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# Partial purification and characterization of a soluble protein kinase from *Leishmania donovani* promastigotes

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**A soluble protein kinase from the promastigote form of the parasitic protozoon *Leishmania donovani* was partially purified using DEAE-cellulose, Sephadex G-200 and phosphocellulose columns. The enzyme preferentially utilized protamine as exogenous phosphate acceptor. The native molecular mass of the enzyme was about 85 kDa. $Mg^{2+}$ ions were essential for enzyme activity; other metal ions, e.g. $Ca^{2+}$, $Co^{2+}$, $Zn^{2+}$ and $Mn^{2+}$, could not substitute for $Mg^{2+}$. cAMP, cGMP, $Ca^{2+}$/calmodulin and $Ca^{2+}$/phospholipid did not stimulate enzyme activity. The pH optimum of the enzyme was 7·0–7·5, and the temperature optimum 37 °C. The apparent $K_m$ for ATP was 60 μM. Phosphoamino acid analysis revealed that the protein kinase transferred the γ-phosphate of ATP to serine residues in protamine. The thiol reagents *p*-hydroxymercuribenzoic acid, 5-5′-dithio-bis(2-nitrobenzoic acid) and *N*-ethylmaleimide inhibited enzyme activity; the inhibition by *p*-hydroxymercuribenzoic acid and 5-5′-dithio-bis(2-nitrobenzoic acid) was reversed by dithiothreitol.**

## Introduction

*Leishmania* are characterized by existing in two main forms – as an amastigote when in the cells of the vertebrate host and as a promastigote in the gut of the insect vector. *Leishmania donovani*, the aetiological agent of visceral leishmaniasis (also known as kala-azar) replicates in mononuclear phagocytic cells of the spleen, liver, lymph glands and bone marrow and produces a chronic disease which usually results in death in untreated cases.

Phosphorylation/dephosphorylation has long been recognized as a process of reversible, covalent protein modification. These reactions are thought to serve a regulatory function in the short-term control of a variety of cellular processes. Protein kinases have been classified according to the specific effector molecules that directly interact with them and regulate their function. Thus distinct classes of protein kinases are known to exist whose actions depend upon second messengers – cAMP, cGMP, $Ca^{2+}$/calmodulin and $Ca^{2+}$/phospholipid. In addition, there is a group of protein kinases which are apparently independent of control via specific regulatory effectors. Besides numerous reports describing protein kinases in animals, there are reports of protein kinase activity in protozoa (Majumder *et al.*, 1973), fungi (Pall, 1981), plants (Keates, 1973) and bacteria (Rahmsdorf *et al.*, 1974). Reports of protozoal protein kinases are few, but such enzymes have been characterized from *Plasmodium berghei* (Wiser *et al.*, 1983; Wiser & Schweiger, 1985), *Trypanosoma brucei* (Walter & Opperdoes, 1982), *T. gambiense* and *T. cruzi* (Walter, 1976; Gomez *et al.*, 1989). Das *et al.* (1986) characterized a cyclic-nucleotide-independent protein kinase in *L. donovani*. This enzyme is localized on the outer surface of the parasite and preferentially uses histone as exogenous phosphate acceptor. Berman (1988) recently isolated a protein kinase from *L. mexicana* promastigotes and amastigotes and compared it with the *L. donovani* enzymes reported by Das *et al.* (1986). Inosine analogues and their triphosphates compete with ATP for the enzyme. Gundersen & Nelson (1987) demonstrated a novel $Ca^{2+}$-dependent protein kinase from the ciliated protozoon *Paramecium tetraurelia*.

We have characterized and partially purified a soluble cyclic-nucleotide-independent protein kinase from *L. donovani* promastigotes and we report some of its properties in this paper.

*Abbreviations*: DTNB, 5,5′-dithio-bis(2-nitrobenzoic acid); NEM, *N*-ethylmaleimide.

## Methods

*Materials.* [$\gamma$-$^{32}$P]ATP [3000 Ci mmol$^{-1}$ (111 TBq mmol$^{-1}$)] was purchased from Bhaba Atomic Research Centre, Bombay, India. Sephadex G-200 and calmodulin were from Pharmacia. Mixed histones, protamine, casein, phosvitin, cAMP, cGMP, kemptide, PMSF, benzamidine.HCl, ATP, diolein, phosphatidylinositol and *p*-hydroxymercuribenzoic acid were obtained from Sigma. DTNB and NEM were obtained from Pierce. All other chemicals were of analytical grade and were purchased from Merck.

*Parasite.* Promastigotes of *Leishmania donovani* (MHOM/IN/1978/UR6), an Indian strain, highly subpassaged on modified Ray's blood-agar slants, were grown at 24–26 °C for 72 h (Ray, 1932). The cells were harvested when at late exponential phase, washed three times in isotonic cold phosphate-buffered saline (10 mM-potassium phosphate, 150 mM-NaCl, pH 7·4) containing 0·5 mM-PMSF, 1 mM-iodoacetic acid, 5 mM-benzamidine.HCl, and kept at −20 °C until use.

*Protein kinase assay.* Protein kinase activity was assayed routinely with protamine as the phosphate acceptor. The standard reaction mixture (0·2 ml) contained 50 mM-Tris/maleate buffer, pH 7·0, 20 mM-$MgCl_2$, 0·25 mg protamine, 150 $\mu$M-[$\gamma$-$^{32}$P]ATP (80–100 c.p.m. pmol$^{-1}$), 10 mM-DTT, 2·5 mg BSA ml$^{-1}$ and an appropriate amount of protein kinase and was incubated at 37 °C. After 10 min, 1 ml ice-cold 10% (w/v) trichloroacetic acid (TCA) was added and the mixture filtered through a glass microfibre filter paper (GF/C, Whatman) using a Millipore manifold apparatus fitted with a vacuum pump. The filter paper was washed twice with 5 ml ice-cold 5% TCA and finally analysed for radioactivity as described by Rubin *et al.* (1974). The units of protein kinase activity were defined as the incorporation into protamine of 1 pmol $^{32}$P min$^{-1}$ (except Table 1: 1 nmol min$^{-1}$).

When mixed histones and phosvitin were used as substrate, reactions were terminated with 1 ml ice-cold 10% TCA and phosphorylated products isolated as described above. The amount of $^{32}$P incorporated into casein was measured as described by Hathaway & Traugh (1983) after terminating the reaction by spotting 50 $\mu$l of the assay mixture on to ET31 (2 × 2 cm, Whatman) papers. When kemptide was used as substrate the reaction was terminated with 90 $\mu$l glacial acetic acid. The phosphorylated kemptide was measured according to the method of De La Houssaye and Masaracchia (1983). Membrane-bound histone kinase activity was determined using a high concentration of mixed histones as phosphate acceptor as suggested by Das *et al.* (1986).

*Purification of the protein kinase.* Unless otherwise stated all operations were done at 2–4 °C.

(*a*) *Extraction.* Frozen packed cells (about 10 ml) were suspended in 50 ml 20 mM-potassium phosphate buffer, pH 6·8, containing 1 mM-EGTA, 1 mM-iodoacetic acid, 5 mM-benzamidine.HCl, 10 mM-2-mercaptoethanol, 0·5 mM-PMSF, 0·02% sodium azide and 10% (v/v) glycerol (buffer I). The resulting suspension was homogenized [15 strokes at 1200 r.p.m. in a Potter S homogenizer (Braun Instruments)] and sonicated (45–60 W) for 20 s on a Labsonic 2000 sonifier, with 1 min intervals between each sonication. The homogenate was centrifuged at 100000 *g* for 90 min and the supernatant used for further purification.

(*b*) *DEAE-cellulose chromatography.* The pH and conductivity of the supernatant was adjusted and the sample applied to a DE-52 DEAE-cellulose column (2·5 × 20 cm) pre-equilibrated with buffer I. The column was washed extensively with 300 ml buffer I and the protein kinase was eluted with 500 ml of a linear (0–0·3 M) NaCl gradient in buffer I. Fractions (7 ml) of the eluate were collected. Fractions 24–30, which contained most of the protein kinase activity, were pooled and concentrated in an Amicon nitrogen pressure cell using a PM-30 membrane.

(*c*) *Sephadex G-200 filtration.* The concentrated pooled fractions from the DEAE-cellulose column were dialysed extensively against 20 mM-potassium phosphate buffer, pH 6·8, containing 150 mM-NaCl (buffer II) for 16–18 h. The dialysed sample was layered on to a Sephadex G-200 column (1·6 × 60 cm) pre-equilibrated with buffer II. The sample was eluted with the same buffer and fractions (about 8 ml) were collected. Fractions 8–12, which contained the protein kinase activity were pooled and concentrated.

(*d*) *Phosphocellulose column chromatography.* The pooled fractions from the Sephadex G-200 filtration were desalted through Sephadex G-25 pre-equilibrated with 20 mM-potassium phosphate buffer, pH 6·0, containing 1 mM-EGTA, 1 mM-iodoacetic acid, 5 mM-benzamidine.HCl, 10 mM-2-mercaptoethanol, 0·5 mM-PMSF, 0·02% sodium azide and 10% (v/v) glycerol (buffer III), and the desalted material was applied to a phosphocellulose column (0·9 × 15 cm). The column was washed extensively and the protein kinase was eluted with 100 ml of a linear (0–0·7 M) NaCl gradient in buffer III. Fractions of about 3 ml were collected. Fractions 8–10, which contained most of the protein kinase activity, were pooled and dialysed extensively against buffer I containing 50% (v/v) glycerol. This partially purified protein kinase preparation lost less than 5% of its activity when stord for 2–3 months at 0–4 °C.

*Gel filtration.* This was done on a Sephadex G-200 (1·6 × 60 cm) column, pre-equilibrated with buffer II. A sample (2 ml) of the purified protein kinase preparation was applied and the column was run as described above. The column was standardized with aldolase, BSA, ovalbumin and chymotrypsinogen A. Dextran blue and Orange G were used to determine the void volume and total column volume, respectively. Molecular mass was determined according to the method of Andrews (1964).

*SDS-PAGE.* Electrophoresis in 10% (w/v) polyacrylamide slab gels containing 0·1% SDS was done by the method of Laemmli (1970). Gels were fixed, stained and destained according to Fairbanks *et al.* (1971). Estimates of subunit molecular masses were obtained from plots of log molecular mass versus relative mobilities of six standard proteins: albumin (66 kDa), ovalbumin (45 kDa), glyceraldehyde-3-phosphate dehydrogenase (36 kDa), carbonic anhydrase (29 kDa), trypsinogen (24 kDa) and lactalbumin (14·2 kDa).

*Phosphoamino acid analysis.* Protamine (250 $\mu$g) was phosphorylated in the presence of leishmanial protein kinase under standard assay condition except that the incubation time was extended to 1 h. The $^{32}$P-labelled product was precipitated with 15% (w/v) TCA. The precipitate was washed twice with 1:1 (v/v) acetone/diethyl ether, dried and hydrolysed in 0·5 ml 6 M-HCl at 110 °C for 4 h. The hydrolysate was air-dried and dissolved in 0·1 ml formic acid/acetic acid/$H_2O$ (25:78:897, by vol.) pH 1·9. Samples (10 $\mu$l) were mixed with authentic internal standards (phosphoserine, phosphothreonine and phosphotyrosine), applied to the cathode edge of a cellulose thin-layer sheet (Eastman 13250) and electrophoresed at 450 V for 90 min in a pH 3·5 buffer system (acetic acid/pyridine/$H_2O$; 50:5:945, by vol.) (Cooper *et al.*, 1983). Radioactive spots were located by radioautography, whereas phosphoamino acid markers were visualized by spraying with ninhydrin reagent.

*Protein determination.* Protein concentration was estimated by the method of Bradford (1976) with BSA as standard.

## Results

### *Purification of the protein kinase*

The results of the purification are summarized in Table 1. About 0·9 mg of partially purified enzyme was obtained from 10 ml of frozen packed cells. The

Table 1. *Purification of protein kinase from L. donovani promastigotes*

The units of protein kinase activity are nmol $^{32}P$ incorporated $min^{-1}$ using protamine as substrate. The data are representative of three experiments.

| Purification step | Vol. (ml) | Protein (mg $ml^{-1}$) | Specific activity [units (mg protein)$^{-1}$] | Total activity (units) | Purification (-fold) | Yield (%) |
|---|---|---|---|---|---|---|
| 100000 *g* supernatant | 80 | 3·95 | 4·3 | 1359 | 1 | 100 |
| DEAE-Cellulose | 6·0 | 5·10 | 23·8 | 753 | 5·5 | 55 |
| Sephadex G-200 | 15·0 | 0·68 | 50·0 | 510 | 10·8 | 48 |
| Phosphocellulose | 6·0 | 0·15 | 390 | 351 | 91 | 26 |

procedure resulted in a 91-fold purification of the enzyme with a recovery of 26%. This enzyme preparation was stable for at least 3 months when stored in buffer I containing 50% glycerol at 0–4 °C. Fig. 1 shows the chromatographic profile of the protein kinase as eluted from the DE-52 column, with a major peak of activity at 40–70 mM-NaCl. When this preparation was subjected to gel filtration through a Sephadex G-200 column, a single relatively sharp and symmetrical peak was obtained (Fig. 2), indicative of a single protein kinase. The enzyme preparation eluted from the Sephadex column was further chromatographed through a phosphocellulose column (Fig. 3). A single peak of protein kinase was eluted from this column at 110–200 mM-NaCl. The purity of this enzyme was examined by SDS-PAGE. Staining with Coomassie Blue revealed at least three bands, with molecular masses of 53 kDa, 48 kDa and 32 kDa, with the 53 kDa protein the most prominent (Fig. 4).

During the purification of the protein kinase, we also looked for the histone kinase activity described by Das *et al.* (1986). We found that most of the histone kinase activity remained in the 100000 *g* pellet (see Methods) indicating that this activity was mostly membrane-bound.

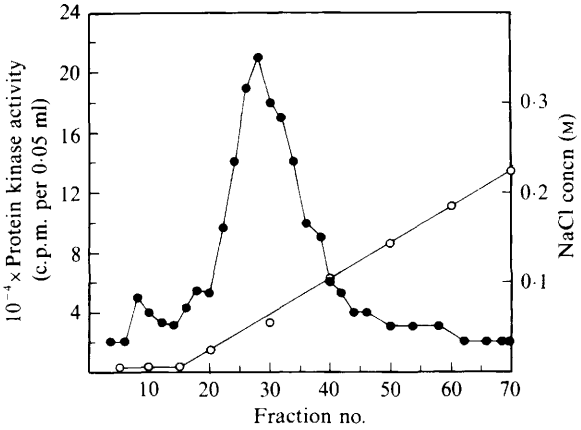


Fig. 1. Chromatography of *L. donovani* cell extract on a 2·5 × 20 cm DE-52 DEAE-cellulose column. The 100000 *g* supernatant from the *L. donovani* extract was diluted, adsorbed on to the DEAE cellulose column and eluted with 500 ml of a linear (0–0·3 M) NaCl gradient in buffer I, pH 6·8; fractions of 7 ml were collected. The experiment was done three times; values shown are from a typical experiment (the other experiments showed similar trends). ●, Protein kinase activity; ○, NaCl concn.

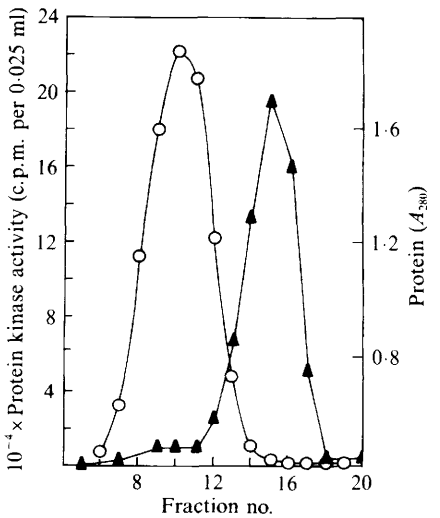


Fig. 2. Gel-filtration chromatography of *L. donovani* protein kinase on Sephadex G-200. The DE-52 eluate (Fig. 1) containing the protein kinase activity was concentrated and dialysed, and applied to a Sephadex G200 column (1·6 × 60 cm); fractions of about 8 ml were collected. The experiment was done three times; values shown are from a typical experiment (the other experiments showed similar trends). ○, Protein kinase activity; ▲, protein ($A_{280}$).

*Properties of the protein kinase*

The partially purified leishmanial protein kinase eluted from the phosphocellulose column was used to characterize some of the physicochemical and kinetic properties of the enzyme.

*Size.* The protein kinase eluted from the Sephadex G-200 column between aldolase and BSA, at an apparent molecular mass of 85 kDa.

*Substrate specificity.* A variety of commonly used protein substrates, namely protamine, mixed histones, phosvitin, dephosphorylated casein and the synthetic peptide kemptide (Leu-Arg-Arg-Ala-Ser-Leu-Gly) were compared in the standard protein kinase assay (Table 2). Highest activity was observed with protamine as exogenous phosphate acceptor, with, surprisingly, kemp-

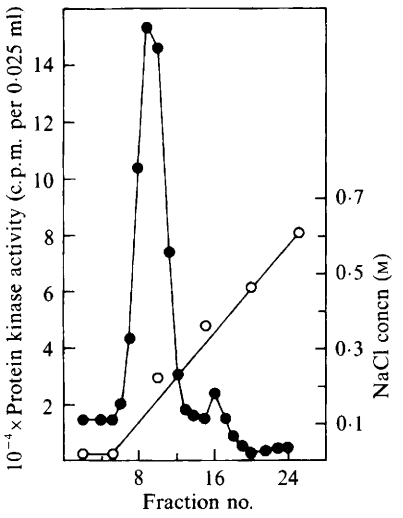


Fig. 3. Chromatography of *L. donovani* protein kinase on phosphocellulose. Fractions from the Sephadex column (Fig. 2) that contained protein kinase activity were pooled and desalted, and were then applied to a 0·9 × 15 cm phosphocellulose column. Protein kinase activity was eluted with a linear (0–0·7 M) NaCl gradient in buffer III; fractions of 3 ml were collected. The experiment was done three times; values shown are from a typical experiment (the other experiments showed similar trends). ●, Protein kinase activity; ○, NaCl concn.

Table 2. *Substrate specificity of L. donovani protein kinase*

The assay was done with the specified amount of acceptor in a 0·2 ml assay mixture as described in Methods. Values are means ± SD of four sets of experiments.

| Substrate | Protein kinase activity (pmol $^{32}P$ transferred $min^{-1}$) |
|---|---|
| Protamine sulphate (1·25 mg $ml^{-1}$) | 70·0 ± 3·5 |
| Kemptide (0·25 mg $ml^{-1}$) | 19·5 ± 0·8 |
| Mixed histone (1·25 mg $ml^{-1}$) | 11·0 ± 0·5 |
| Phosvitin (1·25 mg $ml^{-1}$) | 5·0 ± 0·2 |
| Casein (dephosphorylated) (1·25 mg $ml^{-1}$) | 5·0 ± 0·4 |

tide as the second best acceptor. Phosvitin and dephosphorylated casein were poor substrates.

When protamine phosphorylated by the protein kinase was subjected to acid hydrolysis and the phosphoamino acids separated on cellulose thin-layer plates, a considerable amount of the radioactivity ran with the phosphoserine standard (Fig. 5). We conclude that the protein kinase transfers the γ-phosphate of ATP to serine residues in protamine.

*Metal-ion requirements.* Omission of $Mg^{2+}$ ions from the assay medium drastically reduced enzyme activity (Table 3). Activity was highest at 15–20 mM-$MgCl_2$, but concentrations higher than 30 mM were inhibitory (not

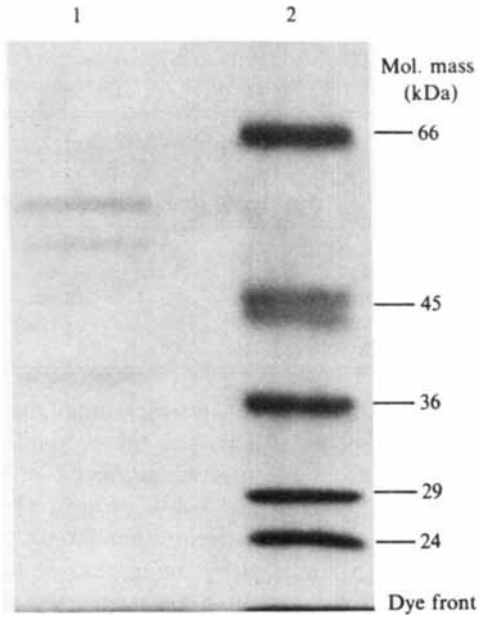


Fig. 4. SDS-PAGE of the partially purified *L. donovani* protein kinase. Approximately 6 μg of the final preparation from the phosphocellulose column (Fig. 3) was subjected to SDS-PAGE (10%, w/v, acrylamide) and stained with Coomassie Brilliant Blue (lane 1). Lane 2, molecular mass standards as described in Methods (lactalbumin ran with the dye front).

shown). $Mn^{2+}$ had a slightly stimulatory effect on enzyme activity, but divalent metal ions, e.g. $Ca^{2+}$, $Co^{2+}$ and $Zn^{2+}$, were inhibitory. Addition of low concentrations of NaCl had no effect on enzyme activity, but 40 mM-NaCl was slightly inhibitory. This is in contrast to the observation of Das *et al.* (1986), who found that omission of NaCl from the standard assay reduced the protein kinase activity by about 80%.

*Effector molecules.* None of the following potential effectors had any effect on protein kinase activity: cAMP or cGMP (0·01 and 0·05 mM); $Ca^{2+}$ (0·2 mM); $Ca^{2+}$ (0·2 mM)/calmodulin (1·8 μg); diolein (15 μM); phosphatidylinositol (10 μg); phosphatidylinositol (10 μg)/$Ca^{2+}$ (10 μM)/diolein (15 μM) (all tested on 0·3 μg protein kinase in the standard assay).

*Effect of thiol reagents.* DTNB, *p*-hydroxymercuribenzoate and NEM all inhibited the protein kinase (Table 4). Inhibition by DTNB and *p*-hydroxymercuribenzoic acid could be reversed by DTT, whereas inhibition by NEM was irreversible.

*ATP requirement.* The effect of ATP concentration (0·005–0·15 mM) on kinase activity was examined. A Lineweaver–Burk plot of the data (not shown) indicated an apparent $K_m$ for ATP of 60 μM.

*Effect of pH.* The pH optimum of the protein kinase using protamine as substrate was pH 7·0–7·5.

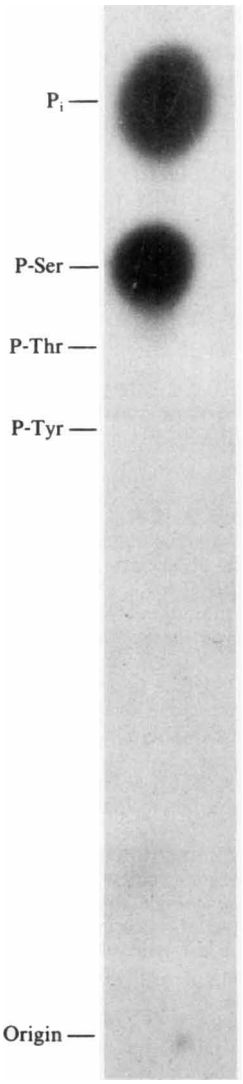


Fig. 5. Identification of the phosphorylated amino acid. Protamine was phosphorylated and the $^{32}P$-labelled product analysed by cellulose thin-layer chromatography, as described in Methods. $^{32}P$ was detected by autoradiography. The positions of authentic internal standards are indicated (P-Ser, phosphoserine; P-Thr, phosphothreonine; P-Tyr, phosphotyrosine).

*Effect of temperature.* Protein kinase activity was highest at 37 °C; most of the activity was lost after heating to 50 °C.

*Localization of protein kinase activities*

The protamine-utilizing protein kinase activity described in this paper could be distinguished from the histone-utilizing protein kinase activity described by Das *et al.* (1986) by exposing leishmanial promastigote cells to controlled hypotonic shock. For this purpose, we adopted a procedure recently developed in our laboratory by Sarkar (1989). When about $10^8$ intact promastigote cells were suspended (1:50) in 5 mM-Tris/HCl buffer, pH 7·4, with gentle shaking, there was a rapid and nearly complete (>95%) release of the cytoplasmic marker enzymes glucose-6-phosphate dehydrogenase and UDP-glucose-4-epimerase within 10 min. In contrast, the washed, pelleted cell ghosts, which kept their original polarity (Sarkar, 1989) retained almost complete activity of the plasma-membrane marker enzymes 3′-nucleotidase and acid phosphatase, and nearly 50% of the activity of the kinetoplast–mitochondria membrane-marker enzyme succinate dehydrogenase. Under the same conditions, more than 90% of protamine kinase activity was released into the supernatant within 10 min, whereas more than 95% of the histone kinase activity remained with the pelleted ghost preparation at the end of this period. This clearly indicates that the protamine kinase is a soluble enzyme whereas histone kinase is membrane-associated.

## Discussion

Our results show that a protein kinase is present in the soluble fraction of *L. donovani* promastigotes that is capable of phosphorylating protamine and, less efficiently, mixed histones, casein and phosvitin. The activity of this protein kinase is not regulated by cAMP, cGMP, $Ca^{2+}$/calmodulin or $Ca^{2+}$/phospholipid.

Das *et al.* (1986) described an *L. donovani* protein kinase that preferentially phosphorylates histone. This enzyme has a number of properties in common with the protein kinase described in the present paper; both have similar pH optima, $Mg^{2+}$ requirements and temperature optima, both phosphorylate exclusively serine residues of their protein substrates, and neither is stimulated by cAMP, $Ca^{2+}$/calmodulin etc. The enzymes differ in their $K_m$ for ATP, substrate requirement, molecular mass and NaCl requirement, but the most striking distinction between the two is their localization. The histone kinase activity described by Das *et al.* (1986) is tightly bound to the outer surface of the parasite and can be released only by repeated freezing/thawing and/or treatment with bile salts, whereas the protamine kinase reported in the present study is soluble, and easily released by controlled hypotonic shock.

The protein kinase characterised in this study was inhibited by reagents that react with thiol groups. Inhibition by *p*-hydroxymercuribenzoic acid and DTNB was dose-dependent and could be reversed by DTT, whereas inhibition by NEM was irreversible. This suggests that the cysteine residue(s) in the protein kinase might be located at or near the active site of the enzyme. This is in agreement with the observation made by

Table 3. *Effect of metal ions on L. donovani protein kinase activity*

Protein kinase activity was assayed as described in Methods in the presence of the metal ion(s) indicated (added as the chloride). The results are representative of two experiments done in duplicate.

| Metal ion | Concn (mM) | Protein kinase activity (pmol $^{32}P$ transferred $min^{-1}$) | Metal ion | Concn (mM) | Protein kinase activity (pmol $^{32}P$ transferred $min^{-1}$) |
|---|---|---|---|---|---|
| None | — | 2·5 | $Ca^{2+}$ | 20 | 0·2 |
| $Mg^{2+}$ | 20 | 22·0 | $Ca^{2+}$ | 40 | ND |
| $Mn^{2+}$ | 20 | 4·2 | $Zn^{2+}$ | 20 | ND |
| $Mn^{2+}$ | 80 | 4·1 | $Zn^{2+}$ | 40 | ND |
| $Co^{2+}$ | 20 | ND | $Na^{+}$ | 10 | 20·9* |
| $Co^{2+}$ | 40 | 0·9 | $Na^{+}$ | 40 | 16·9* |

ND, No protein kinase activity detected (i.e. <0·01 pmol $^{32}P$ transferred $min^{-1}$).
* $Mg^{2+}$ (20 mM) was also present.

Table 4. *Effect of thiol reagents on L. donovani protein kinase activity*

| Thiol reagent | Concn (mM) | Protein kinase activity (pmol $^{32}P$ transferred $min^{-1}$) −DTT | +DTT (10 mM) |
|---|---|---|---|
| None | | 15·6 ± 2·1 | 17·0 ± 2·3 |
| *p*-Hydroxymercuri-benzoic acid | 0·01 | 11·2 ± 1·6 | 17·0 ± 1·6 |
| | 0·05 | 6·3 ± 0·9 | 16·4 ± 1·1 |
| | 0·10 | 3·3 ± 0·5 | 16·9 ± 1·1 |
| DTNB | 0·10 | 11·0 ± 2·0 | 21·3 ± 2·5 |
| | 0·20 | 4·1 ± 0·5 | 20·4 ± 2·5 |
| NEM | 0·40 | 1·1 ± 0·2 | 2·4 ± 0·5 |

Bechtel *et al.* (1977) for the catalytic subunit of soluble cAMP-dependent protein kinase of muscle.

The role of *L. donovani* protein kinase is not yet known. It is possible that it participates in the phosphorylation of the intracellular protein(s) required for growth and differentiation. It will be of interest to discover the physiological substrate(s) of this protein kinase and whether phosphorylation of this substrate(s) has any effect on the transformation process. Such questions will be addressed once the protein kinase has been isolated in a pure form.

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