KINETICS AND INHIBITION STUDY OF ACID PHOSPHATASES FROM CAMEL LIVER

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ABSRACT
The camel liver contained two acid phosphatases and these were separated by Sephadex G-100 column chromatography. The first activity peak was designated as high molecular weight acid phosphatase (HM-AP)while the second peak was referred as low molecular weight acid phosphatase (LM-AP). Both enzymes were inactivated by 10 mM Cu^{++} and Hg^{++}. HM-AP was inhibited by fluoride and tartrate and unaffected by iodoacetate but LM-AP showed opposite behaviour. Phosphate, vanadate, molybdate, tartrate and fluoride were found competitive inhibitors. Their K_i values for HM-AP were 0.6 mM, 0.8µM, 0.18µM, 0.1mM and 0.06mM , respectively while K_i values for LM-AP were1.8mM, 183µM and 317µM, respectively and no inhibitions were observed with tartrate and fluoride.
KEY WORDS: Acid phosphatase, Camel liver, Kinetics and inhibition

INTRODUCTION

Hydrolysis of phosphate monoesters is very important in animal and plants tissues which is linked to many biological processes such as energy metabolism, metabolic regulation and cellular signal transduction pathways (Shan, 2000). This hydrolysis is catalysed by diverse group of enzymes called phosphatases. Five classes of phosphatases exist, the alkaline phosphatases, purple acid phosphatases, low molecular weight acid phosphatases, high molecular weight acid phosphatases and protein phosphatases (Rodrigues et al., 2006; Ostanin et al., 1994;).

Acid phosphatases, APases (EC 3.1.3.2) catalyse the hydrolysis of various phosphomonoesters in acid medium (pH 5-6) to release an inorganic phosphate (Guimaraes et al., 2001; Vincent et al., 1992). These occur in multiple forms (Fujimoto et al.,1984) and can be differentiated according to structure, catalysis, tissue distribution and localization (Suter et al., 2001). Mammalian tissues contains mainly two forms of APases, HM-AP (Mr 80-120 kDa) and LM-AP (Mr 10-30 kDa)(Panara et al., 1992). These forms are clearly vary in their substrate specificity, localization within cells, and sensitivity to activators or inhibitors (Caselli et al.,1996; Panara et al., 1990; Naz et al., 2001).

A large number of compounds have been found to inhibit the phosphatases. It has been reported that okadaic acid was found a well known inhibitor of protein ser/thr phosphatases (Senna et al., 2006; Smith and Walker,1996) while vanadium has a use as an inhibitor of proteintyrosine phosphatases. Levamisole and tetramisole are alkaline phosphatase inhibitors (Belle, 1972). Likewise, fluoride and tartrate are the inhibitors of HM-AP (Hollander, 1971) and reducing agents such as β-mercaptoethanol, iodoacetate and formaldehyde are the inhibitors of LM-AP (Igarashi and Hollander, 1968).

In this study the comparison of the inhibitions by various compounds

between high molecular weight acid phosphatase and low molecular weight acid phosphatase has been reported.

MATERIALS AND METHODS

Chemicals.

The camel liver was obtained from slaughter house. Sephadex G-100 were supplied by Sigma Chemical Co. (USA) and p-nitrophenyl phosphate from Fluka Chemical Co. (Switzerland).

METHODS

Preparation of crude extract and chromatography

Camel liver was collected from slaughter house. Following rinsing with water, it is homogenized in Waring Blender with 3 ml of 0.3 M acetate buffer pH 5.0 containing 1mM EDTA, 0.1mM PMSF and 2mM β-mercaptoethanol per gram of liver. The homogenate was agitated for 1h and centrifuged at 5000 rpm for 30 min. The supernatant obtained was salted out with ammonium sulphate (80% saturation) and placed on Sephadex G-100 column (0.9 x 60 cm), which was previously equilibrated and eluted with 0.01 M acetate buffer pH 5.0 containing 1mM β-mercaptoethanol and 0.1 M NaCl at flow rate of 15 ml/h. Fractions (about 1.5 ml each) were collected at unit gravity. Pooled fractions from both peaks were stored at low temperature (-20°C) in aliquots. When needed aliquots were removed and brought at 4°C for kinetics experiments.

Enzyme assay

Enzyme activity was determined by measuring production of p-nitrophenol as described by Ramponi et al. (1989).Reaction mixture contained 900 µl of 0.1M acetate buffer pH 5.0 containing 4 mM p-nitrophenyl phosphate as substrate and 50 – 100 µl enzyme samples. Mixture was incubated at 37°C for 5 min. Reaction was stopped by the addition of 1.0 ml of 0.1M NaOH. Amount of p-nitrophenol formed was determined spectrophotometrically at 405 nm. The non-enzymatic hydrolysis of p-nitrophenyl phosphate was corrected by measuring the control without added enzyme.

Determination of K_m and K_i values

Kinetic studies were carried out in acetate buffer, pH 5.0 as described (Siddiqua et al., 2009). The K_m and K_i values were determined using double reciprocal plots. Six concentrations ranging from 0.06mM to 4 mM *p*-nitrophenyl phosphate were used in absence and presence of two or three fixed concentrations of inhibitors to caculate the values of K_m and $K_{i.}$. Straight lines were drawn by applying least square rule. Each point was the average of at least three readings

RESULTS AND DISCUSSION

The enzyme extract after ammonium sulphate precipitation was passed through Sephadex G-100. The elution profile of acid phosphatase activities from Sephadex G-100 chromatography is shown in fig.1. The two types of acid phosphatases were separated which may be distinguished in term of their molecular size. The first peak refered to HM-AP and second peak was designated as LM-AP. The same results were obtained from chicken liver of chicken and bovine brain (Panara,et al., 1985; Heinrikson,1969). In addition to LM-AP and HM-AP enzymes, intermediate molecular weight acid phosphatase has also been reported in an appreciable amount in bovine kidney and rat liver

(Fujimoto et al., 1984). In all tissues of mammalian origin, LM-AP is the predominant of all three activities with the exception of few cases such as rat liver, porcine kidney and bovine spleen where HM-AP predominate slightly in percentage compositions over LM-AP.

LM-AP and HM-AP showed a different behaviour with various compounds. Both types of AP were tested with reagents at concentration of 10 mM. These were inactivated by Cu^{++} and Hg^{++}. Fluoride and tartrate were potent inhibitors of HM-AP as found in many other similar enzymes (Zhang and Van Etten, 1990) while LM-AP was unaffected. Formaldehyde and iodoacetate showed opposite behaviour. The HM-AP were not inhibited by these compounds while LM-AP was inhibited. The Zn^{++} ion showed inhibitory action on LM-AP enzyme while a slight activation was observed for high molecular weight enzymes.
Phosphate, vanadate, molybdate, tartrate and fluoride were found purely competitive inhibitors. Their inhibition constants (K_i) were determined by Lineweaver-Burk plots, employing linear regression method. Each value is the mean value of three determinations (Fig. 2 and 3). The HM-AP were found more sensitive to all these inhibitors than LM-AP enzyme. Their K_i values for HM-AP are 0.6 mM, 0.8 µM, 0.18 µM, 0.1 mM, 0.06 mM, respectively and K_i values for LM-AP are 1.8 mM, 183 µM and 317 µM, respectively. No inhibitions were observed with tartrate and fluoride. Almost same results were also obtained from other phosphatases (Siddiqua et al., 2012 and 2008; Naz et al., 2006; Khan et al., 1997).

CONCLUSIONS

Low and high molecular weight acd phosphatases were isolated from camel liver extract. Phosphate, vanadate, molybdate, tartrate and fluoride were found purely competitive inhibitors. Their K_i values were determined. HM-AP was found more sensitive to these inhibitors than LM-AP enzyme.

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Fig. 1 Elution profile from Sephadex g-100 chromatography.

Ordinates: Protein at 280 nm ((•—•); acid phosphatase activity, U/ml(o···o).

Fig. 2 Competitive inhibition of fish liver HM-AP. Lineweaver-Burk plots of 1/v versus 1/S.
(A) phosphate. (**B)** vanadate. (**C**) molybdate. (**D)** tartrate. **(E)** sodium fluoride.

Fig. 3 Competitive inhibition of fish liver LM-AP. Lineweaver-Burk plots of 1/v versus 1/S.
(A) phosphate. (**B)** vanadate. (**C**) molybdate.

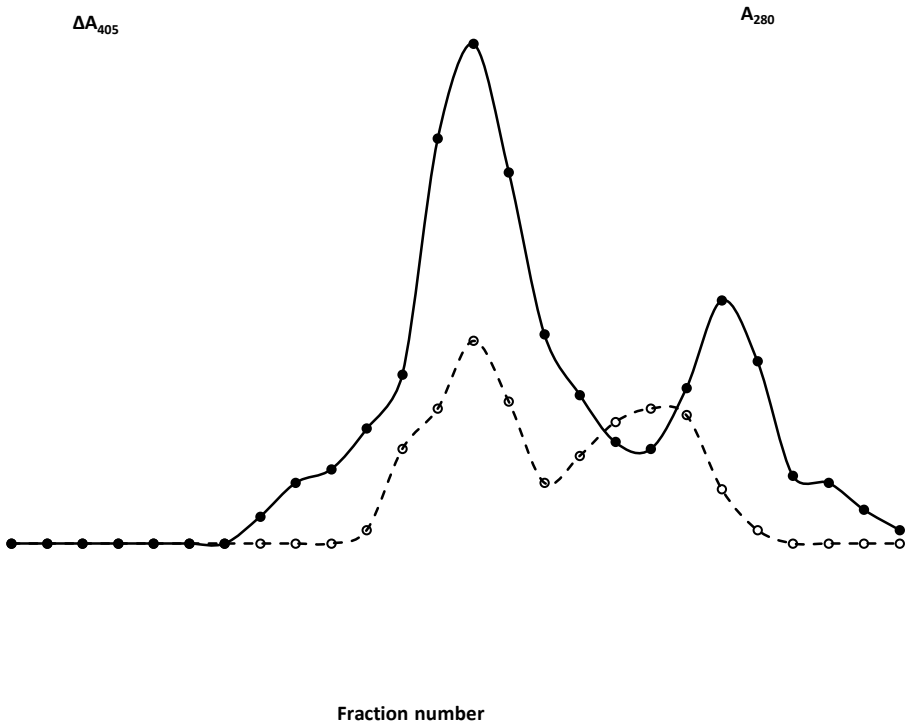
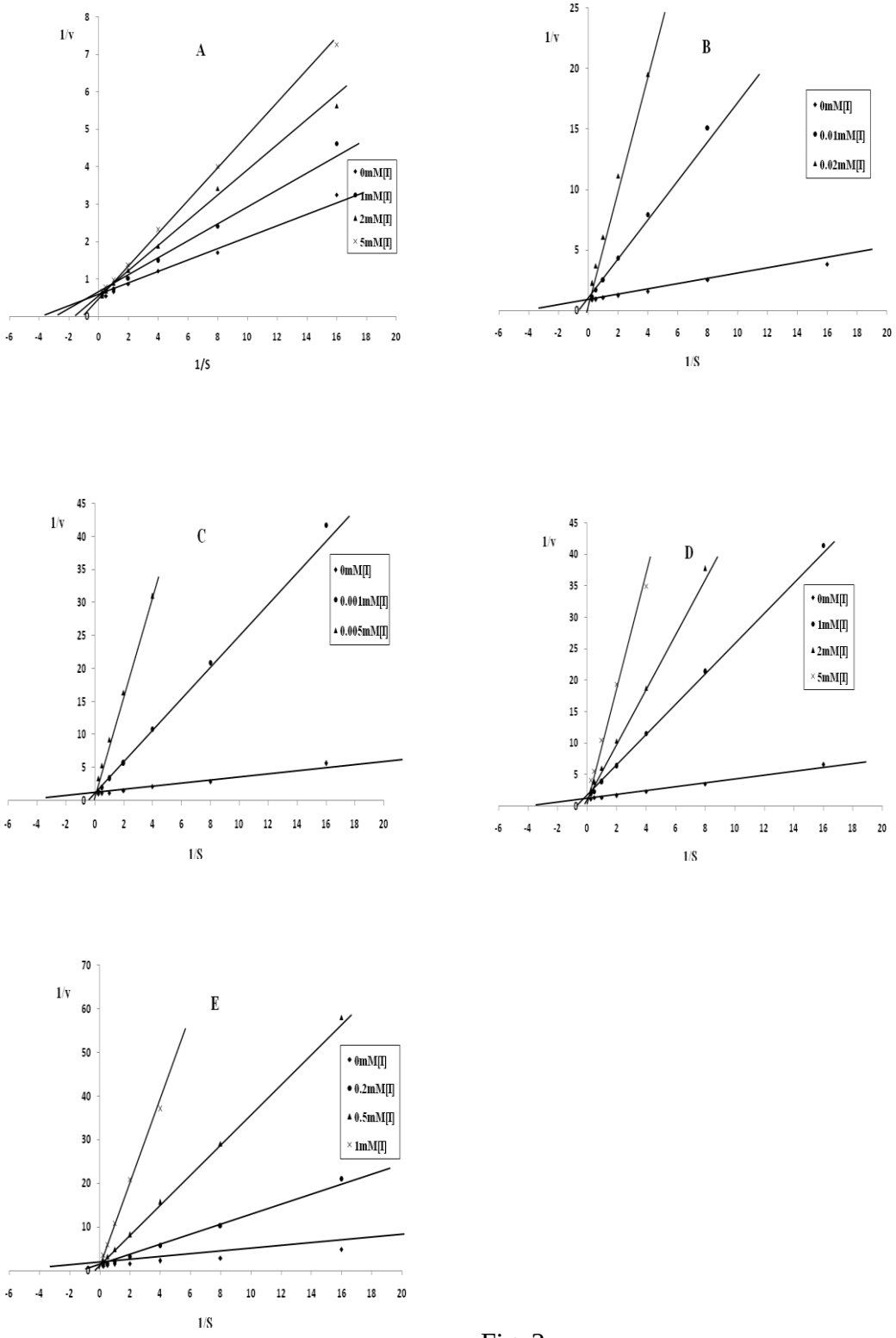


Fig.1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B
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C
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D
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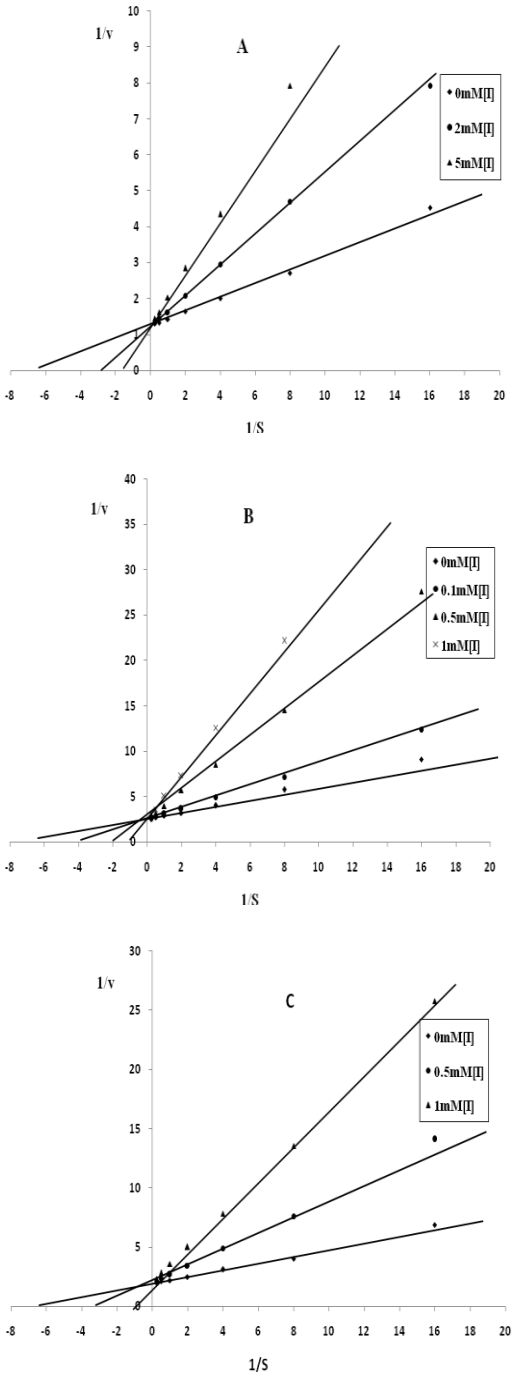
Fig. 2


A
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B
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Fig.3