CHAPTER 4

ARGININE KINASE
Contracting muscle fibers and impulse conducting neurons require extremely large stores of ATP for rapid consumption at times of demand. Such large stores of ATP, however, are likely to throw the cellular metabolic regulatory mechanisms out of gear. Consequently, the limited ATP stores in these cells are supplemented by stores of phosphagen. Phosphagens and their kinases together constitute an efficient ATP-regenerating system of cells, particularly in muscle and brain (Watts, 1971, 1975). Phosphagens are N-phosphorylated guanidino compounds and at present seven different naturally occurring phosphagens have been reported: phosphocreatine, phosphoarginine, phosphotaurocyamine, phosphoglycocyamine, phospholombricine, phosphohypotaurocyamine and phosphoopheline (Watts, 1971, 1975). The enzymes involved in the formation of these phosphagens have been given the generic name phosphagen kinases or ATP : guanidino phosphotransferases. They catalyze the reversible transfer of the terminal phosphoryl group of ATP to a guanidino compound according to the following reaction:
Guanidine + ATP $\xrightarrow{\text{Mg}^{++}}$ Phosphoguanidine + ADP

The distribution of phosphagens and the respective phosphagen kinases in the animal kingdom and its evolutionary implications have been reviewed by Watts (1971, 1975). While all vertebrates contain phosphocreatine as the phosphagen, invertebrates other than echinoderms and annelids have phosphoarginine. Both phosphocreatine and phosphoarginine are present in echinoderms, but all the seven phosphagens occur in different species of annelids.

Arginine kinase (ATP : L-arginine phosphotransferase, E.C. 2.7.3.3), the phosphagen kinase that catalyzes the reversible transfer of the terminal phosphoryl group of ATP to arginine, has been purified and characterized in molluscs and echinoderms (Morrison, 1973). Among the arthropods arginine kinase has been characterized either in pure preparations or in crude extracts from the muscles of the crustaceans Homarus vulgaris (Pradel et al., 1964; Virden et al., 1965, 1966; Virden and Watts, 1966; Der Terrasian et al., 1969; Regnouf et al., 1969; Oriol et al., 1970), Homarus americanus (Klethen and Kaplan, 1967, 1968), Jasus verreauxi (Morrison et al., 1957; Griffiths et al., 1957; Uhr et al., 1966), Callinectus sapidus (Klethen and
Kaplan, 1963) and Cancer pagurus (Oriol et al., 1970), of
the insects Calliphora erythrocephala (Lewis and Fowler, 1962),
Arvis melliphera (Carlson et al., 1971; Cheung, 1971, 1973),
Melanopus bruneri, Partheria dispar and Sympetrum
rubicundulum (Blethen and Kaplan, 1968) and the xiphosuran
Limulus polyphemus (Blethen and Kaplan, 1968; Blethen, 1972).
The only study on arachnid arginine kinase was that of
Blethen and Kaplan (1968) who found two arginine kinase
isozymes of similar molecular weight in the tarantula
Dugesiella bentzi and the spider, Pholcus phalangoides.

The present study deals with the distribution and
properties of arginine kinase in the tissues of the scorpion.

MATERIALS AND METHODS

Chemicals:

Some of the chemicals used in the present study were
obtained as follows: L-arginine, creatine and glycocyanine
from the British Drug Houses, Poole, England; agmatine and
adenosine triphosphate (ATP) from Sigma Chemicals Co.,
London, U.K.; acrylamide from the CSIR Center for Biochemicals,
Delhi; N N' N'-tetramethylethlenediamine from E. Merck
(India) and N N'-methylene bisacrylamide from Koch-Light
Bicine (N,N-Di-(2-hydroxyethyl)glycine) was prepared by the reaction of sodium chloroacetate with diethanolamine and recrystallized (Kumudavalli et al., 1970).

Preparation of homogenates:

Tissue extracts were prepared according to Watts and Moreland (1970). The whole animal was frozen in the deep-freeze for 30 minutes. Then tissues were excised rapidly and homogenized in cold 0.002 M bicine-NaOH buffer (pH 8.5) containing 1 mM mercaptoethanol. The homogenates (10%, w/v) were appropriately diluted with the same buffer and centrifuged at 20,000 g for 20 minutes at 5°C. The supernatants were used for arginine kinase assay.

Arginine kinase assay:

The activity of the enzyme in tissue extracts was assayed in the direction of phosphoarginine synthesis by the method of Watts and Moreland (1970). The reaction mixture consisted 25 mM L-arginine, 0.05 ml enzyme extract, 1 mM of ATP and 4 mM magnesium acetate in a total volume of 0.4 ml. L-Arginine, ATP and magnesium acetate solutions were prepared in 0.1 M bicine-NaOH buffer (pH 8.5). The reaction
was started by adding ATP/Mg$^{++}$ mixture (freshly prepared). After incubation at 37°C for 10 minutes, the reaction was terminated by adding 0.5 ml of an ice-cold solution of sodium chloroacetate (0.16 M), the pH of which was adjusted to 2.5 with perchloric acid. The tubes were immediately transferred to an ice bath. The phosphoguanididine formed in the reaction was hydrolyzed by heating the tubes for exactly one minute in a boiling water bath and then returning them back to the ice bath. After 5 minutes of temperature equilibration, phosphate liberated was colorimetrically determined according to Watts and Moreland (1970). Minus substrate and minus enzyme controls were set up to correct for the contribution of ATPase in tissue extracts and of ATP to the inorganic phosphate. Enzyme activity was expressed as µmoles product formed per hour per gram tissue or mg protein.

**Polyacrylamide gel electrophoresis**

For electrophoretic study, pedipalp muscle homogenates (10%, w/v) were prepared in 0.002 M tris-chloride buffer (pH 8.5) containing 10 mM mercaptoethanol. The homogenate was centrifuged at 20,000 g and 5°C for 20 minutes and supernatants were subjected to electrophoresis.
Polyacrylamide gel electrophoresis was carried out according to Truman (1968). The gel solution was prepared by dissolving 6.82 gms of acrylamide and 0.184 gms of N,N'-methylene bisacrylamide in 99 ml of 0.38 M tris-chloride buffer (pH 8.9) containing 10 mM mercaptoethanol. N,N,N',N'-Tetramethylethylenediamine (TEMED) was added and the solution was filtered. Polymerization of the filtered solution was accomplished by adding 1 ml of 7% (w/v) ammonium persulphate. The gels were polymerised at room temperature in glass tubes (0.5 cm x 11.5 cm).

The tank buffer consisted of 0.038 M glycine, the pH of which was adjusted to 8.3 by the addition of Tris. Mercaptoethanol was added to the tank buffer to give a final concentration of 10 mM. Electrophoresis was carried out in a rectangular electrophoresis chamber (Toshniwal Bros. Pvt. Ltd., Bombay) that holds the tube gels vertically.

The gels were subjected to pre-electrophoresis for 30 minutes at 4°C and 6 mA/tube (270 V). 50 µl of muscle extract containing 10% sucrose and traces of bromophenol blue (as marker dye) was applied on top of the gel with the help of a microsyringe (Hamilton Micromeasure, Boradur, Switzerland). The space above the homogenate was filled
with tank buffer slowly with syringe without disturbing the sample layer.

Anodal electrophoresis (top cathode and bottom anode) was carried out at 5°C initially for 15 minutes at 1.5 mA/tube and then at 6 mA/tube (270 V) till the marker dye reached the lower end. This usually took 2.5 hours.

After electrophoresis the gels were removed on a clean glass plate and cut into 0.2 mM sequential slices starting from the bromophenol blue (anodal) end of the gel. Considering the gel segment containing bromophenol blue as zero the most anodal slice adjacent to it was denoted as 1 according to the recommendations of the Enzyme Nomenclature (Herzfeld and Raper, 1976). Each slice was homogenised in 0.5 ml of 0.002 M tris-chloride buffer (pH 3.5) containing 10 mM mercaptoethanol. 0.1 ml of this gel suspension was added to 0.7 ml of a reaction mixture containing L-arginine, ATP and magnesium acetate at final concentrations of 25 mM, 4 mM and 4 mM respectively. After incubation for 10 minutes at 37°C the reaction was terminated by adding 1 ml of stopping buffer (Watts and Moreland, 1970). The reaction mixtures were centrifuged and phosphoguanidine in 0.5 ml aliquots of supernatants was hydrolysed by immersing the
tubes in boiling water bath for 1 minute. 0.4 ml distilled water was added to each tube and phosphate determined as described by Watts and Moreland (1970).

Molecular weight of arginine kinase:

Molecular weight of scorpion muscle arginine kinase was determined by gel filtration on Sephadex G-100 (Andrews, 1964, 1965). The preparation and calibration of the gel column has been described in Chapter 2 (pages 43-44).

Homogenates (10%, w/v) of scorpion pedipalp muscle and rat sartorius muscle, prepared in 0.05 M tris-chloride buffer (pH 7.5) containing 0.1 M KCl, were centrifuged at 20,000 g and 5°C for 10 minutes and the supernatants loaded on the Sephadex G-100 column after appropriate dilution with the same buffer. Fractions (2 ml) were collected. Aliquots of the fractions were assayed for arginine kinase and creatine kinase activities. Arginine kinase assay has been described above. For creatine kinase assay, L-arginine in the reaction mixture was replaced by creatine (Watts and Moreland, 1970). Otherwise the assay methods for both enzymes were identical (Watts and Moreland, 1970).
RESULTS

In the arginine kinase assay with scorpion pedipalp muscle extracts, product (phosphoarginine) formation was linear up to 15 minutes of incubation. The product formation also increased linearly up to 0.05 ml (0.1%, w/v) homogenate as a function of enzyme concentration (Fig. 4.1).

Appearance of inorganic phosphate under the assay conditions was absolutely dependent on ATP, but omission of L-arginine, Mg^{++} or homogenate reduced inorganic phosphate appearance to 16-18% suggesting non-specific release of inorganic phosphate from ATP (Table 4.1). Hence minus-arginine and minus-enzyme controls were routinely employed to correct for this non-specific appearance of inorganic phosphate in the reaction.

Of the four magnesium salts tested, magnesium acetate (MgAc) was the most effective in the reaction (Table 4.2). MgSO_4 and MgCl_2 gave only 91-92% as much activity as with MgAc. Mg(NO_3)_2 gave 24% less activity than MgAc. Calcium acetate was only one-fourth as effective as magnesium acetate (Table 4.3).

No significant phosphorylation of agmatine, glyco- 
cysteine and creatine was observed with scorpion pedipalp
Effect of enzyme concentration (A) and incubation time (B) on scorpion muscle arginine kinase activity. Different volumes of 0.1% (w/v) homogenate (0.01, 0.02, 0.03, 0.04 and 0.05 ml corresponding to 0.0007, 0.0014, 0.0021, 0.0028 and 0.0035 mg protein respectively) were incubated for 10 minutes at 37°C. Similarly, 0.05 ml of 0.1% (w/v) homogenate of scorpion pedipalp muscle was incubated at 37°C for different periods. In both cases phospho-arginine formed was measured.
FIG. 4.1
0.05 ml of 0.1% homogenate was incubated for 10 minutes in the standard assay system and phosphoarginine formation was measured. Relative activities are shown in the parentheses.

<table>
<thead>
<tr>
<th>Reaction mixture</th>
<th>μmoles product/hr/mg protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete</td>
<td>852 (100)</td>
</tr>
<tr>
<td>Minus arginine</td>
<td>150 (13)</td>
</tr>
<tr>
<td>Minus homogenate</td>
<td>136 (16)</td>
</tr>
<tr>
<td>Minus ATP</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Minus Mg^{++}</td>
<td>154 (18)</td>
</tr>
<tr>
<td>Minus ATP and Mg^{++}</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>
In the assay system Mg acetate was replaced by MgSO₄, MgCl₂ and Mg(NO₃)₂ and product formed was measured. Relative activities are given in parentheses.

<table>
<thead>
<tr>
<th>Mg salts</th>
<th>μmoles product/hr/mg protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mg acetate</td>
<td>768 (100)</td>
</tr>
<tr>
<td>Mg sulphate</td>
<td>706 (92)</td>
</tr>
<tr>
<td>Mg chloride</td>
<td>699 (91)</td>
</tr>
<tr>
<td>Mg nitrate</td>
<td>583 (76)</td>
</tr>
</tbody>
</table>
Effect of Mg acetate and Ca acetate on arginine kinase activity of scorpion pedipalp muscles.

In the assay system Mg acetate was replaced by Ca acetate and product formation measured. Relative activities are given in parentheses.

<table>
<thead>
<tr>
<th>Acetate</th>
<th>μmoles product/hr/mg protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mg acetate</td>
<td>1037 (100)</td>
</tr>
<tr>
<td>Ca acetate</td>
<td>259 (25)</td>
</tr>
</tbody>
</table>
muscle extracts in the presence of ATP (Table 4.4). The activity with these guanidine compounds was less than 0.5% of that with L-arginine.

The arginine kinase activity in scorpion pedipalp muscle extracts, assayed in the direction of phosphoarginine formation, showed a pH optimum of 8.5 (Fig. 4.2).

The arginine saturation curve of the enzyme is shown in Fig. 4.3. The 25 mM L-arginine concentration employed in the assays routinely, was sufficient to saturate the enzyme. Increasing the concentration of L-arginine from 25 mM to 50 mM caused slight inhibition (9%) of the enzyme. The apparent Michaelis-Menten constant of the enzyme for L-arginine determined from the Lineweaver-Burk double reciprocal plot was 1.0 mM. This Km was determined at a ATP:Mg++ concentration ratio of 1:1 (4 mM concentration of both).

The saturation curve of arginine kinase for its cosubstrate, ATP, at fixed concentrations of L-arginine (25 mM) and Mg++ (4 mM) is shown in Fig. 4.4. The 4 mM concentration of ATP used in routine assays gave maximum activity which decreased by 40-50% when ATP concentration
Substrate specificity of scorpion muscle arginine kinase.

L-arginine in the reaction mixture was replaced by other guanidino compounds and product formation measured. Relative activities are given in parentheses.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>μmoles product/hr/mg protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-arginine</td>
<td>1017 (100)</td>
</tr>
<tr>
<td>Agmatine sulphate</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Glycocystamine</td>
<td>3 (0.3)</td>
</tr>
<tr>
<td>Creatine</td>
<td>2 (0.2)</td>
</tr>
</tbody>
</table>
Effect of pH on arginine kinase activity in scorpion pedipalp muscles. 0.05 ml of 0.1% (w/v) homogenate of scorpion pedipalp muscle was incubated at 37°C for 10 minutes. The reaction mixture contained 88 mM bicine-NaOH buffer of different pH. The pH values indicated represent those measured in the complete reaction mixtures during incubation.
Paper electrophoretogram showing the presence of spermidine in scorpion venom. A: standard mixture of (1) spermine (2.5 nmoles), (2) spermidine (2.5 nmoles) and (3) putrescine (5.0 nmoles); B: 5 nmoles spermidine; C: a 20 μl venom sample equivalent to 5 μl whole venom; D: a mixture of 2.5 nmoles spermidine and 10 μl venom sample equivalent to 2.5 μl whole venom. The points of sample application, anode and cathode are indicated respectively by X, (+) and (-).
Fig. 4.2

$\mu$ moles/hr/mg protein

pH

7.0 7.5 8.0 8.5 9.0 9.5 10.0
Effect of L-arginine concentration on arginine kinase activity in scorpion muscle. The enzyme activity (V) was assayed in the presence of different concentrations of L-arginine (S) at fixed concentrations of ATP (4 mM) and magnesium acetate (4 mM). S versus V plot is shown in Fig. A and Lineweaver-Burk double reciprocal plot in Fig. B.
Effect of ATP concentration on arginine kinase activity in scorpion muscles. The arginine kinase activity (V) was assayed in the presence of different concentrations of ATP (S) and at fixed concentrations of L-arginine (25 mM) and magnesium acetate (4 mM). S versus V plot is shown in Fig. A and double reciprocal plot in Fig. B.
was increased from 3-16 mM. The apparent Km values of the enzyme for ATP determined from Lineweaver-Burk double reciprocal plots in two experiments were 0.08 mM and 0.17 mM.

The Mg\(^{++}\) saturation curve of arginine kinase is shown in Fig. 4.5. The 4 mM Mg\(^{++}\) concentration routinely used in assays was adequate to saturate the enzyme. From double reciprocal plots the apparent Km of the enzyme for Mg\(^{++}\) was calculated to be 0.88 mM. This value was determined at fixed concentrations of L-arginine (25 mM) and ATP (4 mM).

The gel filtration behaviour of scorpion muscle arginine kinase is shown in Fig. 4.6A. The enzyme emerged out as a single peak with a molecular weight of about 33,500. Rat muscle creatine kinase yielded a molecular weight of 80,000 on this column (Fig. 4.6A) which agreed well with the mol.wt. reported for vertebrate muscle creatine kinase by many workers (Watts, 1973). When a mixture of scorpion muscle extract and rat thigh muscle extract was passed through the Sephadex G-100 column (Fig. 4.6B), the arginine kinase and creatine kinase activity profiles were exactly similar to the profiles obtained when the two extracts were loaded on the column separately (Fig. 4.6A). The molecular
Effect of magnesium acetate concentration on arginine kinase activity. The enzyme activity (V) was assayed in the presence of different concentrations of Mg acetate (S) but at fixed concentrations of L-arginine (25 mM) and ATP (14 mM). S versus V plot is shown in Fig. A and Lineweaver-Burk double reciprocal plot in Fig. B.
Sephadex G-100 chromatography of scorpion muscle arginine kinase (open circles and continuous line) and rat muscle creatine kinase (closed circles and broken line). Scorpion (1 ml of 2.5%) and rat (1 ml of 10%) muscle extracts were separately loaded on the gel column and the elution profiles of the two enzymes are shown in Fig. A. Fig. B shows the elution profiles of the two enzymes when a 1 ml mixture of scorpion (0.5 ml of 4%) and rat (0.5 ml of 7.5%) muscle extracts was loaded on the gel column. In both cases the fraction containing highest enzyme activity was arbitrarily assigned a value of 100 and the activities in other fractions were plotted relative to this. The elution volumes of dextran blue (1), rabbit muscle aldolase (2), bovine serum albumin (3), ovalbumin (4) and horse heart cytochrome c (5) are indicated by arrows.
Fig. 4.6

Volume (ml)
weight of scorpion muscle arginine kinase and rat muscle creatine kinase again turned out to be 33,500 and 30,000 respectively (Fig. 4.7). As a further check on the reliability of the molecular weight determined here for scorpion muscle arginine kinase, a mixture of scorpion muscle extract and horse heart cytochrome c were subjected gel filtration on the Sephadex G-100 column and their elution profiles are shown in Fig. 4.8. While cytochrome c eluted out at its usual position, arginine kinase activity peak lied between ovalbumin and cytochrome c. The molecular weight of scorpion muscle arginine kinase again turned out to be 33,500. Thus the molecular weight of scorpion muscle arginine kinase determined independently and in the presence of internal standards proved to be 33,500 in repeated experiments.

On polyacrylamide gel electrophoresis, arginine kinase activity in scorpion muscle extracts moved towards the anode suggesting that the enzyme is anionic at alkaline pH (8.3). Only a single peak of activity was found on the gels by the slicing method (Fig. 4.9).

**DISCUSSION**

Arginine kinase, like creatine kinase and other phosphagen kinases, is a thiol enzyme (Morrison, 1973).
Calibration of the Sephadex G-100 column for molecular weight determination of scorpion muscle arginine kinase (SAK) and rat muscle creatine kinase (RCK). Proteins used for calibration of the column were rabbit muscle aldolase (1), bovine serum albumin (2), ovalbumin (3) and horse cytochrome c (4).
Fig. 4.7

![Graph showing elution volume vs. molecular weight.](image-url)
Gel filtration behaviour of scorpion muscle arginine kinase (APK) on a column of Sephadex G-100 with horse heart cytochrome c as an internal standard. A mixture of 0.5 ml of scorpion muscle homogenate (4% w/v) and 0.5 ml of cytochrome c solution (10 mg/ml) was loaded on the column and arginine kinase activity was assayed in fractions (open circles). Cytochrome c was detected in fractions by its absorbance at 660 nm (closed circles). The elution volumes of dextran blue (1), rabbit muscle aldolase (2), bovine serum albumin (3), ovalbumin (4) and horse heart cytochrome c (5) are indicated by arrows. The fraction containing highest arginine kinase activity was arbitrarily assigned a value of 100 and the activities in other fractions were plotted relative to this.
Fig. 4.8

[Graph showing APK Relative Activity and Cytochrome c O.D. against Volume (ml) with peaks labeled 1, 2, 3, 4, and 5.]
Polyacrylamide gel electrophoretic behaviour of scorpion muscle arginine kinase. The gel was cut into slices after electrophoresis and the slices were numbered 1, 2, 3, 4 etc. from the bromophenol blue end (anodal end) to the cathodal end. The slice containing bromophenol blue was treated as 'C'. The anode and cathode are indicated by (+) and (−). The direction of electrophoresis is shown in the form of an arrow at the top. In the experiment shown here, 83% of the enzyme activity loaded was recovered on the gel after electrophoresis. In two other experiments the recoveries were lower (59% and 60%) but the enzyme activity profiles were similar to the one shown here.
Activity (% of total recovered)

Fig. 4.9
Table 4.5

Distribution of arginine kinase activity in scorpion tissues.

Values given are means ± standard deviations with ranges in parentheses below.

<table>
<thead>
<tr>
<th>Tissues (No. of observations)</th>
<th>μmoles product/hr/gm tissue</th>
<th>μmoles product/hr/mg protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pedipalp muscle (10)</td>
<td>53,045 ± 19,961</td>
<td>345 ± 180</td>
</tr>
<tr>
<td></td>
<td>(46,000 - 77,400)</td>
<td>(533 - 1138)</td>
</tr>
<tr>
<td>Hepatopancreas (7)</td>
<td>1,864 ± 339</td>
<td>17 ± 4</td>
</tr>
<tr>
<td></td>
<td>(1,380 - 2,220)</td>
<td>(15 - 25)</td>
</tr>
<tr>
<td>Alimentary canal (6)</td>
<td>2,125 ± 743</td>
<td>23 ± 10</td>
</tr>
<tr>
<td></td>
<td>(1,200 - 3,420)</td>
<td>(10 - 36)</td>
</tr>
<tr>
<td>Nervous system (6)</td>
<td>1,775 ± 514</td>
<td>17 ± 8</td>
</tr>
<tr>
<td></td>
<td>(1,200 - 2,400)</td>
<td>(11 - 31)</td>
</tr>
<tr>
<td>Heart (6)</td>
<td>5,005 ± 1,523</td>
<td>56 ± 22</td>
</tr>
<tr>
<td></td>
<td>(2,820 - 6,300)</td>
<td>(33 - 86)</td>
</tr>
</tbody>
</table>
Covalent modification of the thiol groups by alkylating agents results in inhibition of enzyme activity (Morrison, 1973; Reddy and Watts, 1979).

Phosphagen kinases are also activated by Mg$^{++}$ and this activation involves the non-enzymic formation of metal-nucleotide complexes (Mg$^{++}$-ATP or Mg$^{++}$-ADP) which act as substrates together with the free form of the guanidino compounds (Morrison, 1973; Watts, 1973). While Ca$^{++}$ can activate creatine kinase to some extent, its effect on arginine kinase is not certain and Morrison (1973) has pointed out that "further investigations of the ability of Ca$^{++}$ to activate arginine kinases would seem to be warranted". While Ca$^{++}$ activates, though to a smaller extent than Mg$^{++}$, arginine kinase from Homarus vulgaris (Virden et al., 1965), Limulus polyphemus (Blethen, 1972) and Apis mellifera (Cheung, 1971), it has no effect on the enzyme from the sea crayfish Jasus verreauxi (Morrison et al., 1957). Ca$^{++}$ is only 25% as effective as Mg$^{++}$ in activating scorpion arginine kinase (Table 4.3). However the non-specific agmatine-arginine kinase from the protozoans Euglena gracilis and Ochromonas danica is activated more by Ca$^{++}$ than by Mg$^{++}$ (Piccini and Coppellotti, 1977, 1978, 1979).
Of the four magnesium salts tested, magnesium acetate was the most effective in activating scorpion arginine kinase followed by MgSO₄, MgCl₂ and Mg(NO₃)₂ (Table 4.2). This is probably explained in the light of the observations of Watts and coworkers that acetate is an activator while sulphate and nitrate are inhibitors of rabbit muscle creatine kinase (Milner-White and Watts, 1971) and holothurian and lobster muscle arginine kinases (Anosike and Watts, 1975).

Arginine kinase appears to be the only phosphagen kinase present in scorpion muscle extracts. No phosphorylation of agmatine, glycocyamine and creatine could be detected, suggesting that scorpion muscle extracts do not possess agmatine kinase, glycocyamine kinase and creatine kinase activities and that scorpion arginine kinase is highly specific towards L-arginine. The presence of arginine kinase is in accordance with the report that phosphoarginine is the muscle phosphagen in the scorpion Androctonus australis (Lissitzky, 1954). Arginine kinase in the muscle extracts of many malacostracan crustaceans were reported to be highly specific for L-arginine among the natural guanidines (Virden and Watts, 1964). Purified arginine kinase from Simpunculus nudus (Lacombe et al., 1969), crustaceans (Morrison et al., 1957; Virden et al., 1965) and
honeybee (Cheung, 1971) also do not phosphorylate creatine, agmatine and glycocyamine. However, protozoans like Eulena gracilis and Ochromonas danica have a non-specific arginine kinase that can phosphorylate agmatine very well, but not creatine or glycocyamine (Piccini and Coppellotti, 1977, 1978, 1979).

Phosphagen kinases are known to show different pH optima for the forward and backward reactions (Morrison, 1973). For example, arginine kinase from the sea crayfish (Morrison et al., 1957) and the honeybee (Cheung, 1973) have pH optima respectively at 6.8-7.2 and 7.1-7.2, in the direction of ATP synthesis and at 8.4 and 8.3 in the direction of phosphoarginine synthesis. Scorpion muscle arginine kinase, like that from the blowfly (8.6, Lewis and Fowler, 1962) and the honeybee (8.3; Cheung, 1971), has a sharp pH optimum at pH 8.5 (Fig. 4.2) when measured in the direction of phosphagen synthesis. Arginine kinases from the lobster Homarus vulgaris (Virden et al., 1965) and the protozoan Stentor coeruleus (Watts et al., 1968) have broad pH optima for phosphoarginine formation.

The Michaelis constants of arginine kinase for either substrate is a function of the concentration of the
other substrate (Virden et al., 1965). A 1:1 ratio of ATP/Mg$^{++}$ is considered to be optimal for arginine kinases of lobster (Virden et al., 1965) and honeybee (Cheung, 1971). Hence the apparent Km for scorpion arginine kinase for L-arginine has been determined at this molar ratio of ATP/Mg$^{++}$. This apparent Km (1.0 mM) is about the same as those reported for arginine kinases from lobster, sipunculid, sea urchin (0.7-1.25 mM; Thoai et al., 1966) and sea crayfish (3.2 mM; Griffiths et al., 1957), but slightly higher than those reported for the enzyme from Limulus polyphemus (Blethen, 1972) and Anis melliphera (Cheung, 1973). The apparent Kms of the scorpion enzyme for ATP are comparable to those of Limulus polyphemus (Blethen, 1972) and Anis melliphera (Cheung, 1973). Increasing the ATP/Mg$^{++}$ ratio to 1:10 is known to inhibit arginine kinase from Homarus vulgaris (Virden et al., 1965) and Limulus polyphemus (Blethen, 1972), but no such inhibition has been noticed with the scorpion enzyme. However, significant inhibition of the enzyme has been found at higher concentrations of ATP (> 8 mM).

Extensive investigations have been made on the molecular weight of arginine kinases from a variety of invertebrate species using preparations with various
degrees of purity (Morrison, 1973). The results indicate that these enzymes can be divided into three classes, monomeric (mol.wt. 40,000), dimeric (mol.wt. 30,000) and tetrameric (mol.wt. 160,000), according to their molecular weights. Arginine kinases with molecular weights in the vicinity of 40,000 have been found in arthropods and molluscs, while 30,000 molecular weight forms occur in echinoderms and annelids (Morrison, 1973). The tetrameric arginine kinases (mol.wt. 150,000-166,000) are present in the polychaete worms Sabella pavonia and Spirographis spallanzani (Robin et al., 1969). The evolutionary and physiological significance of the distribution of different molecular forms of arginine kinase is not apparent at present. The molecular weight of 33,500 determined here by Sephadex G-100 chromatography for scorpion muscle arginine kinase is in agreement with the molecular weights (35,000-38,000) reported by Helethon and Kaplan (1968) for arginine kinase from several arthropods classes by the same technique. Thus the molecular weight of scorpion arginine kinase conforms to the rule that arthropod arginine kinases are monomeric (Morrison, 1973).

Based on a starch gel electrophoretic study, Virden and Watts (1964) suggested the presence of arginine
kinase isoenzymes in the muscle extracts of many invertebrates particularly crustaceans. Two forms of arginine kinase, negative and neutral arginine kinases (at pH 8.6), have been subsequently purified from the muscle of *Limulus polyphemus* (Blethen and Kaplan, 1968; Blethen, 1972). The two isoenzymes, which were suggested to be produced by different genes (Blethen, 1971), differed from each other only in their charges and stability, but have similar molecular weights (37,000-38,000), amino acid composition and kinetic properties. From a study of several arthropod species (five crustaceans, four insects, two arachnids and *Limulus polyphemus*), Blethen and Kaplan (1968) concluded that the presence of the neutral and negative forms of arginine kinases is characteristic of chelicerate arthropods (arachnids and *Limulus polyphemus*) while no such isoenzymes are found in crustaceans and insects. By polyacrylamide gel electrophoresis at pH 3.3-3.9, only the negative form of arginine kinase was detected in scorpion muscle extracts. There was no indication of the presence of the neutral form. Since the neutral arginine kinase is quantitatively smaller (Blethen and Kaplan, 1968) and also less stable (Blethen, 1972) than the negative form, it might have escaped detection on polyacrylamide gels.
The specific activity of arginine kinase in crude pedipalp muscle extracts of the scorpion (Table 4.5) is comparable to that in the claw and leg muscles reported by Blethen and Kaplan (1968) for several arthropods. Similarly, the activity of the enzyme in the hepatopancreas and alimentary canal of the scorpion are comparable to those reported by these workers in Homarus americanus. However, the activity of the enzyme in the heart muscle and nervous system are very low compared to the activity in the corresponding tissues of Homarus americanus (Blethen and Kaplan, 1968).

In general, arginine kinase of scorpion appears to be similar in its characteristics to the enzyme from other arthropods.