

Acid Phosphatases in Skeletal Muscles of Uromastix

R. NAZ, N. IKRAM†, A. SAEED, Z.A. KHAN AND A.R. KHAN

Departments of Chemistry, Gomal University, D.I. Khan and †Bahauddin Zakarya University, Multan-Pakistan

ABSTRACT

Two major types of acid phosphatases, high and low molecular weights, are reported in the extract of Uromastix's skeletal muscles, which can be separated by gel chromatography on Sephadex G-150. These enzymes differ from each other with regards to activation, inhibition and substrate specificity.

Key Words: Acid phosphatases; Skeletal muscles; Uromastix

INTRODUCTION

Acid phosphatase (Ac Pase, EC 3.1.3.2) classes have been studied in plants and animals (Hollander, 1971). Vertebrate tissues contain two major acid phosphatase forms of Mr. 90,000-102,000 and 13,400-30,000, which can be separated by gel chromatography (Heinrikson, 1969; Campbell *et al.*, 1973; Rehlop & Van Etten, 1975). The effect of inhibitors, substrate requirements, optimum pH and localization within cells and other kinetic parameters also distinguish between these enzymes (Shibko & Tappel, 1963; Heinrikson, 1969; Saini & Van Etten, 1978; Taga & Van Etten, 1982; Baldijao *et al.*, 1995).

Knowledge of the structure, properties and physiological role of skeletal muscle Ac Pase is sparse. The presence of different acid phosphatase activities in the vain skeletal muscle has been demonstrated by histochemical technique (Trout *et al.*, 1981). Baxter and Suelter (1984) reported presence of different Mr. Ac Pase form in avian pectoralis muscle. The purification of low Mr. Ac Pase from chicken and frog skeletal muscles has been reported (Baxter & Suelter, 1985; Panara *et al.*, 1991). The enzymes are composed of two different isoenzymes which differ in isoelectric and molecular size.

In this paper, isolation of low and high Mr. Ac Pase forms from muscles of *Uromastix hardwickii* and comparison of some biochemical properties between these forms have been reported.

MATERIALS AND METHODS

Uromastix hardwickii, a spiny tailed lizard (Reptile), captured from plane regions of N.W.F.P. was killed by al blow to the head. The skeletal muscles were removed immediately for extraction of enzymes.

Enzyme assay. Acid phosphatase activity was determined at 37°C in 1 mL of 125 mM acetate buffer pH 5.0 containing 1 mM EDTA and 4 mM p. nitrophenyl-1-phosphate as described by Ramponi *et al.* (1989). The reaction was started by the addition of enzyme solution and was terminated by the addition of 4 mL of 0.1 M KOH solution. The absorbance was read at 405 nm. One unit of enzyme

was defined as the amount of enzyme required to produce 1 μmol of p. nitrophenol per min. Hydrolysis rates of various phosphoric esters were determined at 37°C under above conditions by estimation of inorganic phosphate (Pi) liberation. The liberated Pi was determined by Black and Jones method (Black & Jones, 1983). Optimum temperature for enzyme catalyzed reaction was determined in range of 0-70°C at intervals of 10°C by the incubation of enzyme in the presence of acetate buffer pH 5.0 and mM p. nitrophenyl phosphate for 5 min. Thermal stability was studied by incubating the enzyme solution at temperatures ranging from 40 to 70°C in the presence of 0.1 M acetate buffer (pH 5.0) for various intervals of time. The reaction was stopped by immersion in ice bath and residual activity was assayed as described above. Optimum pH was determined in 0.1 M acetate buffer (pH 4.0-6.0), 0.1 M barbital buffer (pH 6.0-6.7) and 0.1 M Tris-HCl buffer (pH 7.0-8.0).

Effect of metal ions and some modifiers at various concentrations on the enzyme activities were determined as described earlier. Km was determined graphically (Lineweaver-Burk plot) using seven substrate concentrations ranging from 0.06 to 4.0 mM.

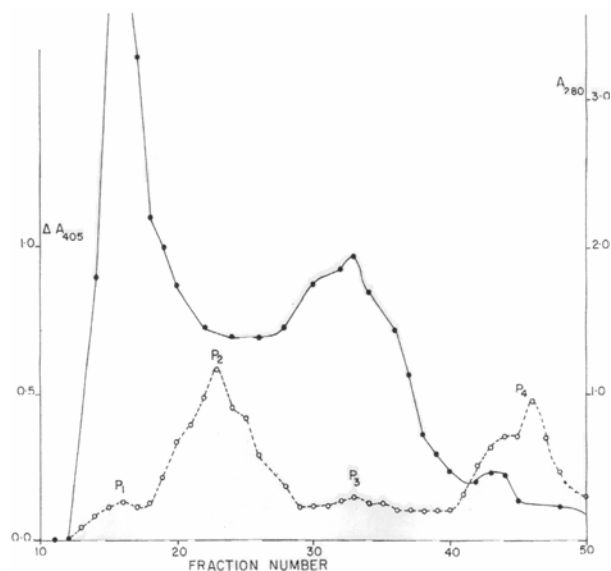
Isolation of low and high molecular weight acid phosphatases. Skeletal muscles from *Uromastix hardwickii* were washed with cold 10 mM Tris-HCl buffer (pH 7.4) containing 0.25 M sucrose and 1.0 mM EDTA and cut into small pieces. It was then homogenized into Edmund Bühler homogenizer model HO-4 in the same buffer. After homogenization the volume was adjusted to 3 mL of buffer/g of original tissues and then gently stirred for 1 h. The whole content was centrifuged at 45,000 x g for 20 min. The supernatant was treated with 30% saturated ammonium sulphate. After 15 min, the precipitate was discarded by centrifugation at 12,000 x g for 20 min and the supernatant was brought to 70% saturation with ammonium sulphate and left to stand for 1h at 4°C. The precipitate obtained after centrifugation at 12,000 x g for 20 min was dissolved in 0.1 M acetate buffer (pH 5.0), 0.14M NaCl, 1.0 mM EDTA and 0.1 mM PMSF and placed on Sephadex G-150 column (1.8 x 80 cm), equilibrated and eluted with the same buffer at flow rate of 9-12 ml/h. Chromatographic fractions about 3-4 mL were assayed for protein concentration (O.D. 280 nm)

and enzyme activity. The enzymes thus obtained were used for biochemical analysis.

RESULTS AND DISCUSSION

Fig. 1 shows the elution profile from the Sephadex G-150 column of acid phosphatases extracted from the muscles of *Uromastix*. Four activity peaks are obtained namely P1, P2, P3 and P4. P1 and P2 peaks are seemed to be of high molecular weight acid phosphatases, while P3 and P4 are intermediate and low molecular weight acid phosphatases, respectively. P2 and P4 are major peaks and are designated as high molecular weight acid phosphatases and low molecular weight acid phosphatases.

Fig. 1. Sephadex G-150 chromatography. 1-1.5 mL sample after $(\text{NH}_4)_2\text{S}_0_4$ precipitation was applied onto the column (1.8 x 80 cm) with flow rate 10-15 mL/h, 3-4 mL fraction was collected.



These results are similar to that of phosphatases in other mammalian tissues (Panara *et al.*, 1992). High molecular weight form is present in two molecular species: a microsomal associated enzyme (HMW-I) of about 200 kDa and 80-120 kDa Ac pase (HMW-II), which is of lysosomal origin (Baxter & Suelter, 1984). The existence of an acid phosphatase with intermediate molecular weight (IMW 35-50 kDa) was reported in rat liver (Murakami *et al.*, 1986), sea urchin eggs and embryos (Yokota & Nakano, 1984).

Low molecular weight form (10-30 kDa) is not a particle associated enzyme and seems to be of cytosolic origin (Waheed *et al.*, 1988; Zhang & Van Etten, 1990). High molecular weight acid phosphatases (P2) and low

molecular weight acid phosphatases (P4) have been used for further biochemical studies.

Biochemical properties of low and high molecular weight acid phosphatases. The enzyme is not stable at room temperature, and activity starts decreasing after 5-6 h, but it remains active if stored at -4°C for 1-2 weeks at pH 7.0. The enzyme does not require divalent ions for activity. Table I shows that Zn^{2+} , Mg^{2+} , and Mn^{2+} had little or no effect on the activity while Cu^{2+} , Hg^{2+} and Cd^{2+} were found to be inhibitors of the enzyme.

Table I. Effect of divalent metal ions on Ac Pase activities

Cation	High Mr. Ac Pase (%)	Low Mr. Ac Pase (%)
None	100	100
CaCl_2	99	97
MgCl_2	98	95
ZnCl_2	92	89
MnCl_2	95	83
MuSO_4	35	39
HgCl_2	11	17
CdCl_2	65	71

Low molecular weight acid phosphatases of *Uromastix* muscle were insensitive to NaF and tartrate, but strongly inhibited by formalin while high molecular weight acid phosphatases were strongly inhibited by fluoride and tartrate and insensitive to formaldehyde. The inorganic phosphate, vanadate and molybdate were found to be effective inhibitors for both enzymes as shown in Table II.

Table II. Effect of inhibitors on high and low Mr. Ac Pases

Inhibitors	Conc.	High Mr. Ac. Pase (%)	Low Mr. Ac. Pase (%)
Control	-	100	100
NaF	10 mM	10	100
Sodium potassium tartrate	10 mM	12	99
Formaldehyde	0.5%	85	13
KH_2PO_4	1 mM	55	70
Na_2VO_4	0.1 mM	2	39
Na_2MoO_4	0.5 mM	0	7

A marked difference between high and low molecular weight acid phosphatases is the substrate specificity. Table III shows the hydrolysis rates of various phosphate esters. High molecular weight acid phosphatase hydrolyzed all substrates tested, whereas only p. nitrophenyl phosphate, phenyl phosphate, riboflavin mononucleotide were hydrolyzed by low molecular weight acid phosphatase at significant rate.

Low molecular weight acid phosphatase was stimulated in the presence of glycerol, methanol, ethanol, and acetone (Table IV). Glycerol and methanol at 10% concentrations caused 2-fold increase in the activity, while these modifiers did not affect high molecular weight acid

phosphatases. These activations were also observed in other low molecular weight acid phosphatases (Taga & Van Etten, 1982; Murakami et al., 1986; Pnara et al., 1992). Thermal stability of the acid phosphatase (Fig. 2) showed that enzyme was not inactivated at 40°C for 1 h, but inactivated in some time dependent manner with a half life of 20 min at 50°C and of about 3 min at 60°C.

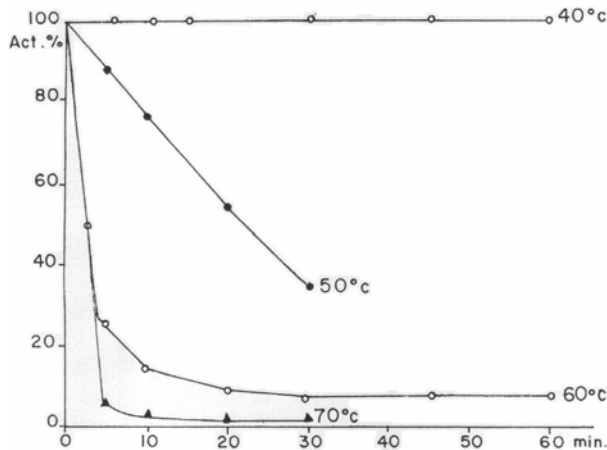
Table III. Substrate specificity of Ac Pase

Substrate	High Mr. Ac Pase (%)	Low Mr. Ac Pase (%)
p. nitrophenyl phosphate	100	100
Phenyl phosphate	98	90
α-glycerol phosphate	21	10
β-glycerol phosphate	56	15
G-1-phosphate	20	0
ATP	30	0
AMP	42	0
FMN	35	98
G-6-phosphate	15	0

Table IV. Effect of modifiers on Mr. Ac Pase activity

Compounds	Concentration (%)	High Mr. Ac Pase (%)	Low Mr. Ac Pase (%)
Water	10	100	100
Glycerol	10	100	235
Methanol	10	98	205
Ethanol	10	98	147
Acetone	10	93	151

Fig. 2. Thermal inactivation of acid phosphatase. The enzymes were heated at 40, 50, 60, and 70 °C in 0.1M acetate buffer (pH 5.0) for times indicated in abscissa. The residual activities were assayed as usual.



Km values for low and high Mr. Ac Pase were found to be 0.36 mM and 0.22 mM as shown from Lineweaver-Burk plot (Figs. 3 & 4).

Fig. 3. Lineweaver Burk plot for the effect of substrate concentrations on the Low Mr. Ac-Pase activity. Km was calculated using linear regression analysis.

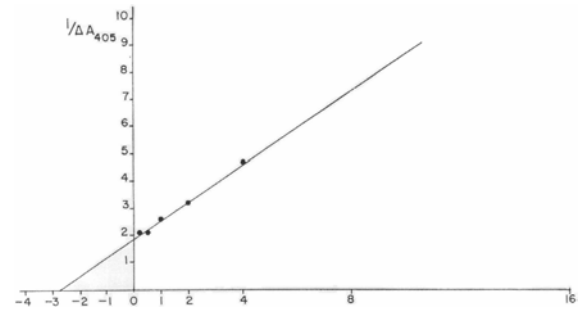
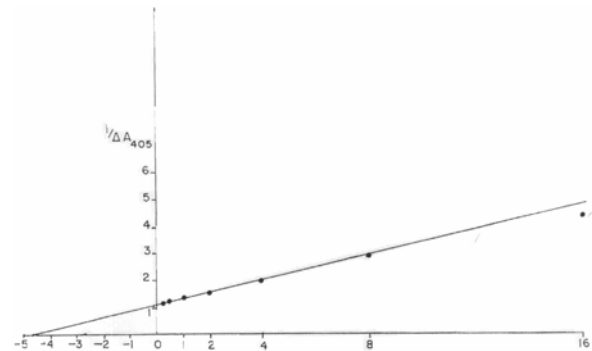


Fig. 4. Lineweaver Burk plot for high Mr. Ac-Pase.



REFERENCES

Baldijao, C.E., H.M.S. Bittencourt and H. Chaimovich, 1975. Study state kinetics and effect of SH inhibitors on acid phosphatase from bovine brain. *Biochem. Biophys. Acta*, 391: 316–25.

Baxter, J.H. and C.H. Suelter, 1984. Multiple acid phosphatase in avian pectoral muscle; the post microsomal supernatant acid phosphatase is elevated in avian dystrophic muscle. *Arch. Biochem. Biophys.*, 288: 397–406.

Baxter, J.H. and C.H. Suelter, 1985. Resolution of the low molecular weight acid phosphatase in avian pectoral muscle into distinct enzyme form. *Arch. Biochem. Biophys.*, 239: 29–37.

Black, M.J. and M.J. Jones, 1983. Inorganic phosphate determination in the presence of a labile organic phosphate: assay for carbonyl phosphatase activity. *Anal. Biochem.*, 135: 233–8.

Cambell, N.D., N.P.B. Dudman and B. Sermer, 1973. On the multiple forms of acid phosphatase in pig liver. *FEBS Lett.*, 31: 123–6.

Fujimoto, S., K. Murakami, A. Ishikawa, K. Himi and A. Ohara, 1988. Two distinct low molecular weight acid phosphatase from rat liver. *Chem. Pharm. Bull.*, 36: 3020–26.

Heinrikson, R.L., 1969. Purification and characterization of low molecular weight acid phosphatase from bovine liver. *J. Biol. Chem.*, 244: 299–307.

Hollander P.V., 1971. *The Enzymes*. Vol. 4. Academic Press, New York.

Murkami, K., S. Fujimoto and A. Ohara, 1986. Subcellular localization and some properties of intermediate molecular weight acid phosphatase from rat liver. *Chem. Pharm. Bull.*, 34: 3320–27.

- Panara, F., A. Angiolillo, T. Sacca, A. Fagotti, I. Di-rosa, S. Francesca and R. Pascolini, 1992. Acid phosphatase in mammalian tissue evidence for the existence of a 57 kDa Zn²⁺ dependent acid phosphatase form. *Int. J. Biochem.*, 24: 1619–23.
- Panara, F., A. Angiolillo, T. Seca, I. Di Rosa, A. Fagotti and R. Pascolini 1991. Acid phosphatase in the frog (*Rana esculenta*) skeletal muscle; purification and some properties of low molecular weight enzyme. *Int. J. Biochem.*, 23: 1115–22.
- Ramponi, G., G. Manao, G. Camici, G. Cappugi, M. Rugiero and D.P. Bottaro, 1989. The 18 kDa cytosolic acid phosphatase activity on the auto-phosphorylated epidermal growth factor receptor. *FEBS Lett.*, 250: 469–73.
- Rehlop, D.M. and R.L. Van Etten, 1975. Human liver acid phosphatases. *Hoper Syeler's Z. Physiol. Chem.*, 356: 1725–82.
- Saeed, A., E. Tremori, G. Camici, G. Cappugi and G. Ramponi, 1990. Bovine brain low Mr. Acid phosphatase: Purification and properties. *Physiol. Chem. Physiol. Med. NMR.*, 22: 81–94.
- Saini, M.S. and R.L. Van Etten, 1978. A homogeneous isozyme of human liver acid phosphatase. *Arch. Biochem. Biophys.*, 191: 613–8.
- Shibko, S. and A.L. Tappel, 1978. Acid phosphatase of the lysosomal and soluble fraction of rat liver. *Biochem. Biophys. Acta*, 73: 76–86.
- Taga, E.M. and R.L. Van Etten, 1982. Human liver acid phosphatase: purification and properties of low molecular weight isozymes. *Arch. Biochem. Biophys.*, 214: 505–15.
- Trout, J.J., W.T. Stauber and B.A. Schottelius, 1981. Further studies on unique T-tubular acid phosphatase in avian skeletal muscle. *Histochem. J.*, 13: 445–52.
- Waheed, A., P.M. Laidler, Y.P. Wo and R.L. Van Etten, 1988. Purification and physicochemical characterization of human placental acid phosphatase activity. *Biochemistry*, 27: 4265–73.
- Yokota, Y. and E. Nakano, 1984. Two acid phosphatases in sea urchin eggs and embryos. *Comp. Biochem. Physiol.*, 79B: 17–22.
- Zhang, Z.Y. and R.L. Van Etten, 1990. Purification and characterization of low molecular weight acid phosphatase-A phosphotyrosyl-protein phosphatase from bovine heart. *Arch. Biophys.*, 282: 39–49.

(Received 22 August 2001; Accepted 05 September 2001)