain other phosphagens, which can function as substrates for phosphotransferases, although vertebrate kinases appear to be limited to creatine as the phosphate acceptor (Ellington, 1989). The distribution data for local species complement the table assembled by Morrison (1973), which includes listing the number of polypeptide subunits. Interestingly, all species investigated that failed to cross-react with antibodies against shrimp AK are in Phyla that contain organisms possessing only dimeric AK (Morrison, 1973). The broad distribution of AK in the invertebrate world attests to its essential role in biological energy transduction. The ubiquity of phosphagen transferase activities in invertebrates suggests a role analogous to that of the more well-characterized creatine kinases found in vertebrates. Metabolic functions proposed include participation in a high energy buffer system (Bessman and Geiger, 1981) and in the regulation of glycolytic flux (Ouellet and Shoubridge, 1992 ). Results show that equivalent activities of AK from the cockroach and shrimp stain much more intensely with Coomassie blue for the shrimp AK protein after protein separation by SDS-PAGE. This result is consistent with a preliminary conclusion that AK is a potentially more efficient enzyme for the cockroach, possibly related to high metabolic demands for rapid ATP production.

The pH optima for the forward and reverse catalyzed reactions for AK from the cockroach are in the range reported for other phosphagen kinases. These include AK from the shrimp (France et al., 1997) and the sea cucumber (Seals and Grossman, 1987) as well as several species summarized by Morrison (1973). Numerous structural and catalytic features of AK contribute to this pH dependence of activity, and as pointed out by Morrison, the effects of pH on the substrate ADP and cofactor magnesium ion are significant. The narrower pH optimum in the forward direction, compared to the more acidic and broader pH optimum in the reverse direction of catalysis, has been observed in other species (Virden and Watts, 1964; France et al., 1997). The fact that the optimum pH for AK from numerous species falls within the same range, suggests that the catalytic mechanism is conserved over a range of organisms populating different environments.

In comparison with AK from other species, AK from the cockroach is relatively thermostable. Several species, including the sea cucumber (Seals and Grossman, 1987) and shrimp (France et al., 1997) are totally inactivated after heating for 10 min at 50°C, while under these conditions the enzyme from cockroach retains about 50% of its activity. On the other hand, AK from the tarantula is still nearly fully active when heated under these conditions (Blethens and Kaplan, 1968). The thermal stability of AK in the cockroach may be an adaptation consistent with its well-known tolerance of different environments.

The number of reactive sulfhydryl groups in the native AK is closest to one for the cockroach but may range up to six in other species, as in Sipunculid (Raghupathi et al., 1991). The finding of one reactive SH group per polypeptide chain is consistent with that observed for dimeric CK (Watts, 1973; Grossman, 1983) and it generally appears to be the case that phosphagen kinases contain at least one highly reactive sulfhydryl at or near the active site. The role of the reactive sulfhydryl is unclear, but at least one report for AK suggests that it is essential for catalysis (Roustan et al., 1970). The biphasic kinetics observed for the reaction between AK and 5,5″-dithiobis[2-nitrobenzoic acid] suggests that more than one conformer of the enzyme may exist under the reaction conditions. Previous studies (France and Grossman, 1996) have provided evidence for the occurrence of two conformational states of shrimp AK under native conditions.

The value of the extinction coefficient for AK ($A^{1\%,1cm}$ = 7.0) from cockroach is similar to that for the enzyme from the shrimp (France and Grossman, 1996) and lobster (Roustan et al., 1971), though somewhat lower than that of the creatine kinases (Grossman and Mollo, 1979). The value of 12% α-helical content for cockroach AK is obtained from calculations based on the circular dichroic spectrum. Together with the 16% value determined for the shrimp enzyme (France and Grossman, 1996), these results indicate that these monomeric phosphagen kinases are lower in α-helical content than dimeric CK-MM (18%), CK-BB (22%), and CK-MB (25%) (Grossman and Sellers, 1998). Preliminary investigations indicate that dimeric AK from the sea cucumber (*Isostyopus badontus*) has 23% α-helix. Lastly, the fluorescence emission spectrum of native cockroach AK exhibits a maximum (340 nm) wavelength usually interpreted to indicate that the tryptophan residues, on average, are shielded from the solvent.

The allergic reaction by individuals to shrimp is, in part, a response to ingestion of AK (Crespo et al., 1995), even though AK is apparently strongly susceptible to chymotryptic hydrolysis (Yu et al., 2003; Ercan and Grossman, 2003). Cockroach AK is also a widespread allergen in humans (Binder et al., 2001) responsible for asthmatic reactions among children residing in areas of high infestation. The simple, high yield and rapid purification of AK will allow for additional studies of physical, kinetic, and immunological properties. Since arginine kinase catalytic activity is essential for motility of the cockroach, a domestic and agricultural pest, and since arginine kinase protein is a serious antigen, this protein seems a reasonable target for development of agents useful in population control. Ongoing investigations are aimed at targeting arginine kinase activity through substrate and transition state analogs.

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## LITERATURE CITED

Anosike EO, Watts DC. 1976. Effects of arginine and some analogues on the partial adenosine triphosphate-adenosine diphosphate exchange reaction catalyzed by arginine kinase. Biochem J 155:689–693.

Bessman SP, Geiger PJ. 1981. Transport of energy in muscle: the phosphorylcreatine shuttle. Science 211:448–452.

Binder M, Mahler V, Hayek B, Sperr WR, Scholler M, Prozell S, Wiedermann G, Valent P, Valenta R, Duchene M. 2001. Molecular and immunological characterization of arginine kinase from the indiameal moth, *Plodia interpunctella*, a novel cross-reactive invertebrate pan-allergen. J Immunol 167:5470–5477.

Blethen SL, Kaplan NO. 1968. Characteristics of arthropod arginine kinases. Biochemistry 7:2123–2135.

Bradford MM. 1976. A rapid and sensitive method for the quantitation of microgram quantities for protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254.

Chen YH, Yang JT, Martinez HM. 1972. Determination of secondary structure by circular dichroism and optical rotatory dispersion. Biochemistry 11:4120–4131.

Crespo JF, Pascual C, Helm R, Snachez-Pastor S, Ojeda I, Romualdo L, Martin-Esteban M, Ojeda JA. 1995. Cross-reactivity of IgE-binding components between boiled Atlantic shrimp and German cockroach. Allergy 50:918–924.

Dunbar BS. 1994. Protein blotting: a practical approach. In: The practical appraoch series. New York: Oxford Press. p 242.

Dunbar BS, Schwoebel ED. 1990. Preparation of polyclonal antibodies. Methods Enzymol 182:663–670.

Elligton WR. 1989. Phosphocreatine represents a thermodynamic and functional improvement over other muscle phosphagens. J Exp Biol 143:177–179.

Ellman GL. 1959. Tissue sulfhydryl groups. Arch Biochem Biophys 82:70.

Ercan A, Grossman SH. 2003. Proteolytic susceptibility of creatine kinase isozymes and arginine kinase. Biochem Biophys Res Commun 306:1014–1018.

Florini JR. 1989. Assay of creatine kinase in microtiter plates using thio-NAD to allow monitoring at 405nm. Anal Biochem 182:399–404.

France RM, Grossman SH. 1996. Denaturation and urea gradient gel electrophoresis of arginine kinase: evidence for a collapsed-state conformation. Arch Biochem Biophys 326:93–99.

France RM, Sellers DS, Grossman SH. 1997. Purification, characterization, and hydrodynamic properties of arginine kinase from Gulf Shrimp (*Penaeus aztecus*). Arch Biochem Biophys 345:73–78.

Grossman SH. 1983. Interaction of creatine kinase from monkey brain with substrate: analysis of kinetics and fluorescence polarization. J Neurochem 41:729–736.

Grossman SH, Mollo E. 1979. Physical and serological comparison and hybridization of isozymes of creatine kinase from primates. Int J Biochem 10:367–381.

Grossman SH, Sellers DS. 1998. Subunit conformation and dynamics in a heterodimeric protein: studies of the hybrid isozyme of creatine kinase. Biochim Biophys Acta 1387:447–453.

Laemmli UK. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680–685.

Moreland G, Watts DC, and Virden R. 1967. Phosphagen kinases and evolution in the Echinodermata. Nature 214:458–462.

Morrison JF. 1973. Arginine kinase and other invertebrate guanidino kinases. In: The enzymes, vol. 8. New York: Academic Press. p 457–486.

Morrison JF, O'Sullivan J, Ogston AG. 1961. Kinetic studies of the activation of creatine phosphoryltransferase by magnesium. Biochim Biophys Acta 52:82–96.

Ouellet M, Shoubridge EA. 1992. Phosphocreatine-dependent protein phosphorylation in rat skeletal muscle. Bochem J 284:115–122.

Raghupathi RR, Roustan C, Benyamin Y. 1991. Purification and properties of two molecular forms of arginine kinase from the adductor muscle of the scallop, *Pecten maximus*. Comp Biochem Physiol 99:387–394.

Robyt JF, White BJ. 1987. Theory and Practice. In: Biochemical techniques. Montery, CA: Brooks/Cole Publishing Co. p 242–244.

Rosalki, SB. 1967. An improved procedure for creatine phosphokinase determination. J Lab Clin Med 69:696–705.

Rosenthal GA, Dailman DL, Robinson GW. 1977. L-Arginine kinase from tobacco hornworm, *Manduca sexta* (*L.*). J Biol Chem 352:3679–3583.

Roustan C, Pradel L, Kassab R, Fattoum A, Thoai NV. 1970. Spectrometric investigations of the interaction of native and chemically modified ATP: Guanidinophosphotransferases with their substrates. Biochim Biophys Acta 206:369–379.

Roustan C, Pradel L, Kassab R, Nguyen T. 1971. Studies on the partial exchange and overall reactions catalyzed by native and modified arginine kinases from *Homarus vulgaris* muscle. Biochim Biophys Acta 250:103–106.

Seals JD, Grossman SH. 1987. Purification and characterization of arginine kinase from the Sea Cucumber *Caudina arenicola*. Comp Biochem Physiol B 250:103–116.

Suzuki T, Yamamoto Y, Umekawa M. 2000. *Stichopus japonicus* arginine kinase: gene structure and unique substrate. Biochem J 351:579–585.

Watts DC. 1973. Creatine kinase (adenosine 5′-triphosphate-creatine phosphotransferase), vol. 8. In: Boyer PD, editor. The enzymes. New York: Academic Press. p 383–455.

Virden R, Watts DC. 1964. The distribution of guanidine adenosine triphosphate phosphotransferase and adenosine triphosphatase in animals from several phyla. Comp Biochem Physiol 13:161–177.

Yu CJ, Lin YF, Chaing BL, Chow LP. 2003. Proteomics and immunological analysis of a novel shrimp allergen, Pen m 2. J Immunol 170:445–453.