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Characterization of a Phosphomonoesterase from *Brucella abortus*

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Brucellae are facultative intracellular bacterial pathogens that reside primarily in cells of the reticuloendothelial system. The high-speed supernatant obtained after centrifuging a suspension of *Brucella abortus* that had been frozen-thawed and sonicated contained abundant phosphomonoesterase activity, determined by using 4-methylumbelliferylphosphate as the substrate; this enzyme was purified 2,900-fold (yield, 570%) by chromatography on DE-52 cellulose and hydroxylapatite columns and high-performance liquid chromatography-gel filtration. The native enzyme had a molecular mass of 120,000 daltons (±10,000 daltons), as determined by gel filtration chromatography, and resolved into two bands (60,000 and 66,000 daltons) when subjected to polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate. The *B. abortus* phosphomonoesterase had the following properties: pH optimum, 6.0 to 6.5; isoelectric point, 3.0; substrate specificity, 5′-AMP > 3′-AMP > 3′-GMP > 5′-GDP > 5′-CDP > 5′-CTP > 5′-UTP > phosphotyrosine > phosphoserine > phosphothreonine. The K_m for 5′-AMP was 0.37 mM. Phosphatidylinositol 4,5-bisphosphate and *myo*-inositol 1,3,4-trisphosphate were poor substrates for the *B. abortus* enzyme. The phosphomonoesterase did not inhibit superoxide anion production by human neutrophils stimulated with formylmethionyl-leucyl-phenylalanine. The phosphomonoesterase may be one of the bacterial enzymes in the pathway leading to the production of adenine, which is secreted by *B. abortus* and blocks the activation of neutrophils.

Brucellosis is a disease of considerable importance for humans and domesticated animals (1). The causative agent of bovine brucellosis, *Brucella abortus*, is a facultative intracellular bacterial pathogen which is capable of surviving within polymorphonuclear neutrophils and nonactivated macrophages (6, 14, 19, 24, 31). The mechanisms and virulence factors responsible for the capacity of *B. abortus* to escape the bactericidal properties of host phagocytes are not well understood. Kreutzer et al. (14) and Riley and Robertson (24) examined the ability of human and bovine neutrophils to ingest and kill smooth and rough strains of *B. abortus*. Both bacterial strains were ingested, and both resisted killing by neutrophils; however, the smooth strain was more resistant to intraphagocytic killing than was the rough strain. By electron microscopy, it was shown that degranulation of both primary and secondary granules was inhibited by *B. abortus* and that viable organisms were not required for the inhibition of granule fusion (5). Canning and co-workers (2, 5) subjected the culture media in which *B. abortus* was grown to high-performance liquid chromatography (HPLC) and isolated two nucleotidelike substances which inhibited the ability of neutrophils to iodinate proteins, a measure of neutrophil myeloperoxidase-H_2O_2-halide activity, which requires primary granule fusion. These investigators showed that the two inhibitory substances were 5′-GMP and adenine and that 5′-GMP and adenine independently suppressed neutrophil iodination activity (4).

Our interest in Brucella acid hydrolases, in particular, acid phosphatases, as possible virulence factors was stimulated by the observation that a pure preparation of acid phosphatase derived from *Legionella micdadei* (27) and another from the outer surface of *Leishmania donovani* (21, 22) could block the production of superoxide anion (O_2^-) by stimulated neutrophils. *B. abortus* resembles *L. micdadei* and *L. donovani* in that all three are intracellular parasites which proliferate inside macrophage phagosomes; furthermore, *B. abortus* (5, 14, 24), like *L. micdadei* (33), is able to survive in the phagosomes of neutrophils. The ability of these microbial enzymes to block neutrophil oxidative metabolism may play an important pathogenetic role, since O_2^- and H_2O_2 generated by activated phagocytic cells play a central role in the killing of bacteria and parasites (20, 26).

In view of the paucity of information regarding the concentrations and properties of hydrolases with acidic pH optima which brucellae might contain, we measured the activity of a variety of hydrolases in *B. abortus* extracts. We found that *B. abortus* does contain intense acid phosphatase activity; however, the phosphomonoesterase, which is actually a nucleotidase, does not block the activation of neutrophils. Furthermore, unlike the *Legionella* (29) and *Leishmania* (11) phosphatases, the *B. abortus* enzyme does not hydrolyze either phosphatidylinositol 4,5-bisphosphate (PIP_2) or *myo*-inositol 1,4,5-trisphosphate (IP_3).

MATERIALS AND METHODS

Chemicals. DE-52 cellulose (Whatman, Inc., Clifton, N.J.) was obtained from Reeve Angel, Inc. (Clifton, N.J.). Hydroxylapatite and ampholytes were obtained from Bio-Rad Laboratories (Richmond, Calif.). 4-Methylumbelliferyl derivatives of phosphate and various sugars and 5′-AMP and the other nucleotides were obtained from Sigma Chemical Co. (St. Louis, Mo.). Heteropolymolybdate complex A ($[C(NH_2)_3]_4[(C_3H_7O_3PO_3)_2Mo_5O_{15}] \cdot 3H_2O$), complex D ($\alpha$-$(NH_4)_4[SiMo_{12}O_{40}] \cdot H_2O$), and complex E ($\alpha$-$(NH_4)_6[P_2Mo_{18}O_{62}] \cdot 9H_2O$) were gifts from Michael T. Pope, Department of Chemistry, Georgetown University, Washing-

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TABLE 1. Comparison of the activities of various glycosidases in different strains of *B. abortus*

Enzyme	Sp act (U/mg of protein) in *B. abortus*[a]:			
	1900	0800	2000	5100
Acid phosphatase	12,400 ± 105[b]	6,780 ± 62	5,040 ± 70	3,300 ± 50
β-D-Glucosidase	0.3 ± 0.01	0.1 ± 0.01	1.0 ± 0.2	0.3 ± 0.01
β-D-Glucuronidase	2.5 ± 0.5	0.2 ± 0.02	<0.1	0.4 ± 0.05
β-D-Galactosidase	8.1 ± 1.0	0.5 ± 0.05	0.3 ± 0.01	0.3 ± 0.01
α-D-Mannosidase	2.9 ± 0.2	1.6 ± 0.1	<0.1	<0.1
β-Hexosaminidase	0.5 ± 0.05	0.8 ± 0.06	0.6 ± 0.01	<0.1

[a] The values are the means ± standard errors of the means for two analyses.

ton, D.C. 3'-AMP was a gift from James K. Lovelace, Walter Reed Army Institute of Research, Washington, D.C. The other chemicals were of reagent grade and were purchased from Fisher Scientific Co. (Pittsburgh, Pa.).

Bacteria and growth conditions. The *B. abortus* strains were obtained from Billy Deyoe, National Animal Disease Center, Ames, Iowa (12). Brucellae were cultivated on potato infusion agar (Difco Laboratories, Detroit, Mich.). The bacterial cells were washed from the plates in 2 ml of Tris-buffered saline (50 mM Tris hydrochloride in 150 mM NaCl [pH 8.0]), pelleted at 5,000 × *g*, washed twice, and suspended at 10^9 to 10^{10} cells per ml in Tris-buffered saline.

Protein determination. Protein concentration was determined by the method of Bradford (3) with bovine serum albumin as the standard.

Enzyme assays. Acid phosphatase activity was determined fluorometrically with 4-methylumbelliferylphosphate (MUP) serving as the substrate (25). The standard acid phosphatase assay was carried out for 15 min at 37°C in 0.1 ml of 0.2 M sodium acetate buffer (pH 5.5) containing 7 mM MUP. One unit of enzyme activity is defined as the amount of enzyme required to convert 1 nmol of substrate to product per h. α-Mannosidase, β-glucuronidase, β-galactosidase, β-hexosaminidase, α-fucosidase, and β-glucosidase activities were determined as described elsewhere (13).

The enzyme-catalyzed release of phosphate from various nucleotides, phospholipids, and IP_3 was estimated by using the malachite green-dependent assay of Lanzetta et al. (16).

Substrate saturation kinetics were determined with different concentrations of MUP and 5'-AMP; the K_m and V_{max} values of the enzyme preparation were determined by the method of Cleland (8).

Purification of acid phosphatase. A washed pellet of *B. abortus* cells was subjected to three freeze-thaw cycles and sonication of 30 s. The extract was centrifuged at 100,000 × *g* for 30 min, and the resulting supernatant was applied to a DE-52 cellulose column (2 by 10 cm) equilibrated in 10 mM sodium phosphate buffer (pH 6.0). The column was developed with a 50-ml, 0 to 0.5 M linear NaCl gradient prepared in 10 mM sodium phosphate buffer (pH 6.0). The pooled enzyme from the DE-52 column was dialyzed against the same sodium phosphate buffer and applied to a hydroxylapatite column (2 by 8 cm). Approximately 20% of the acid phosphatase activity appeared in the column breakthrough fractions. The column was developed with a 50-ml, 0 to 0.5 M linear ammonium sulfate gradient prepared in 10 mM sodium phosphate buffer (pH 6.0). The salt-eluted acid phosphatase peak was pooled, and aliquots were subjected to HPLC-gel filtration performed on a Protein Pak 300 SW column (7.5 by 600 mm; Waters Associates, Inc., Milford, Mass.) (28). The column was equilibrated in 20 mM sodium citrate buffer (pH 6.0) and developed at a flow rate of 0.5 ml/min. Fractions of 1 ml were collected and analyzed for acid phosphatase activity.

Polyacrylamide gel electrophoresis. Proteins were subjected to electrophoresis in sodium dodecyl sulfate-containing gels by the method of Laemmli (15) with a 3% polyacrylamide stacking gel and a 10% separating gel at pHs 6.8 and 8.8, respectively. Gels were stained with Coomassie brilliant blue R-250.

Isoelectric focusing. Isoelectric focusing was performed at 4°C by the procedure of Vesterberg and Stevenson (32) with an 8101 electrofocusing column (LKB Instruments, Inc., Gaithersburg, Md.). Electrofocusing was carried out for 19 h at 3 W in a 110-ml sucrose density gradient (0 to 28% [wt/vol]) containing 0.63% (wt/vol) ampholytes (pHs 3 to 10).

Measurement of O_2^- production by neutrophils. Cell suspensions containing 98% ± 1% neutrophils were prepared from human blood by dextran sedimentation, density gradient centrifugation on Ficoll-Hypaque gradients, and isotonic NH_4Cl lysis (18). The generation of O_2^- by neutrophils was measured as superoxide dismutase-inhibitable cytochrome *c* reduction by a continuous assay method described elsewhere (22).

RESULTS

Content of hydrolytic enzymes in *B. abortus* extracts. We estimated the specific activities of several hydrolytic enzymes in the crude extracts obtained by freezing-thawing and sonicating four strains of *B. abortus* (Table 1). In each case, for the enzymes that were analyzed, the specific activity of acid phosphatase was by far the highest; the specific activity of acid phosphatase in the extracts of the various strains ranged from 3,300 to 12,400 U/mg of protein.

With regard to the location of the phosphatase activity in *B. abortus* cultures, we first compared the amounts of cell-associated and secreted activities. From 100 ml of a *B. abortus* culture at 48 h, we found 850 U of phosphatase in the extracellular medium (supernatant from centrifugation at 4,500 × *g* for 15 min) and 2,500 U of phosphatase in the cell sonicate. When the extracellular medium was filtered through a 0.22-μm-pore-diameter (Millipore Corp., Bedford, Mass.) to remove the last traces of bacterial cells, we found that all of the 850 U of acid phosphatase passed through the filter; thus, about 75% of the total acid phosphatase activity in the culture, as determined at pH 5.5 with MUP as the substrate, was associated with the bacterial cells. We do not know if the 25% of the total acid phosphatase activity that appeared in the extracellular medium was secreted or if it was the result of some degree of cell lysis. When we fractionated the cells into a cell envelope fraction and a soluble fraction by the method of Lutkenhaus (17), we found 70% of the cellular acid phosphatase activity in the nonsed-

TABLE 2. Purification of the acid phosphatase from *B. abortus*

Purification step	Total activity (U[a])	Total protein (mg)	Sp act (U/mg)	Recovery (%)	Purification (fold)
High-speed supernatant	16,500	6.5	2,500	100	1
DE-52 cellulose	15,700	4.5	3,490	95	1.4
Hydroxylapatite	71,200	0.10	712,000	430	285
HPLC-gel filtration	94,300	0.013	7,260,000	571	2,900

[a] One unit is defined as 1 nmol of MUP hydrolyzed per h at 37°C.

imentable fraction (data not shown). We do not know if this soluble activity arose from the periplasmic space or if it was localized to the cytoplasm of the cell.

Purification of acid phosphatase. The phosphatase was isolated and purified from *B. abortus* 1900. In our large-scale purification, approximately 60% of the total acid phosphatase activity in the crude homogenate prepared by freezing-thawing and sonication was contained in the high-speed supernatant fraction (Table 2). This soluble acid phosphatase was purified by chromatography on DE-52 cellulose, and the acid phosphatase-containing fractions from the DE-52 cellulose column were pooled and chromatographed on hydroxylapatite. Finally, the hydroxylapatite pool was subjected to HPLC-gel filtration (Fig. 1); this step yielded a 10-fold purification. Noteworthy is the fact that acid phosphatase and 5'-nucleotidase activities cochromatographed on this column. The elution position of the enzyme corresponded to a native molecular mass of 120 ± 10 kilodaltons. When the final preparation of the phosphatase was subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis, a pair of sharp, intense bands was detected at positions which corresponded to 60- and 66-kilodalton proteins (Fig. 2). The sum of the molecular masses of these two proteins (126 kilodaltons) was close to the native molecular mass of the acid phosphatase (120 ± 10 kilodaltons). On the basis of these results, the native enzyme appears to be a dimer composed of nonidentical subunits. The final yield of activity was 571%, and the specific activity was enriched 2,900-fold relative to that of the crude, high-speed supernatant. The

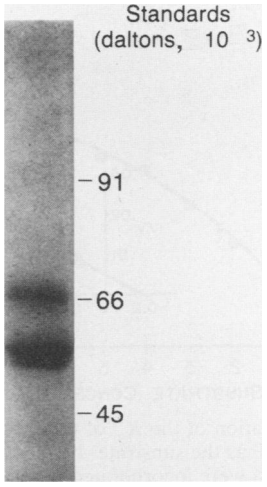

FIG. 2. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of the *B. abortus* phosphatase. Purified phosphatase (10 μg) after the HPLC-gel filtration step was electrophoresed in a polyacrylamide gel and stained for protein as described in the text.

fact that we recovered more than five times the activity we started with in the initial high-speed supernatant indicates that the crude extract may contain an inhibitor of the phosphatase. By mixing fractions from the hydroxylapatite column with the phosphatase peak fraction, we attempted to identify the putative inhibitor; however, this effort was not fruitful.

Isoelectric point. The acid phosphatase from the hydroxylapatite column was subjected to isoelectric focusing (Fig. 3); the acid phosphatase activity did not focus as a single sharp peak; the peak focused at a pI of 3.0, with a shoulder on the acid side. This result indicates there may be some heterogeneity present in the enzyme; however, the basis for this apparent microheterogeneity is unclear.

Inhibitors. We determined the effect of various potential phosphatase inhibitors on the activity of the *B. abortus* phosphatase (data not shown). At a 0.5 mM concentration

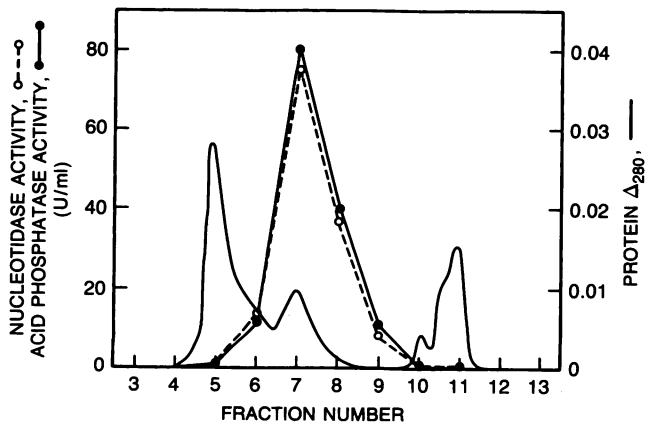

FIG. 1. HPLC-gel filtration. The pooled fractions from the hydroxylapatite column were applied to an HPLC-gel filtration column. The column was equilibrated in 20 mM sodium citrate buffer (pH 6.0) and developed at a flow rate of 0.5 ml/min. One-milliliter fractions were collected and analyzed for acid phosphatase (●) and 5'-nucleotidase (○) activities. The solid line indicates the A_{280} of each fraction.

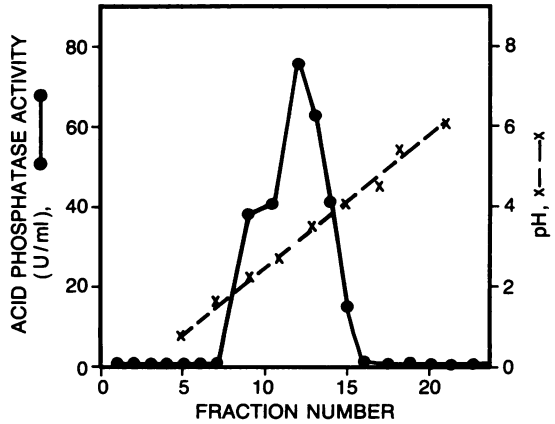

FIG. 3. Determination of the isoelectric point of the *B. abortus* phosphomonoesterase by isoelectric focusing. Isoelectric focusing was performed with an 8101 focusing column containing a 100-ml sucrose gradient (0 to 50%, [wt/vol]) and 2% (wt/vol) ampholytes in the pH range of 3 to 10. Two-milliliter fractions were collected, analyzed for pH (×), and assayed for acid phosphatase activity (●). The pI of the phosphomonoesterase was 3.0.

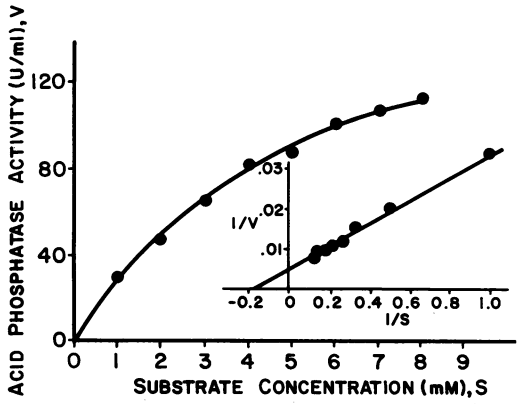

FIG. 4. Determination of the K_m of the *B. abortus* phosphomonoesterase with MUP as the substrate. Increasing concentrations of MUP (1.0 to 8.0 mM) were incorporated in the standard incubation mixture, and acid phosphatase activity was determined. The K_m of the phosphomonoesterase was 5.5 mM.

neither L-(+)-sodium tartrate nor sodium fluoride inhibited phosphatase activity. In addition, at a 0.5 mM concentration, 12 heteropolymolybdate complexes (including complexes A, D, and E) inhibited enzyme activity by less than 50%. Since most true acid phosphatases are inhibited by one or more of these compounds, the insensitivity of the *B. abortus* enzyme to these inhibitors compels us to designate the enzyme as a nucleotidase.

Kinetic properties of the acid phosphatase. The Michaelis-Menten constant (K_m) of the acid phosphatase, estimated at pH 5.5 with MUP as the substrate, was 5.5 mM (Fig. 4). Compared with the K_m values of other, more extensively characterized acid phosphatases (13) which utilize MUP as a substrate, this K_m is relatively high. For example, the K_m values for MUP of prostatic acid phosphatase and the leishmanial phosphatase are 0.3 to 0.4 mM, respectively. However, the K_m for MUP of the *B. abortus* enzyme was similar to that of the *L. micdadei* phosphatase (3.8 mM), which also has significant nucleotidase activity (27). In view of these considerations, we analyzed the purified *B. abortus* phosphatase preparation for its ability to catalyze the dephosphorylation of 3'-AMP and 5'-AMP at pH 5.5 and found both to be excellent substrates (Table 3). Furthermore, both 3'-AMP and 5'-AMP were excellent substrates at pH 7.0 as well as pH 5.5. The K_m for 5'-AMP pH 6.5, 0.37 mM (Fig. 5), was much lower than that for MUP at pH 5.5.

We tested several other nucleotides for their ability to serve as substrates for the *B. abortus* phosphatase; at pHs 5.5 and 7.0, 5'-GMP, 5'-GDP, 5'-CDP, 5'-CTP, and 5'-UTP were cleaved at only 10 to 66% the rate for 5'-AMP or 3'-AMP (Table 3). PIP_2, phosphatidylinositol 4-monophosphate (PIP), and IP_3 were not cleaved (<1% of the rate for 5'-AMP) at pHs 5.5 and 7.0 by the purified *B. abortus* phosphatase (Table 3).

pH optimum. The effect of pH on the activity of the *B. abortus* acid phosphatase was determined with MUP as the substrate (Fig. 6); optimum activity was obtained at pHs 5.5 to 6.0. About 10% of the maximum activity was evident at pH 4.0, but the enzyme was nearly inactive at pH 9.0. When 5'-AMP was used as the substrate, the pH optimum of the phosphomonoesterase was found to be in the pH range of 6.0 to 6.5 (Fig. 6).

Effect of acid phosphatase on O_2^- production by neutrophils. Neutrophils were incubated for 10 min at 37°C in the presence of 20 to 350 U (determined at pH 7.2 in Hanks balanced salt solution with 9.0 mM MUP) of purified *B. abortus* phosphomonoesterase before stimulation with formyl-methionyl-leucyl-phenylalanine (fMLP). Phosphatase treatment did not significantly reduce the ability of the stimulated neutrophils to generate O_2^-, as compared with control neutrophils preincubated (15 min, 37°C) with boiled enzyme.

TABLE 3. Hydrolysis of phosphorylated compounds by the *B. abortus* phosphomonoesterase

Substrate	Concn (mM)	Phosphate released (nmol/ml per h) at assay[a] pH:	
		5.5	7.0
MUP	1.0	26.0 ± 1.0 (100)	19.5 ± 0.8 (100)
5'-AMP	1.0	33.6 ± 1.2 (129)	24.0 ± 0.8 (123)
3'-AMP	1.0	28.5 ± 1.0 (109)	26.5 ± 0.7 (136)
5'-GMP	1.0	17.3 ± 0.7 (66)	10.7 ± 0.4 (55)
5'-GDP	1.0	14.0 ± 0.6 (54)	8.4 ± 0.4 (43)
5'-CDP	1.0	6.2 ± 1.0 (24)	2.2 ± 0.2 (11)
5'-CTP	1.0	6.2 ± 0.7 (24)	3.4 ± 0.2 (17)
5'-UTP	1.0	3.8 ± 0.4 (14)	2.3 ± 0.3 (11)
Tyrosine phosphate	1.0	0.3 ± 0.01 (<2)	4.2 ± 0.5 (22)
Serine phosphate	1.0	0.3 ± 0.01 (<2)	0.5 ± 0.01 (<3)
Threonine phosphate	1.0	0.1 ± 0.01 (<1)	0.2 ± 0.02 (<2)
Fructose 1,6-diphosphate	1.0	0.1 ± 0.01 (<1)	0.1 ± 0.01 (<1)
Glucose 1-phosphate	1.0	0.1 ± 0.01 (<1)	0.7 ± 0.02 (<4)
PIP_2	0.02	0.05 ± 0.01 (<1)	0.2 ± 0.01 (<1)
PIP	0.02	0.12 ± 0.02 (<1)	0.03 ± 0.01 (<1)
IP_3	0.02	0.22 ± 0.02 (<1)	0.13 ± 0.01 (<1)

[a] The various phosphomonoesters were incubated for 15 min to 4 h at 37°C with 5 to 100 U of purified enzyme in a 0.1-ml incubation mixture. Phosphate release was estimated by the malachite green assay with P_i as the standard. The values are the means ± standard errors of the means for two analyses. Numbers in parentheses indicate the rate of hydrolysis (as a percentage) relative to that of MUP.

DISCUSSION

The hydrolase activities revealed by analyzing crude extracts of *B. abortus* for their ability to cleave fluorogenic

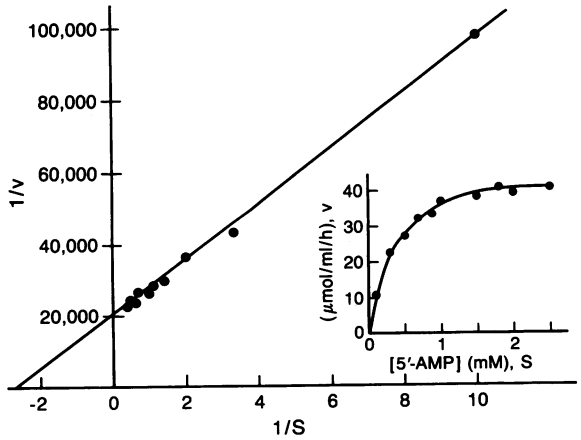

FIG. 5. Determination of the K_m of the *B. abortus* phosphomonoesterase with 5'-AMP as the substrate. The 5'-AMP concentration in 0.2 M sodium acetate buffer (pH 6.5) was varied from 0.1 to 2.5 mM. The reaction was conducted for 15 min at 37°C, and nucleotidase activity was measured by the malachite green assay. The K_m was estimated to be 0.37 mM.

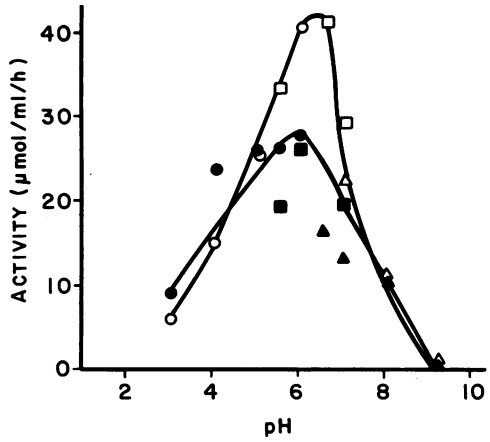

FIG. 6. Effect of hydrogen ion concentration on the activity of the *B. abortus* phosphomonoesterase. The activity of the enzyme at various pHs was determined with the following buffers: 0.2 M sodium acetate (pHs 3.0 to 6.0) (○); 0.2 M sodium cacodylate (pHs 5.5 to 6.5) (□); and 0.2 M Tris hydrochloride (pHs 7 to 9.2) (△). The substrates were MUP (● and ■) and 5′-AMP ○, □, and △).

glycoside and phosphomonoesters are remarkably similar to the profiles we obtained with extracts of *L. micdadei* (27) and *L. donovani* (13) promastigotes. *B. abortus*, *L. micdadei*, and *L. donovani* all contain large amounts of acid phosphatase activity, and none of these organisms possesses other acid hydrolase activities in significant quantity. However, unlike the *L. micdadei* (27) and *L. donovani* (21, 22) phosphatases, which also block the production of O_2^- by human neutrophils, the *B. abortus* phosphomonoesterase does not inhibit O_2^- production by neutrophils stimulated by the formylated peptide fMLP. Thus, although *B. abortus* is a microorganism which shares with *L. donovani* and *L. micdadei* the property of being able to establish itself inside phagosomes of macrophages and neutrophils and which produces a phosphomonoesterase, its phosphatase is not capable of inhibiting superoxide anion production by host phagocytes.

Also, in contrast to the *L. micdadei* and *L. donovani* phosphatases, the *B. abortus* enzyme possesses essentially no PIP_2 or IP_3 phosphomonoesterase activity, as these compounds were hydrolyzed at <1% the rate for MUP or 5′-AMP. Like the *L. micdadei* enzyme (27), the *B. abortus* phosphomonoesterase possesses considerable nucleotidase activity. Furthermore, since our most pure preparation of the acid phosphatase showed coelution of phosphatase and 5′-nucleotidase activities on the HPLC-gel filtration column (Fig. 1), we believe that both activities probably reside in a single enzyme.

While the mechanisms and virulence factors responsible for the capacity of brucellae to escape the bactericidal properties of host phagocytes are not completely understood, the production by *B. abortus* of 5′-GMP and adenine, which independently suppress neutrophil iodination activity (4), is believed to play a role. However, it must be noted that the amounts of adenine and 5′-GMP released by brucellae growing in glucose-peptone M medium were insufficient to cause a significant reduction in the iodination activity of bovine neutrophils in suspension in vitro (5). *Haemophilus somnus* also produces adenine, GMP, and guanine extracellularly as virulence factors (7). By virtue of its high nucleotidase activity, the *B. abortus* phosphomonoesterase characterized in this report may be one of the bacterial enzymes in the pathway leading to the secretion of adenine by the bacterium.

There is evidence that adenosine, as well as adenine, may adversely affect host phagocyte function. Riches et al. (23) reported that lysosomal enzyme secretion by murine macrophages was inhibited in the presence of micromolar concentrations of adenosine, AMP, ADP, and ATP. Their studies indicated that adenine nucleotides are converted to adenosine through the action of cell-associated phosphohydrolases and 5′-nucleotidase and that it is the adenosine that is responsible for the inhibition of phagocyte function. At physiologic concentrations, adenosine markedly inhibited O_2^- and H_2O_2 generation by neutrophils stimulated with various soluble stimuli (9). Adenosine did not inhibit neutrophil granule fusion (9, 30), although adenosine agonists inhibited degranulation (30). Neither neutrophil uptake nor the metabolism of adenosine was required for inhibition (9). Unexpectedly, endogenously generated adenosine was present in supernatants of neutrophil suspensions, and the removal of endogenous adenosine by incubation of neutrophils with exogenous adenosine deaminase led to the marked enhancement of O_2^- generation in response to fMLP (9). Extracellular adenosine apparently attenuates neutrophil function by binding to adenosine A_2 receptors; engagement of adenosine A_2 receptors activates adenylate cyclase and raises intracellular cyclic AMP levels (10, 30). Therefore, the phosphatase present in the extracellular medium probably arises by secretion rather than cell lysis. We do not know if the phosphatase present in the ultrafiltrate of the *B. abortus* culture medium is the same as the cellular phosphatase that we have isolated and characterized in the present report. Although we do not know if the extracellular phosphatase arises by secretion or cell lysis, the possibility nevertheless remains that the 5′-nucleotidase activity that is released extracellularly may promote bacterial survival in phagocytes by generating purine nucleosides, in particular, adenosine. The isolation and characterization of the extracelluar *B. abortus* phosphatase are in progress.

ACKNOWLEDGMENTS

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