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Characterization of alkaline phosphatases activities in uterine secretion of buffaloes

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ABSTRACT

Alkaline phosphatases is reported as a predominant protein in buffalo uterine luminal secretion during the late-luteal phase of the cycle. Since the biochemical properties of this important protein are not known, an experiment was designed to purify and characterize the alkaline phosphatase from buffalo uterine secretion. The enzyme could be purified from uterine luminal fluid by 2 chromatography steps using DEAE cellulose ion-exchange and Sephacryl S-200 HR gel-filtration columns. The molecular weight of the purified protein was 179 kDa by non-reducing SDS-PAGE. Reduction by ß-metcaptoethanol yielded 136kDa and 43kDa bands indicated presence of 2 separable subunits of this protein. Complete inhibition of activity by ß-mercaptoethanol and partial inhibition by iodoacetamide indicated that both the subunits are essential for bioactivity. Three isoforms with pI values 5.9, 6.0 and 6.2 were detected by iso-electric focusing. Purified enzyme showed optimum activities at 37°C in pH ranges 10.5 to 11.0. It is a temperature sensitive enzyme as evidenced by a loss of 91% enzyme activity within 28 h of room temperature storage. The loss of activity was slow (12% per day) at 4p C storage indicating importance of cold chain maintenance while processing. The SDS proved to be a better detergent compared to Triton X100 as less inhibition (46% vs 76%) of enzyme activities was observed with the former. Inhibition of activities by metal chelators confirmed that it was metallo-phosphatase. Further study would help exploring its role in buffalo uterine microenvironment.

Key words: Alkaline phosphatases, Buffalo, Uterine secretory proteins

Recently there has been an increasing interest on defining the biochemical nature of uterine luminal secretion because of the problems encountered in the success of embryo transfer experiments and issues related to the early embryonic loss. Even the 1 day asynchrony of uterine environment for the developing embryos were found detrimental for survival during transfer in cattle (Rowson *et al*. 1972). In sheep, the hatched embryo or the trophoblastic vesicle does not elongate *in vitro* unless transferred into the uterus (Heyman *et al.* 1984, Flechon *et al.* 1986). The protein mileu of uterine secretion changes during different stages of estrous cycle in cattle (Guise and Gwazdauskas 1987), pig (Zavy *et al.* 1984), sheep (Moffatt *et al.* 1987, Ing *et al.* 1989) and buffaloes (Roy *et al.* 2006).

Protein phosphorylation is the most important reversible post translational modification which controls the function of many proteins. Switching on and off of certain protein functions is obvious and are noticed in these cells. The kinases are effectors of phosphorylation whereas phosphatases are responsible for dephosphorylation of proteins. Reversible phosphorylation of proteins is now recognized to be the major mechanism by which intracellular events are controlled through neural and hormonal stimuli. More than 30 enzymes were found regulated in this manner (Ingebritsen and Cohen 1983). Generality emerged that phosphorylation activates the enzymes in bio-degradation pathways and inactivates the enzymes in biosynthetic pathways (Ingebritsen and Cohen 1983). Phosphatases are the key enzymes in liberating and recycling the phosphate molecules that are necessary for many fundamental biological processes. Phosphatases produce high-energy phosphate compounds and owe their energetic properties to anhydride bonds. The role of phosphatases is implicated in the process of implantation in rodents (Emadi and Salehnia 2004). Roy *et al.* (2006) reported the presence of acid and alkaline phosphatases enzymes in buffalo uterine secretion; and alkaline phosphatases is the predominant enzyme found secreted during luteal phase of estrous cycle (Ghosh *et al.* 2012). Potential information on their function has been obtained from their induction and localization; but no unifying theme has been identified. Therefore in this study we purified and characterized the biochemical properties of buffalo uterine alkaline phosphatases, which were found present abundantly in late luteal phase uterine luminal secretion.

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MATERIALS AND METHODS

Collection of uterine secretions

Reproductive tract of cycling buffalo having bilaterally

symmetrical uterine horn with no gross ovarian or uterine pathology were collected immediately after slaughter from the slaughterhouse, Bengaluru, and brought to the laboratory in ice cold temperature. The late-luteal phase (days 11–17) uterine wash (n=5) in 20mL NaCl was collected for classifying stages of the cycle as per Roy *et al.* (2006). The washed fluid was centrifuged at 10,000 g for 30 min to remove the cell and cell debris and the supernatant was stored frozen at –20°C until further analysis. Uterine washes having cloudiness or with white flakes were discarded and not included for the study.

Determination of protein content and phosphatases activities in sample

The protein concentrations in uterine secretion were determined by Lowry's method (Lowry *et al.* 1951) using purified bovine serum albumin as standard (BSA; fraction V). Alkaline phosphatases activity was determined by colorimetric method in 0.1 mL samples as per Linhardt and Walter (1965). Each sample was prepared in duplicate and read against the control tube which was prepared similarly except that the samples were added after stopping the enzyme reaction. The standard curves were prepared using *p*-nitrophenol in the 11.1mL standard assay buffer specific for alkaline phosphatases (11.1mL) assay. To obtain the unit of alkaline phosphatase micromole value from standard curve was multiplied by 20. Since as per definition 1 unit of alkaline phosphatases is the amount of enzyme that liberates 0.05 micromole *p*-nitrophenol under the assay condition of 0.1ml sample, 11.1ml assay volume and 30 min incubation at 37°C.

Purification of buffalo uterine secretory alkaline phosphatases

The pooled (n = 5) late-luteal phase uterine-washes were processed similarly by dialyses at 4°C using dialysis tubes cut off 1 kDa for 24 h against 10mM Tris HCl, pH 7.8 buffer with 2 changes to remove the low molecular weight substances including NaCl and to equilibrate with the same buffer as used for protein adsorption condition under continuous stirring. The resulting uterine fluid was concentrated to 1/5th of the volume in a vacuum dryer and protein concentration and alkaline phosphatases activity were determined as per the method described above.

Fractionation of uterine proteins by DEAE ion-exchange material

The 10g dry resin of DEAE cellulose were regenerated as per the manufacturer instruction in two 250mL (5g each) beaker and equilibrated with the binding buffer 50mM Tris HCl pH 7.8 containing 0.02% sodium azide. One beaker resin was used for adsorption of uterine protein and the other was used for making an ion-exchange column. The uterine protein of about 143.3 mg (18.5 mL) proteins was allowed to adsorb in the charged DEAE cellulose overnight at 4°C. The slurry with unbound proteins was filtered using funnel with Whatman filter paper no 41 and then washed 2 times with the same resin volume of binding buffer. The resulting unbound fraction were pooled and passed through the DEAE column (20×1.6cm) in tandem to make sure that unbound fraction does not contain protein to remain bound with the DEAE matrix. The unbound fraction was concentrated to about 4mL in a vacuum dryer for further purification.

Final purification by Sephacryl S-200 HR column

Concentrated unbound DEAE cellulose fraction of uterine protein samples of about 42 mg protein were loaded on to a 95 cm × 1.6 cm Sephacryl S200HR column prepared as per the manufacturer instruction at ambient temperature and eluted with 50 mM Tris–HCl, pH 7.8, 0.15 M sodium chloride and 0.02% sodium azide with a flow rate of 24 mL/h. Fractions (3 mL) were collected and screened at 280 nm wavelength. The alkaline phosphatase activities in 0.1mL column eluants of each tube were screened as per Linhardt and Walter (1965).

Poly acrylamide gel electrophoresis (PAGE) analysis for determination of molecular weight and subunits

Denaturing polyacrylamide gel was prepared (Okajima *et al.* 1993) for electrophoresis analysis of purified alkaline phosphatase samples. A 10% separating gel (12 cm × 14 cm) and 5% spacer (4 cm × 14cm) gel with 1.0 µm thickness was used. About 50µg protein were mixed with the required quantity 2x sample buffer containing 0.125 M Tris–HCl, pH 6.8, 20% (v/v) glycerol, 4% (w/v) SDS, 10% (v/v) with or without mercapto-ethanol and 0.025% (w/v) bromophenol blue, kept in boiling water for 5 min for complete denaturation of proteins and loaded in each well. The samples were run in 0.05 M Tris–0.384 M glycine buffer, pH 8.3 containing 0.1% SDS at 60 V till the tracking dye reached the separating gel then increased to 90 V till the end. The gels were kept in fixing solution comprising 50% (v/v) ethanol and 10% (v/v) acetic acid solution for 2 h. Protein bands in gel were visualized by staining with 0.25% Comassie brilliant blue containing 40% methanol and 7% glacial acetic and destained by de-staining solution containing 25% methanol and 10% glacial acetic acid. The molecular weight of each band was determined by comparing known molecular weight standards using gel documentation system and Multi Gauge software version 2.2.

Determination of protein isoforms by isoelectric focusing of purified protein

A vertical (8×7cm) denaturing isoelectric-focusing gel (5% final concentration) containing 8 M urea was casted with 1 mm thickness mixing the components, viz. 30% acrylamide 1% bis acrylamide: 0.83mL, ampholyte mixture (ampholine, pH 3.0–10.0): 0.36mL, Triton-x 100: 0.12mL, urea: 2.88g, nanopure water: 1.5 mL, TEMED: 10 µL and freshly prepared 10% ammonium per sulphate: 40 µL. The wells and upper chamber were filled with 25 mM ortho-phosphoric acid (anode solution) and the lower chamber

was filled with 20 mM NaOH (cathode solution). The polarity was reversed by electrode connectivity and electrophoresis performed at room temperature with pre-cooled buffer. Pre-focusing of the gel was done for 1.5 h at a constant voltage of 150 V. The samples were mixed with half the volume of 2X sample buffer (40 mM Tris–HCl, 0.6% SDS, 2% (v/v) Triton X–100, 2% ampholytes (ampholine, pH 3.0–10.0), 50% (v/v) glycerol, 8 M urea) and loaded into the wells. Then the gel was run at a constant voltage of 150 V for 1.5 h followed by the run of 1.5 h at an increased voltage of 400 V. A vertical long gel slice was kept for pH gradient measurement in the gel and rest part used for staining. The vertical gel was horizontally sliced at 0.5 cm distance, crushed and taken individually in the tubes containing 1mL double distilled water kept overnight at 4°C. The pH in each solution was measured using a pH meter and plotted as a function of the distance from the top of the gel and the exact pH where band appeared was extrapolated from the pH versus distance graph. The remaining gel was first washed with 10% (v/v) trichloroacetic acid (TCA), for 10 min, then by 1% TCA overnight to remove the ampholytes. After a brief rinse with distilled water the gel was stained by Coomassie brilliant blue-R 250: 0.25%, methanol: 40% and glacial acetic: 7% solution for minimum 5 h. The background was destained by repeated washing with 25% methanol and 7% acetic acid solution.

Evaluation of substrate concentration effect on enzyme activity

Aliquots (0.1 mL) of purified alkaline phosphatase (~35mg protein equivalent) were incubated at 37°C with increasing concentrations of 0.1mM to 10mM *p*-nitrophenylphosphate substrate in 50mM glycine-NaOH (pH, 10.5) in triplicate for 30 min. Effect of varying substrate concentration on alkaline phosphatase activity was calculated to have Michaelis-Menton equation and Lineweaver-Burk plot.

Biochemical properties of purified enzyme

In all the cases if otherwise not stated, triplicate samples of 0.1 mL enzymes (35mg protein equivalent) were allowed to react with 5mM *p*-nitrophenyl phosphate in 50mM glycine-NaOH (pH: 10.5) at 37°C for 30 min. The reaction was stopped by addition of 10 mL 0.02N NaOH before reading absorbance at 400 nm.

Effect of inhibitors and ions: The enzyme samples were incubated in absence or presence of 1 mM each of sodium orthovanadate, ammonium molybdate, iodo-acetamide, 2-mercapito ethanol, tetramisole hydrochloride, magnesium chloride hexahydrate, EDTA, EGTA, ferrous sulphate and sodium phosphate as per the standard assay condition of alkaline phosphatase. The percent inhibition of alkaline phosphatase activity was calculated comparing the activity in control where none of the salts were added.

Effect of detergents: The enzyme samples were incubated either in the absence and presence of urea (6 M), guanidine hydrochloride (6 M), triton X100 (1%) and SDS (1% or 0.1%) in triplicates. The percent inhibition of alkaline phosphatase activity was calculated comparing activity in control where none of these were added.

Effect of pH: The enzyme activities were assayed at different pH in 50mM citrate buffer (pH 5.5 – 6.5), 50 mM Tris-HCl buffer (pH: 7.0 – 9.0) and 0.2 M glycine- NaOH buffers (pH 9.5 – 11.0) having a difference of 0.5 pH and compared with the control prepared as per the standard assay condition.

Effect of varying incubation temperatures: Enzyme activities were measured at different temperature of 4°, 25°, 37°, 45°, 50°, 60°, 70°, 80°, and 90°C for 30 min to analyze the effect of different temperatures on enzyme activity.

Enzyme activity at different storage temperature: A stock enzyme sample was kept at room temperature and at 4°C for extended period of time and the activity was determined by taking 0.1 mL sample at each time point at different interval as per the standard assay condition. Activity of enzyme was calculated at each time interval for comparison with the initial activity.

RESULTS AND DISCUSSIONS

Purification, molecular weight, subunits and isoforms of purified enzyme

Tandem DEAE cellulose column ensured removal of most of the contaminating protein from the sample as screening could detect maximum alkaline phosphatases activity in the unbound fractions and a very minor activity in the bound fractions eluted with 1M NaCl (data not shown). Further purification of unbound fraction resulted in a single peak of alkaline phosphatases activity that was eluted at about 93 mL volume, which was after the void volume (81 mL) of the column (Fig. 1). Increase in alkaline phosphatases specific activity was observed at different

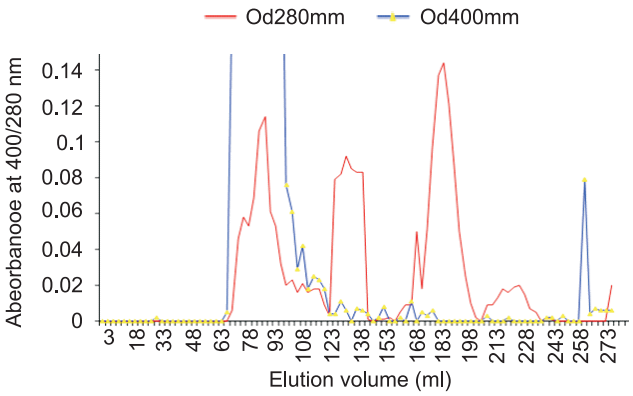

Fig. 1. Sephacyl-S200HR gel filtration column (95×1.6cm) elution profile of DEAE cellulose unbound protein fractions contatining buffalo secretory uterine alkaline phosphatases compared to proteins (OD 280nm). Proteins were eluted in buffer 20mMTris-Cl, pH 7.2 containing 0.1MNaCl and 0.02%NaN_3 with a column flow rate of 24ml/h. Eluants were collected 3mL in each tube and alkaline phosphatases activity was determined taking 0.1mL from each tube following the standard method and plotted as OD400nm.

Table 1. Amount of protein and alkaline phosphatases activities in crude uterine secretion and purified samples at different steps

Parameters	Crude uterine protein	DEAE cellulose column eluted	Sephacyl-S200HR column eluted
Protein (mg)	143.31	42.39	1.41
Enzyme activity (U)	217419.9	106637.55	13631.75
Specific activity (U/mg protein)	1517.13	2515.63	9673.39

steps of purification. At the end of purification procedure the specific activity of 9,673 unit/mg proteins was obtained (Table 1).

The analysis of purified buffalo uterine alkaline phosphatase by denaturing PAGE under non-reducing condition, showed a single band at the high molecular weight range. The calculated approximate molecular weight of this band was 170 kDa when compared with the migration of the standard molecular weight proteins (Fig. 2A). The same protein when subjected to ß-mercaptoethanol treatment and analysed further by electrophoresis, produced 2 bands with molecular weight 136 kDa and 43 kDa (Fig 2B).

The purification of alkaline phosphatases is first time reported from buffalo uterine secretion by two simple steps. The DEAE cellulose bound enzyme eluted with 1M NaCl was not considered for purification because that represented very minor portion of the total enzyme activity. Single band under denaturing SDS-PAGE gel indicated that the sephacryl-S200HR column purified fraction containing alkaline phosphatases activity was a single protein. The approximate molecular weight of the purified enzyme was about 170kDa. Molecular weight ranging from 80 – 200 kDa of this enzyme is reported in literature (Vincent and Crowder 1995). On reduction of disulphide bonds, it yielded 2 bands with molecular weight of 136 and 43kDa indicating that the enzyme is hetero-dimeric protein as reported in literature. Mammalian alkaline phosphatases are multiple isoenzymes, often associated with the cytoplasmic membrane (Fernley 1971) and are also available in hetero-dimer form. The observed difference in molecular weight in non-reducing PAGE as compared to the molecular weights obtained by addition of both the subunits is expected for 2 reasons: first, the percentage of gel (10%) used for calculating molecular weight, and second SDS PAGE only can resolute molecular weight with 90% accuracy. The calculation of molecular weight by the addition of both subunits would be considered more accurate because bands were located in between the bands of standard molecular weight marker proteins. Therefore, suggested molecular weight of this protein by SDS-PAGE method would be about 179kDa. The end product after purification showed 6.4 fold increases in specific activity as compared to initial crude secretion. However, electrophoresis analysis of active fractions at the end of Sephacryl S 200HR chromatography showed a single band indicating 100%

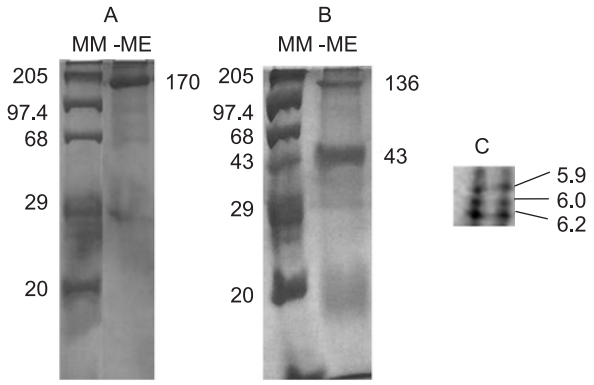

Fig. 2. Gel electrophoresis of purified buffalo uterine secretory alkaline phosphatases in SDS-PAGE showing panel (A) bands without reduction (-ME), panel (B) with reduction by ß-mercaptoethanol (+ME) and denaturing pH gradient (panel C) condition. 10% gels were used for panel (A) and (B) whereas 5% gel was used for panel (C). The separated proteins were detected by Coomassie brilliant blue R250 statining. The marker protein lanes (MM) in panel A and B are indicated by their respective molecular weights. The actual pH gradient in the panel C gel was ascertained from the vertical cut strip.

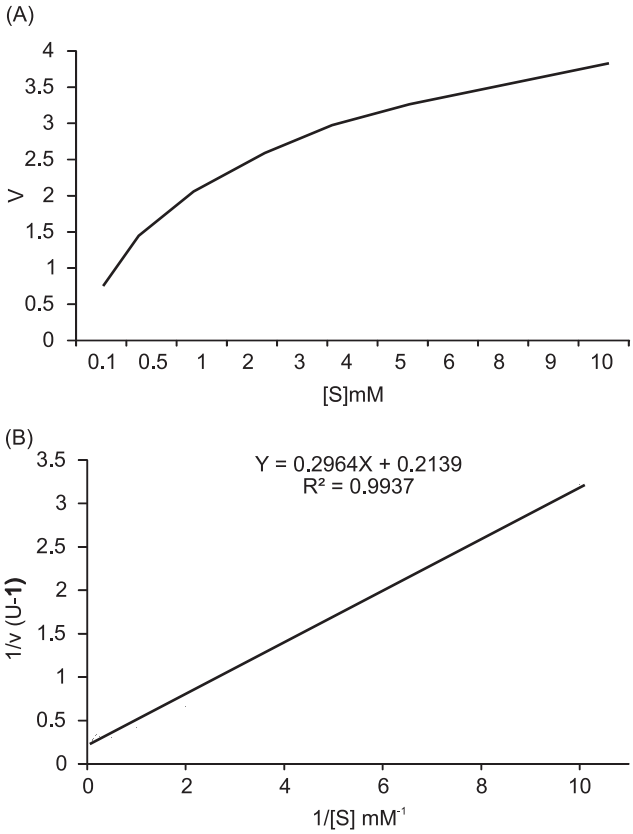

Fig. 3. Alkaline phosphatases activities (V) curve (A) with increasing concentrations of *para*- nitrophenyl phosphate substrate (S) and the Lineweaver-Burk plot (B) with equation. About 35 mg protein-equivalent enzymes was added per tube and the assays were performed using *p*-nitro phenyl phosphate as substrate under standard incubation condition. Each assay for a specific substrate concentration was run in triplicate and values presented were averages.

purity of the protein. Isoelectric-focusing in a vertical denaturing polyacrylamide mini gel system provided a rapid resolution of the purified alkaline phosphatase in presence of 8 M urea within 3 to 10 pH range ampholine. The measurement of pH in the cut strips of gel showed that the gradient was established in the range of 4.3 to 8.6. The staining of gel showed that purified alkaline phosphatase was focused at 3 pH values, viz. 5.9, 6.0 and 6.2 (Fig. 2C) indicating 3 isoforms of this protein.

Effect of increasing substrate concentration, inhibitors, ions and detergents on enzyme activity

Rate of enzyme activity (V) at 30 min incubation with increasing concentrations of *p*-nitrophenyl phosphate substrate (S) showed a typical curve of enzyme activity (Fig. 3A) in 50 mM glycine-NaOH buffer at pH 10.5. A straight line was obtained when 1/[V] was plotted against 1/[S] in Lineweaver-Burk plot (Fig. 3B). Km value calculated from the line equation was 1.6 mM. The Km value obtained from Lineweaver–Burk plot was 1.6 mM. Most purified commercial preparation of porcine intestinal alkaline phosphatase has shown 1.5 mM Km value.

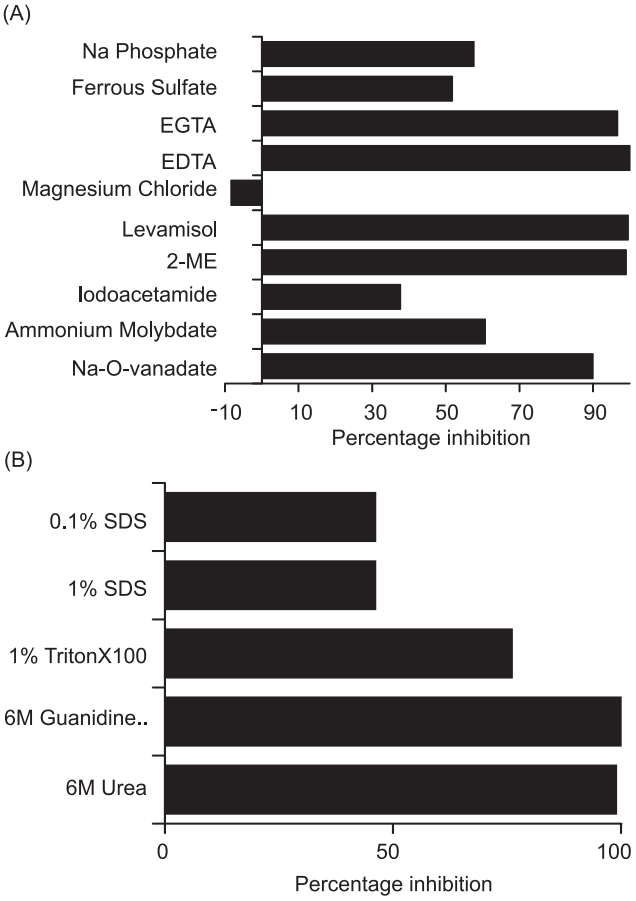

Fig. 4. Per cent inhibition of purified buffalo uterine secretory alkaline phosphatases by 1mM concentration of different inhibitors (panel A), protein denaturing agents (panel B) and effect of 1mM magnesium chloride (panel A). *Para*-nitro phenyl phosphate was used as substrate for enzyme assay under standard incubation condition.

The mercaptoethanol, tetramisole and EDTA at 1mM concentration inhibited the enzyme activity to about 100% (Fig. 4A). The EGTA and sodium orthovanadate at 1mM concentration showed inhibition of about 97 and 90%, respectively. Ammonium molybdate, ferrous sulphate, and sodium phosphate showed 50–60% inhibition. Iodoacetamide showed 38% inhibition. Magnesium chloride at 1 mM concentration increased the activity of the enzyme by 8%.

Protein denaturing agents urea and guanidine hydrochloride at 6M concentrations showed 100% inhibition, SDS at 1% and 0.1% showed only 46% , however 1% TritonX-100 showed 76% inhibition on the enzyme activity (Fig. 4B).

Inhibition of enzyme activity by metal chelator EDTA and EGTA, indicated that metal ions might be the integral component of this enzyme, which is essential for showing the bioactivity and it is a member of metallo protein phosphatases. The increased activities in presence of $MgCl_2$ indicated that the enzyme is dependent on the Mg^{2+}. Determination of exact type of this enzyme needs screening of uterine secretion using specific alkaline phosphtase antibodies on which we are concentrating now, which is not unusual as the presence of 2 Zn (II) and 1 Mg (II) ion per subunit are reported and the presences of Zn ions are shown essential for enzyme activity (Sowadski *et al.* 1985). Complete inhibition of activity by 2-metcapto ethanol and partial inhibition by iodoacetamide indicated that disulphide bonds play an important role and the dimeric form of this protein is essential for showing the enzyme activity. According to Cirri *et al.* (1993) and Zhang (1997) cysteine residues, which are involved in formation of disulfide bond are directly involved in the catalytic mechanism of the hydrolysis of tyrosine-phosphorylated proteins by protein tyrosine acid phosphatase. Difference with protein tyrosine acid phosphatase is that this enzyme is active at alkaline pH and the activity of this enzyme is inhibited by mercaptoethanol whereas, mercaptoethanol stimulates the activity of protein tyrosine acid phosphatase (Wysocki and Strzezek 2003). Sodium orthovanadate at 1mM concentration inhibited the alkaline phosphatase activity. No activity of enzyme in either 6M urea or guanidine hydrochloride. However 24% activities in 1% Triton X100 and 54% activities were retained even 0.1% SDS treated samples. Increase in percentage of SDS to 1% did not affect enzyme activity which has greater significance. In a separate study by Kim *et al* (1989) Triton X100 was used for tissue extraction of phosphatases. However, our study indicated that the SDS would be the better choice as detergent for inclusion in the extraction buffer of tissues. Thus SDS can be used (i) in the final step purification by gel filtration column which would help in removing the inter protein interaction without affecting the bioassay in the column eluant, and (ii) for electrophoresis separation of protein under non-reducing condition if one is interested for direct gel detection of this protein after extraction from the source tissues or secretion.

Optimum activity, stability and storage of purified enzyme

This enzyme had activity in a narrow range of pH. It started showing activity at around pH 7 with a little increase in activity between pH: 9.5 to 10.0. The maximum activity was observed in the pH range of 10.5 to 11.0 (Fig. 5A). An optimum activity of the purified enzyme was found at 37°C (Fig. 5B). The favorable temperature was from 37 to 45°C since up to this temperature range enzyme showed reasonably high activity, but when assay temperature was increased to 50°C about 90% of the enzyme activity reduced as compared to that at 45°C. A sharp decrease in enzyme activity was observed on room temperature storage as about 91% of the enzyme activity was lost within 28h (Fig. 6A). The activity reduction was about 12% daily when kept at 4°C for an extended period of time (Fig. 6B). Although the protein was purified to homogeneity the specific activity increase was only 6.37 times as compared to the crude sample. This might have happened due to loss of enzyme activities during the purification process as the enzyme is proved to be very temperature sensitive. The loss of enzyme activity might have occurred during several steps of purification process. The potential steps where losses of activity might have occurred are: (i) initial dialysis, (ii) DEAE binding, (iii) concentration while vacuum drying (48 h), (iv) Sephacryl S200HR column purification which was done at room temperature, and (v) during final concentration (24 h). Nevertheless the amount of enzyme obtained by this protocol was sufficient enough to carry out all the experiments and did not affect yield for characterizing the properties of the enzyme. Processing of this enzyme requires cold chain maintenance thus by running column and pre-cooling buffer at 4°C, in addition to minimum exposure of sample at room temperature could increase the fold purification and specific activity in the end product. The 50 mM glycine-NaOH buffer pH of 10.5 and temperature 37 to 45°C was optimum for this enzyme activity.

Alkaline phosphatases are of 4 types, viz. placental, intestinal, placental-like and tissue non-specific types. An another type of classification exists in in eukaryote which is based on the action of these enzyme on the amino acid residue such as protein tyrosin phosphatases (PTP) and protein serine/threonine phosphatases (PSP). The PSP are of 3 types (Shi 2009) , viz. phosphoprotein phosphatases (PPPs), metal-dependent protein phosphatases (PPMs), and aspartate-based phosphatases represented by FCP/SCP (TFIIF-associating component of RNA polymerase II CTD phosphatase/small CTD phosphatase). Our results indicated that the secretory uterine alkaline phosphatase is a protein of about179 kDa size, has 2 non-identical subunits with 3 isoforms. Presence of both the subunits is essential for its

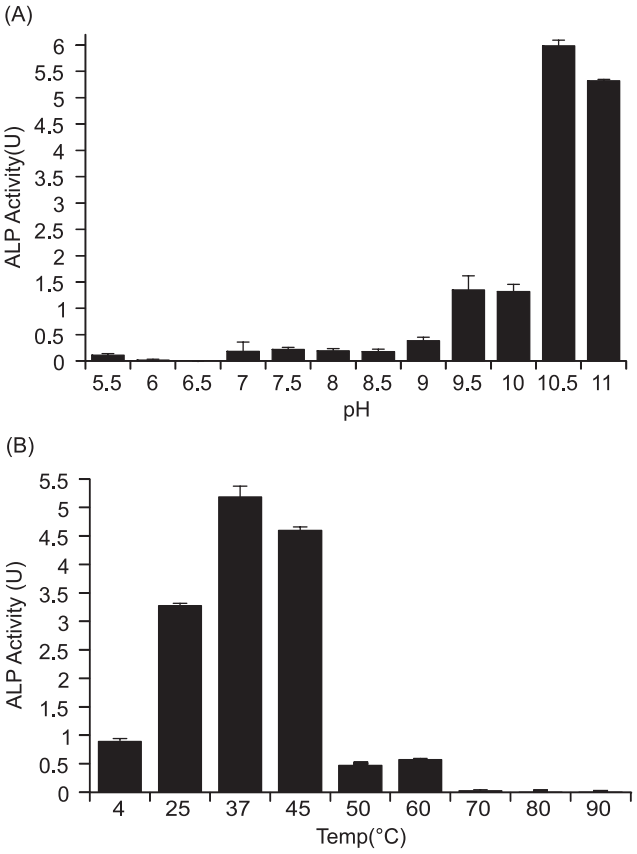

Fig. 5. Effect of buffer pH (panel A) and incubation temperature (panel B) on purified buffalo uterine alkaline phosphatases activity. *Para*-nitrophenyl phosphate was used as substrate for measurement of enzyme activity under standard incubation condition. Values are mean ± SE of three separate assays of triplicate samples

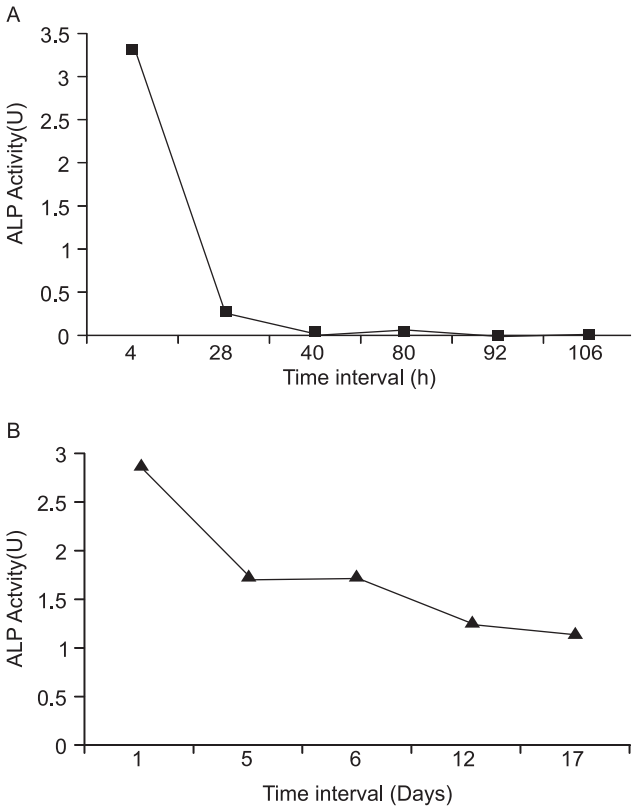

Fig. 6. Changes in alkaline phosphatases activity at different time points of room temperature (panel A) and at 4°C (panel B) storage. At each time point enzyme assay was carried out in duplicate under standard incubation condition.

function. It is a temperature sensitive metal dependant enzyme so is called metalo-phosphatase; show optimum activity at pH 10.5 in the temperature ranges 37 to 45°C. Further study on varying aspects of uterine secretory alkaline phosphatases would help elucidating its precise role in different uterine functions.

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