Molecular and Biochemical Parasitology, 26 (1987) 289–296
Elsevier

MBP 00890

Biosynthesis and secretion of acid phosphatase by *Leishmania donovani* promastigotes

Paul A. Bates and Dennis M. Dwyer

Cell Biology and Immunology Section, Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, U.S.A.

(Received 18 May 1987; accepted 28 July 1987)

Metabolic labeling and immunoprecipitation experiments demonstrated that soluble acid phosphatase (EC 3.1.3.2) was rapidly synthesized and released into culture medium by *Leishmania donovani* promastigotes. The kinetics of release indicated a constitutive secretory process ($t_{1/2}$ = 45 min). Moreover, acid phosphatase was the major secretory protein. The extracellular enzyme is composed of two heterodisperse bands of approximately 110 and 130 kDa in sodium dodecyl sulphate-polyacrylamide gels. It is synthesized as two intracellular precursors of 92.5 and 107 kDa which acquire the heterodisperse form characteristic of the mature extracellular enzyme during biosynthesis. Labeling in the presence of tunicamycin altered the electrophoretic mobility of the acid phosphatase, indicating the presence of several N-linked oligosaccharides on the mature enzyme. However, tunicamycin did not block secretion of the enzyme or its processing to the heterodisperse form. The biosynthetic effect of tunicamycin was mimicked by *N*-glycosidase F treatment of acid phosphatase immunoprecipitates. In contrast to tunicamycin, labeling in the presence of monensin inhibited processing of the phosphatase to its heterodisperse form. This indicates that Golgi processing, probably glycosylation, is responsible for the heterodispersity of the mature enzyme in sodium dodecyl sulphate-polyacrylamide gels. As with tunicamycin, monensin treatment did not prevent secretion of the acid phosphatase. These cumulative results demonstrate that release of this enzyme by *L. donovani* promastigotes occurs via a secretory pathway.

Key words: *Leishmania donovani*; Acid phosphatase; Enzyme biosynthesis; Secretion; Glycosylation

Introduction

The protozoan parasite *Leishmania donovani* is the causative agent of human visceral leishmaniasis [1]. The organism is exposed to hydrolytic environments throughout its life cycle either as extracellular promastigotes within the alimentary tract of the sandfly vector or as intracellular amastigotes within the phagolysosomal system of mammalian macrophages [2–4]. The biochemical mechanisms responsible for the survival of the parasite are poorly understood. However, one unusual property of *Leishmania* parasites is the release of a non-specific acid phosphatase (EC 3.1.3.2) activity by in vitro cultured promastigotes [5,6]. This extracellular soluble acid phosphatase is distinct from a membrane-bound acid phosphatase activity associated with promastigote surface membranes [7–12]. Here we describe the biosynthesis and secretion of the extracellular enzyme by *L. donovani* promastigotes. Some results of this study were presented earlier [13].

Materials and Methods

Parasites. A cloned line [14] derived from the 1S strain [15] of *L. donovani* promastigotes was used in all the experiments described (W.H.O. designation *L. donovani* MHOM/SD/00/1S-2D). Parasites were routinely maintained in tissue culture medium 199 (Gibco, Grand Island, NY) supplemented with 10% (v/v) heat inactivated fetal bovine serum (Biofluids, Rockville, MD) [8].

Correspondence address: Paul A. Bates, Cell Biology and Immunology Section, Laboratory of Parasitic Diseases, Building 5, Room 112, NIAID/NIH Bethesda, MD 20892, U.S.A.

Abbreviations: SDS-PAGE, sodium dodecyl sulphate-polyacrylamide gel electrophoresis.

Antisera. A rabbit antiserum raised against monoclonal antibody-purified soluble acid phosphatase was used [12].

Enzyme assays. Acid phosphatase enzyme activity was assayed using *p*-nitrophenyl phosphate as substrate as previously described [5].

Metabolic labeling. Log-phase promastigotes (1–2 $\times 10^7$ cells ml^{-1}) were harvested from serum containing medium by centrifugation at 6000 $\times$ *g* for 10 min. These cells were washed three times by resuspension and centrifugation at ambient temperature (21°C) using RE-III medium [16], which was prepared lacking methionine and bovine serum albumin, i.e., chemically defined and macromolecule-free. Promastigotes were resuspended in this medium to 2 $\times 10^8$ cells ml^{-1}, L-[^{35}S]methionine (Amersham, Arlington Heights, IL; > 800 Ci $mmol^{-1}$) was added to a final concentration of 25 μCi ml^{-1}, and the cells incubated at 26°C for the desired interval. In chase experiments, unlabeled methionine and cycloheximide were then added to 50 μg ml^{-1} and 1 μg ml^{-1} respectively, to prevent further incorporation of radiolabel into proteins (trichloroacetic acid precipitable counts). In preliminary experiments, it was determined that, aside from its effect on protein synthesis, cycloheximide had no effect on acid phosphatase secretion. Subsequent procedures were performed at 4°C or on ice. Labeled cells were pelleted by centrifugation at 6000 $\times$ *g* for 10 min, the supernatant retained, and the cells washed three times in 10 mM phosphate, 145 mM NaCl, pH 7.4. Final pellets were resuspended to 2 $\times 10^9$ cells ml^{-1} in 38 mM Tris, 100 mM glycine pH 8.5, 1% (v/v) Triton X-100 (Pierce Chemical Co., Rockford, IL), 1.6 mM phenylmethylsulphonyl fluoride, 25 μg ml^{-1} leupeptin (both inhibitors from Sigma Chemical Co., St. Louis, MO) and stirred for 1 h at 4°C to ensure solubilization prior to immunoprecipitation. To the labeled cell-free culture supernatant a 1/10th volume of the same solubilization buffer was added (i.e., to provide protease inhibitors and a detergent concentration appropriate for immunoprecipitation). The labeled cell lysate and culture supernatant were used directly in immunoprecipitation.

In experiments where tunicamycin (Sigma Chemical Co.) was used to prevent N-linked glycosylation, promastigotes were prepared as above, except that 16 h prior to labeling the cultures were subdivided, one half serving as control and the other given tunicamycin at 1 μg ml^{-1} from a 10000-fold stock in 0.1 M NaOH. Tunicamycin had no effect on either the rate of cell growth or overall protein synthesis during pulse labeling as compared to the control culture.

In experiments where monensin (Sigma Chemical Co.) was used, additions were made to cultures 15 min prior to labeling. Monensin was prepared as a 1000-fold stock in absolute ethanol. In parallel experiments the addition of this small amount of ethanol (0.1%, v/v) did not have any deleterious effects on promastigotes as judged by growth, motility, their incorporation of counts into trichloroacetic acid precipitates or overall pattern of protein synthesis on sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE).

Immunoprecipitation. 100 μl aliquots of labeled cell lysates (equivalent to 2 $\times 10^8$ cells) or 1 ml aliquots of labeled culture supernatants (derived from 2 $\times 10^8$ cells) were mixed with 5 μl volumes of antiserum and incubated overnight at 4°C. The 100 μl samples were then diluted to 1 ml by the addition of 900 μl of solubilization buffer prepared as above but containing only 0.1% (v/v) Triton X-100 (0.1% Triton buffer), and all samples (1 ml total volumes) centrifuged at 50000 $\times$ *g*, 30 min, 4°C to pellet non-solubilized material. The supernatants were removed and transferred into 1.5 ml microcentrifuge tubes (Tekmar, Cincinnati, OH) each containing 100 μl packed volume of Sepharose 4B (Pharmacia, Piscataway, NJ) previously washed in 0.1% Triton buffer, and adsorbed for 1 h by rocking at 4°C. The samples were centrifuged briefly in an Eppendorf microfuge and the supernatants transferred to a new set of microcentrifuge tubes, each containing 50 μl of washed protein A-Sepharose CL-4B (Pharmacia). Immune complexes were allowed to bind to these beads by rocking at 4°C for 1 h. To remove unbound proteins, each 50 μl aliquot of protein A-Sepharose was washed three times by resuspending in 1 ml of 0.1% Triton buffer, vortexing briefly, pelleting by centrifugation, and re-

moving the supernatant. The final pellets of washed protein A-Sepharose were resuspended in 200 μl of sample buffer [17], heated in a boiling water bath for 2 min, and the supernatants analyzed by SDS-PAGE.

N-glycosidase F treatment. Immunoprecipitates were prepared as above, except that the final pellets of washed protein A-Sepharose containing bound immune complexes were resuspended in 100 μl of 0.2% (w/v) sodium dodecyl sulphate, 50 mM dithiothreitol, 38 mM Tris, 100 mM glycine, pH 8.5, and heated in a boiling water bath for 2 min. The tubes were centrifuged briefly and the supernatants containing labeled proteins were transferred to new microcentrifuge tubes, to which 6 μl of 20% (v/v) Triton X-100 and 4 μl of 0.5 M ethylenediaminetetraacetic acid pH 8 were added and mixed. 2 μl of peptide *N*-glycosidase F (EC 3.5.1.52) (*N*-glycanase, 250 units ml^{-1}; Genzyme Corp., Boston, MA) was added to each sample and incubated for 1 h at 30°C. An equal volume of 2 × gel sample buffer [17] was then added, samples heated for 2 min in a boiling water bath, and analyzed by SDS-PAGE.

SDS-PAGE. Standard methods were used [17] to prepare 7.5% acrylamide gels, with the exception that 50 mM dithiothreitol was used as reducing agent. Gels were processed for fluorography using EN^{3}HANCE (New England Nuclear, Boston, MA) according to manufacturer's instructions and exposed to X-Omat AR film (Kodak, Rochester, NY) at −70°C. Fluorographs were scanned with a 2202 Ultroscan laser densitometer (LKB Instruments, Gaithersburg, MD). ^{14}C-methylated molecular weight standards and prestained molecular weight standards were used to calibrate fluorographs (Amersham and Bethesda Research Labs, Gaithersburg, MD, respectively).

Results

Pulse labeling. The results of a typical experiment in which promastigotes were pulse labeled for 1 h are shown in Fig. 1. During this period, a wide variety of cellular components were labeled (Fig. 1A, lane 1). Of these, two proteins with apparent molecular masses of 108 and 92.5 kDa were immunoprecipitated by a rabbit antiserum against the soluble acid phosphatase (Fig. 1A, lane 3). In contrast, during the same period, only a limited number of labeled proteins were released by the promastigotes into the culture supernatant (Fig. 1B, lane 1). Immunoprecipitates of these showed that the extracellular enzyme contained diffuse bands of ~110 and 130 kDa (Fig. 1B, lane 3). The presence of these two bands was more visually apparent in underexposed fluorograms and was verified using laser scanning densitometry. Although numerous proteins were synthesized during pulse labeling (Fig. 1A), the soluble acid phosphatase was clearly the major component released by these cells (Fig. 1B). The results of densitometry verified this and showed that it accounted for 60–65% of the total signal in such labeled supernatants.

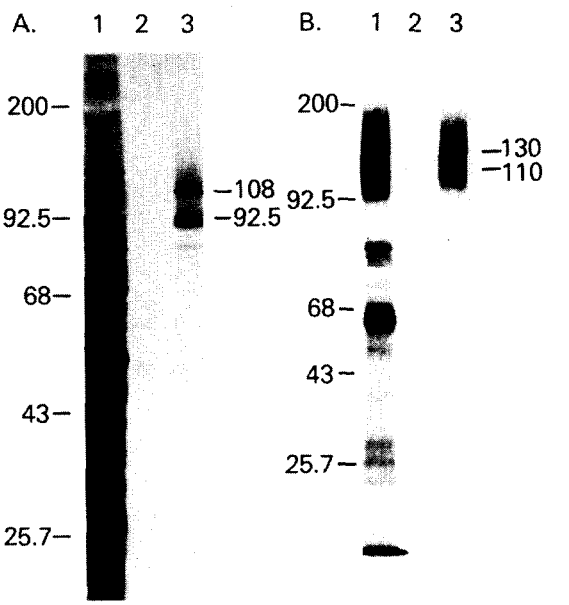

Fig. 1. Metabolically labeled soluble acid phosphatase analyzed by SDS-PAGE and fluorography. (A) Cell associated enzyme. *L. donovani* promastigotes were pulse labeled with [^{35}S]methionine for 1 h and solubilized with Triton X-100. Lane 1, total labeled proteins; lane 2, immunoprecipitate with preimmune rabbit serum; lane 3, immunoprecipitate with antiserum to soluble acid phosphatase. (B) Extracellular/released enzyme. Samples as in (A), but using the cell-free culture supernatant. Results shown were typical of those obtained in ten separate experiments. Apparent molecular masses are shown in kDa.

Pulse-chase experiments. A precursor–product relationship between the intracellular (Fig. 1A) and extracellular (Fig. 1B) forms of the soluble acid phosphatase was demonstrated in pulse-chase experiments (Fig. 2). Cumulative results showed that it was synthesized as two discrete precursor bands which were processed and subsequently released from the cells as the mature, extracellular, soluble enzyme. The two intracellular precursor bands appeared to be processed in parallel, as both diminished equally with time, and no evidence was deduced that one was the precursor/product of the other. The extracellular form, first detectable at 20 min, showed a concomitant increase in signal intensity with all of the enzyme being externalized by 240 min. The time required for release of half of the pulse labeled enzyme ($t_{1/2}$) was approximately 45 min (Fig. 3). A minimum of 10 min was required for de novo production of extracellular acid phosphatase. Presumably this lag reflects the time required for the uptake of radiolabeled methionine, its incorporation into the protein, secondary modifications such as glycosylation, and secretion into the medium. No similar lag phase was observed when extracellular enzyme activity was assayed directly (Fig. 3), indicating that the cells contained a pool of preformed enzyme. Previous experiments showed that the level of extracellular acid phosphatase activity increased in parallel with the growth of promastigotes in vitro [5]. Taken together with the current data, this indicates that the enzyme is released from promastigotes by a constitutive secretory process, i.e., continuously released as opposed to regulated secretion under secretagogue control. As is evident from Figs. 1 and 2, the mature extracellular acid phosphatase possessed higher apparent molecular weight and was heterodisperse relative to its major intracellular precursors. It should be noted, however, that a small amount of the heterodisperse form was also present in the cell-associated enzyme (Figs. 1 and 2). This suggests that some event in biosynthesis, prior to secretion, was responsible for the heterodispersity. Since the purified enzyme

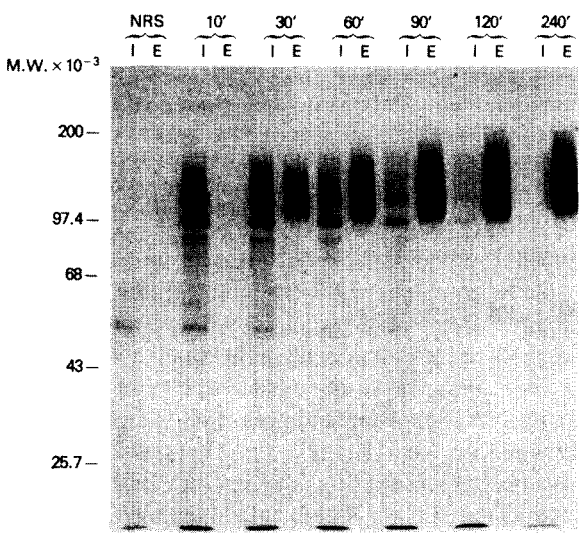

Fig. 2. Analysis by SDS-PAGE and fluorography of immunoprecipitates of intracellular (I) and extracellular (E) soluble acid phosphatase during the time course of a pulse-chase experiment. Cells were pulse labeled for 10 min with [^{35}S]methionine and chased for up to an additional 230 min. The times shown are inclusive of the pulse period, and indicate immunoprecipitates with rabbit anti soluble acid phosphatase serum. NRS is the preimmune serum of this rabbit reacted with a 10 min sample for I and a 240 min sample for E. Virtually identical results to those shown were obtained in three separate experiments.

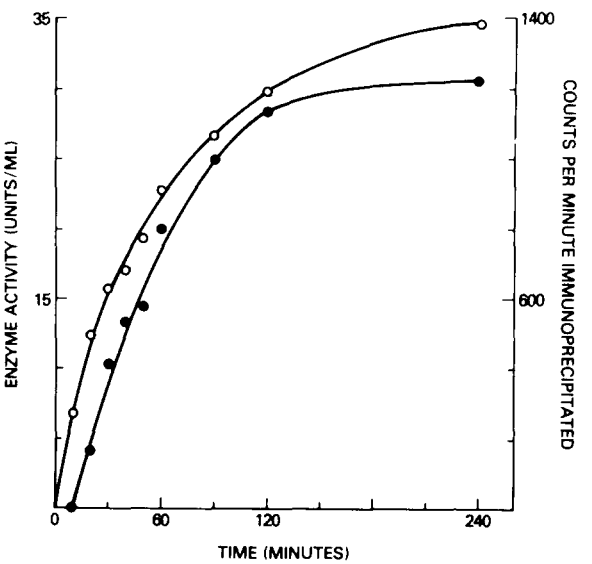

Fig. 3. Kinetics of soluble acid phosphatase release. Radiolabeled enzyme released during a pulse-chase experiment as measured by scintillation counting of immunoprecipitates (●-●) compared with the release of enzymatic activity (○-○). These results were typical of those obtained in three separate experiments.

has a relatively high carbohydrate content, about 30% by weight [18], we examined the effects of glycosylation inhibitors on its biosynthesis.

Effect of tunicamycin. The antibiotic tunicamycin was used to investigate N-linked oligosaccharide side chains in the soluble acid phosphatase. As tunicamycin inhibits the biosynthesis of the dolichol-linked side chain, its inclusion in metabolic labeling experiments results in the synthesis of proteins deficient in N-linked carbohydrates [19]. Tunicamycin was added to promastigotes 16 h prior to pulse labeling to deplete their intracellular pools of oligosaccharide chains. As previously shown, 1 $\mu g\ ml^{-1}$ tunicamycin does not block the secretion of soluble acid phosphatase [13]. However, the secreted protein is enzymatically inactive [20]. Fig. 4 shows the effect of tunicamycin in a 1 h pulse labeling experiment. The apparent molecular weights of both intracellular and extracellular forms of acid phosphatase were reduced. However, the enzyme still acquired heterodispersity, indicating that this property is not caused by extensive N-linked glycosylation or modification of N-linked side chains in the Golgi apparatus. As a result of tunicamycin treatment, the heterodisperse extracellular enzyme exhibited a net shift of approximately 25 kDa [i.e., (130 + 110) − (120 + 95) = 25 kDa]. Similarly the intracellular precursors demonstrated a net shift of 31 kDa [(108 + 92.5) − (92.5 + 77) = 31 kDa] in electrophoretic mobility. These would indicate the presence of several N-linked side chains in the intact enzyme. Although the exact number has not been determined, a single side chain would be expected to contribute about 4 kDa to the apparent molecular mass on SDS-PAGE [21].

Enzymatic deglycosylation. In addition to experiments with tunicamycin, the occurrence of N-linked oligosaccharide side chains in soluble acid phosphatase was assessed using *N*-glycosidase F. Immunoprecipitates of the enzyme from both untreated and tunicamycin treated promastigotes were used as substrates for deglycosylation. Results of *N*-glycosidase F digestion mimicked the biosynthetic effect of tunicamycin, causing a similar shift in the electrophoretic mobility of acid phosphatase (Fig. 5). Further, immunoprecipitates of the enzyme from tunicamycin treated cells were unaffected by *N*-glycosidase F digestion (not shown) indicating the equivalence of these treatments. As is evident in Figs. 4 and 5, both components of the intracellular and extracellular forms of soluble acid phosphatase demonstrated similar shifts in mobility, indicating that each of these components contain N-linked oligosaccharides.

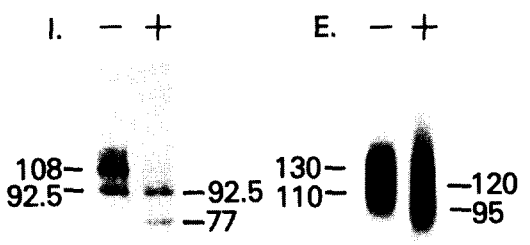

Fig. 4. Effect of tunicamycin on soluble acid phosphatase biosynthesis. Immunoprecipitates of intracellular (I) and extracellular (E) enzyme from promastigotes labeled in the absence (−) or presence (+) of tunicamycin. Apparent molecular masses are shown in kDa. These experiments were repeated several times with similar results.

Effect of monensin. The possible role of the Golgi apparatus in the biosynthesis of *L. donovani* soluble acid phosphatase was investigated by employing the H^+–Na^+/K^+ ionophore monensin. This reagent has been shown to affect the glycosylation/transport of certain glycoproteins in a variety of eukaryotic cells by interfering with Golgi function [22]. Such effects have also been observed in another trypanosomatid, *Trypanosoma brucei* [23]. Monensin treatment had no apparent effect on the biosynthesis of the major intracellular precursors of the acid phosphatase (Fig. 6). However, the ionophore dramatically inhibited its conversion to the heterodisperse form,

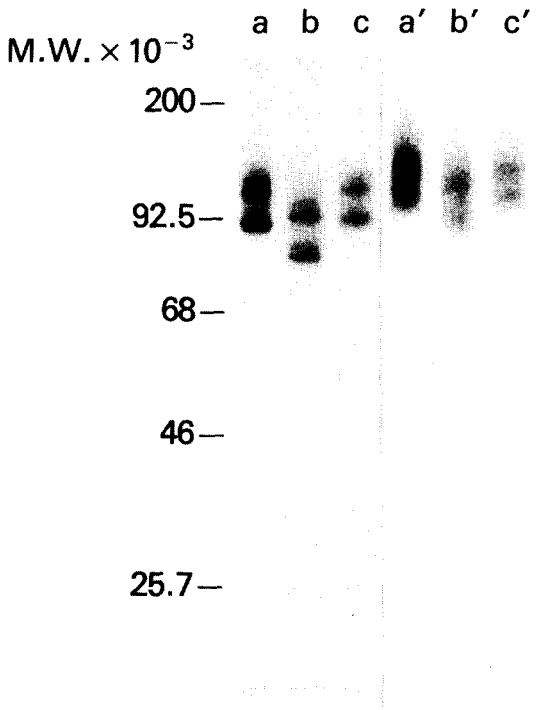

Fig. 5. Treatment of soluble acid phosphatase with *N*-glycosidase F. Intracellular (a,b,c) and extracellular (a′,b′,c′) immunoprecipitates were untreated (a,a′), deglycosylated with *N*-glycosidase F (b,b′), or subjected to control incubation without *N*-glycosidase F (c,c′). The results shown were representative of three separate experiments.

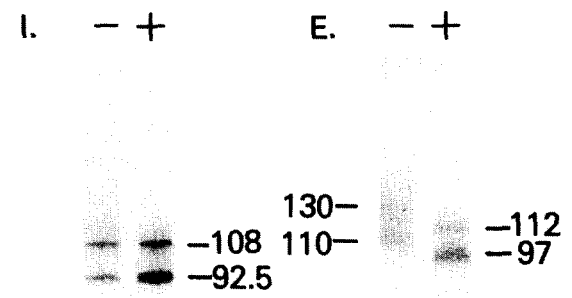

Fig. 6. Effect of 0.1 μM monensin on soluble acid phosphatase biosynthesis. Immunoprecipitates of intracellular (I) and extracellular (E) enzyme from cells labeled in the absence (−) or presence (+) of monensin. Apparent molecular masses are shown in kDa. The results shown were a typical example of three individual experiments.

such that the enzyme was released as two distinct bands of ~97 and 112 kDa on SDS-PAGE. The apparent sizes of these were slightly larger than their intracellular precursors, suggesting that some processing events might have occurred which were not inhibited by monensin. A range of monensin concentrations were employed in these studies (0.1–12.5 μM). All had an identical effect on the biosynthesis of the enzyme. However, above 0.5 μM the rate of secretion was inhibited, presumably due to bioenergetic side effects.

Discussion

The results of the foregoing experiments constitute substantive evidence of a secretory pathway in *L. donovani*. Further, soluble acid phosphatase appears to be the major protein secreted by *L. donovani* promastigotes during their growth in vitro. The levels of enzyme activity released by the cloned line used in this study are similar to those described for other *L. donovani* strains [6] and have remained stable over several years of continuous in vitro cultivation. In addition to *L. donovani*, release of acid phosphatase has been noted in a variety of other species [6], suggesting that this pathway is of widespread occurrence in the genus *Leishmania*. The results obtained with tunicamycin are consistent with cotranslational addition of N-linked side chains as found in other eukaryotes [24]. The precise number and possible modifications of the N-linked side chains in this acid phosphatase remain to be determined. These are important structural considerations to the function of the native enzyme, since tunicamycin treated acid phosphatase is enzymatically inactive [20]. With regard to subsequent processing of the enzyme, monensin treatment clearly

prevented conversion to the heterodisperse form. This effect on the parasite is strong circumstantial evidence for an involvement of the Golgi apparatus in the secretory pathway [22]. In all eukaryotes examined to date, the enzymes responsible for secondary glycosylation reside in the Golgi apparatus [25]. Tunicamycin treatment alone caused a net reduction of only 10% in apparent molecular weight of the extracellular enzyme on SDS-PAGE (from above). Since direct chemical analysis showed that the soluble acid phosphatase had a higher carbohydrate content, about 30% by weight [18], it is likely that the heterodispersity of the mature enzyme results from secondary glycosylation events. The yeast *Saccharomyces cerevisiae* also secretes acid phosphatases which show heterodispersity on SDS-PAGE similar to the leishmanial enzyme [26,27]. In yeast, this property is caused by extensive and variable addition of mannose residues to N-linked oligosaccharides resulting in large branching polymannose chains [26,27]. In higher eukaryotes, heterodipersity has also been ascribed to heterogeneity in Golgi processing of N-linked oligosaccharides [21]. The explanation is clearly different for the *L. donovani* acid phosphatase, since tunicamycin did not interfere with the generation of enzyme heterodispersity. A related process may be occurring in *L. donovani*, however, such as an unusual form of O-linked glycosylation or possibly a completely novel form of secondary glycosylation. The nature of this post-translational modification is under investigation.

The two components present in the soluble acid phosphatase may represent two subunits or coexpressed isozymes. Neither enzyme activity nor the mobility of the two bands on SDS-PAGE were affected by the presence or absence of reducing agents (unpublished observations). The latter indicates that the two components are not disulphide bonded. The native enzyme was immunoprecipitated by each of the four independently derived monoclonal antibodies against the soluble acid phosphatase [12]. On SDS-PAGE, such immunoprecipitates contained both bands of the enzyme. These observations might result from either the immunoprecipitation of a complex containing non-covalently attached subunits or two isoforms containing antigenically cross-reactive epitopes. In SDS-PAGE immunoblots, both components were also recognized by the monoclonal antibodies (unpublished observations). Further, using a variety of non-denaturing physical separation techniques (compatible with retention of enzymatic activity), the two components always co-purified. Although equivocal in differentiating subunits from isozymes, these results further indicate that the two components of acid phosphatase are antigenically and structurally related.

It is clear that the biosynthesis and release of soluble acid phosphatase is a major cellular process of *L. donovani* promastigotes. This suggests that the enzyme must play an important role in the survival of the organism. With regard to function, the soluble acid phosphatase is capable of dephosphorylating a wide variety of simple substrates [5,18] rendering them available for parasite uptake. It also possesses phosphoprotein phosphatase activity [18]. Although the enzyme is active over a broad pH range, its optimum of 4.8 is similar to those of endogenous host lysosomal hydrolases [5]. Further, the soluble acid phosphatase is highly resistant to toxic oxygen metabolites [28]. Therefore, the parasite enzyme could function in both the sandfly alimentary tract and the macrophage lysosomal environment. In the latter regard, we have recently observed that *L. donovani* amastigotes also produce this enzyme (unpublished observations). Pretreatment of *L. donovani* promastigotes with tunicamycin has been reported to adversely affect their subsequent survival as amastigotes within macrophages [29]. These results may be partially explained by the effect of tunicamycin on acid phosphatase biosynthesis, since the enzyme produced by such parasites would be enzymatically inactive [20]. The parasite enzyme could aid the intracellular survival of amastigotes by dephosphorylating critical elements involved in lysosomal function and/or oxidative killing mechanisms. These possibilities would make the soluble acid phosphatase or its secretory pathway a target for immunological or chemotherapeutic intervention.

Acknowledgements

The expert technical assistance of Mary K. Kurtz and Anne L. Hoover is gratefully acknowledged. P.A.B. is a postdoctoral fellow supported by the Fogarty International Center, National Institutes of Health.

References

1 Bray, R.S. (1974) *Leishmania*. Annu. Rev. Microbiol. 28, 189–217.
2 Chang, K.P. and Dwyer, D.M. (1978) *Leishmania donovani*. Hamster macrophage interactions in vitro: cell entry, intracellular survival, and multiplication of amastigotes. J. Exp. Med. 147, 515–530.
3 Dwyer, D.M. (1979) Membrane interactions between *Leishmania* and host cells. In: Microbiology (Schlesinger, D., ed.) pp. 130–134, American Society of Microbiology, Washington, D.C.
4 Berman, J.D., Dwyer, D.M. and Wyler, D.J. (1979) Multiplication of *Leishmania* in human macrophages in vitro. Infect. Immun. 26, 375–379.
5 Gottlieb, M. and Dwyer, D.M. (1982) Identification and partial characterization of extracellular acid phosphatase of *Leishmania donovani* promastigotes. Mol. Cell. Biol. 2, 76–81.
6 Lovelace, J.D. and Gottlieb, M. (1986) Comparison of extracellular acid phosphatase from various isolates of *Leishmania*. Am. J. Trop. Med. Hyg. 35, 1121–1128.
7 Gottlieb, M. and Dwyer, D.M. (1981) Protozoan parasite of humans: surface membrane with externally disposed acid phosphatase. Science 212, 939–941.
8 Gottlieb, M. and Dwyer, D.M. (1981) *Leishmania donovani*: surface membrane acid phosphatase activity of promastigotes. Exp. Parasitol. 52, 117–128.
9 Glew, R.H., Czuczman, M.S., Diven, W.F., Berens, R.L., Pope, M.T. and Katsoulis, D.E. (1982) Partial purification and characterization of particulate acid phosphatase of *Leishmania donovani* promastigotes. Comp. Biochem. Physiol. 72B, 581–590.
10 Dwyer, D.M. and Gottlieb, M. (1983) The surface membrane chemistry of *Leishmania*: its possible role in parasite sequestration and survival. J. Cell. Biochem. 23, 35–45.
11 Remaley, A.T., Das, S., Campbell, P.I., LaRocca, G.M., Pope, M.T. and Glew, R.H. (1985) Characterization of *Leishmania donovani* acid phosphatases. J. Biol. Chem. 260, 880–886.
12 Bates, P.A., Kurtz, M.K., Gottlieb, M. and Dwyer, D.M. (In Press) *Leishmania donovani*: generation of monospecific antibody reagents to soluble acid phosphatase. Exp. Parasitol.
13 Bates, P.A. and Dwyer, D.M. (1987) Secretion of acid phosphatase by *Leishmania donovani*. In: Molecular Strategies of Parasitic Invasion (Agabian, N., Goodman, H. and Nogueira, N., eds.) U.C.L.A. Symposia on Molecular and Cellular Biology, New Series, Vol. 42, pp. 595–604, Alan R. Liss Inc., New York.
14 Dwyer, D.M. (1976) Antibody-induced modulation of *Leishmania donovani* surface membrane antigens. J. Immunol. 117, 2081–2091.
15 Stauber, L.A. (1966) Characterization of strains of *Leishmania donovani*. Exp. Parasitol. 18, 1–11.
16 Steiger, R.F. and Steiger, E. (1977) Cultivation of *Leishmania donovani* and *Leishmania braziliensis* in defined media: nutritional requirements. J. Protozool. 24, 441–443.
17 Laemmli, U.K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227, 680–685.
18 Lovelace, J.D., Dwyer, D.M. and Gottlieb, M. (1986) Purification and characterization of the extracellular acid phosphatase of *Leishmania donovani*. Mol. Biochem. Parasitol. 20, 243–251.
19 Elbein, A.D. (1981) The tunicamycins – useful tools for studies on glycoproteins. Trends Biochem. Sci. 6, 219–221.
20 Lovelace, J.D. and Gottlieb, M. (1987) Effect of tunicamycin on the extracellular acid phosphatase of *Leishmania donovani* promastigotes. Mol. Biochem. Parasitol. 22, 19–28.
21 Green, S.A., Plutner, H. and Mellman, I. (1985) Biosynthesis and intracellular transport of the mouse macrophage Fc receptor. J. Biol. Chem. 260, 9867–9874.
22 Tartakoff, A.M. (1983) Perturbation of vesicular traffic with the carboxylic ionophore monensin. Cell 32, 1026–1028.
23 Bangs, J.D., Andrews, N.W., Hart, G.W. and Englund, P.T. (1986) Posttranslational modification and intracellular transport of a trypanosome variant surface glycoprotein. J. Cell Biol. 103, 255–263.
24 Hubbard, S.C. and Ivatt, R.J. (1981) Synthesis and processing of asparagine-linked oligosaccharides. Annu. Rev. Biochem. 50, 555–583.
25 Tartakoff, A.M. (1983) The confined function model of the Golgi complex: center for ordered processing of biosynthetic products of the rough endoplasmic reticulum. Int. Rev. Cytol. 85, 221–252.
26 Barbaric, S., Mrsa, V., Ries, B. and Mildner, P. (1984) Role of the carbohydrate part of yeast acid phosphatase. Arch. Biochem. Biophys. 234, 567–575.
27 Schonholzer, F., Schweingruber, A.-M., Trachsel, H. and Schweingruber, M.E. (1985) Intracellular maturation and secretion of acid phosphatase of *Saccharomyces cerevisiae*. Eur. J. Biochem. 147, 273–279.
28 Saha, A.K., Das, S., Glew, R.H. and Gottlieb, M. (1985) Resistance of leishmanial phosphatases to inactivation by oxygen metabolites. J. Clin. Microbiol. 22, 329–332.
29 Nolan, T.J. and Farrel, J.P. (1985) Inhibition of in vivo and in vitro infectivity of *Leishmania donovani* by tunicamycin. Mol. Biochem. Parasitol. 16, 127–135.