www.ijrps.pharmascope.org

ISSN: 0975-7538
Review Article

Human Alkaline phosphatases in health and disease: A mini review

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ABSTRACT

Distribution of Alkaline phosphatase (APase) is ubiquitous amongst different cell types and tissues in almost all living organisms. In mammals four isoenzymes with tissue-specific distribution have been well characterized. Several physiological functions have been proposed for APase. In bone, Tissue Non specific Alkaline Phosphatase **(**TNAPase) is thought to mediate phosphate assimilation. TNAPase may also play a role in the renal transport of phosphate. In the intestine, APase has been proposed to participate in the absorption and transport of lipids and nucleotides. Intestinal Alkaline phosphatase (IAPase) also function as a phosphate binding protein under physiological conditions in the brush border membrane vesicles. APases are abundantly expressed in tumor cells, and serum levels of APase isozymes are often used as tumor markers. Placental Alkaline phosphatase (PLAPase) and Germ cell Alkaline phosphatase (GCAPase) have been evaluated extensively in various malignant conditions. Structure and functions of different human APases along with their clinical significance are discussed in this communication.

Keywords: Apase; Tumour markers; B-lymphocyte activation; inhibitors

INTRODUCTION

Alkaline Phosphatase (APase, EC 3.1.3.1), an orthophosphoric-monoester phosphohydrolase, is present in most species from bacteria to man (McComb *et al.* 1979 and Millan, 2006). The enzyme was first isolated from ossifying bone and cartilage (Robinson, 1923). The enzyme catalyzes the hydrolysis of phosphomonoesters, including Tyr/Ser/Thr-phosphates in phosphoproteins with release of inorganic phosphate and alcohol at an alkaline pH between pH 8.2 to 10.7 (Mc Comb *et al.* 1979, Chan and Stinson, 1986, Pezzi *et al.* 1991, Sarroulihe *et al.* 1992 and Coleman, 1992, Coleman et al.1983).

The enzyme is inactivated under acidic conditions (Fishman and Ghosh, 1967). The pH optimum is affected by the type and concentration of the substrate (Ross *et al.* 1951 and Fedde *et al.* 1988). The catalytic activity of APases depends on a multimeric configuration of identical monomers. Each identical subunit possesses one active site, and contains two Zn^{2+} ions and one Mg^{2+} ion that stabilize the tertiary structure (Kim and Wyckoff 1991). APase is a metalloenzyme believed to be homodimeric in serum and membranes, but the tissue non-specific isoform may exist as a homodimeric and/or homotetrameric structure in membranes (Hawrylak and Stinson, 1987). Unlike the bacterial enzymes, mammalian APases are all anchored to the plasma membrane via a glycophosphatidylinositol moiety (Udenfriend and Kodukula, 1995).

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Received on: 23-03-2013
Revised on: 13-06-2013
Accepted on: 17-06-2013

APase – Genomic localization

In humans, three out of the four APase isoenzymes are tissue specific: placental (PLAPase), germ cell (GCAPase) and intestinal (IAPase). They are 90-98% homologous at the protein level with PLAPase and GCAPase differing only by 12 aminoacid substitutions. The genes encoding these enzymes are clustered on chromosome 2q37.1. The fourth isozyme, tissue non-specific APase (TNAPase) is ubiquitous particularly abundant in bone, liver and kidney and is only about 50% identical with the other three APases (Stigbrand, 1984, Harris, 1989 and Millan, 1992). Post-translational modification and differential glycosylation of TNAPase gives rise to tissue-specific APases of liver, bone and kidney (Komoda and Sakagishi, 1978). It was hypothesized that the physiological role of the sugar moieties could be in the protection of the enzyme from rapid removal from circulation through binding to the asialoglycoprotein receptors of the liver. The different isoenzymes of APase can be differentiated based on their structure, immunological properties and sensitivity to heat and different inhibitors.

The gene encoding TNAPase is localized on short arm of chromosome 1 in humans and on chromosome 4 in mice (Terao *et al.* 1990, Greenberg *et al.* 1990). The gene encoding TNAPase consists of 12 exons distributed over 50kb. Intestinal and placental APase genes are present closely on chromosome 2 in both mice as

well as humans (Henthorn *et al.* 1988 and Knoll *et al.* 1989). The tissue specificity is achieved by differential transcription and subsequent co-translational and post-translational modifications controlled by two alternative exons (Matsuura *et al.* 1990, Studer *et al.* 1991). The transcript of exon I-B is found in bone and kidney while the transcript of exon I- L is found in liver. The mechanism of tissue-specific regulation of TNAP is not yet known in detail. All APases share the same core three-dimensional structure, as suggested by the sequence alignment of the three available structures from *E.coli*, human placental and shrimp (Le Du *et al.* 2001 and De Backer *et al.* 2002). However, important differences exist in the peripheral domains of the molecule. The four mammalian isozymes exhibit characteristic differences in a number of functional properties, in particular in their variable heat stability (Bossi *et al.* 1993), allosteric regulation (Holyaerts *et al.* 1997), binding to collagen (Wu *et al.* 1991) and uncompetitive inhibition by various modulators (Holyaerts *et al.* 1992 and Kozlenkov *et al.* 2002).

General Functions of Alkaline Phosphatase

Like many other GPI anchored proteins, APase has also been proposed to be involved in transmembrane signalling function. As the GPI anchored proteins are located on the outer leaflet of the bilayer of cell membrane, they are more mobile than other cell surface proteins and may be involved in cell-cell interaction, reception or transduction of extracellular stimuli. Low and Saltiel (1988) have shown that GPI anchor acts as an apical targeting signal in the polarized epithelial cell and in this way could act as an activation antigen in the immune system. APase has little preference for a particular substrate and will hydrolyze all the phosphomonoesters but not diesters. Catalysis includes phosphorylation of a serine residue at the active site followed by the delivery of the phosphoryl group to either water (phospo hydrolysis) or to an organic acceptor alcohol (phosphor tranferase). However, phosphoester cleavage is faster if the transfer of phosphate is to an acceptor rather than to water.

APase has been shown to be involved in cell adhesion (Hui and Tenenbaum, 1993), proliferation differentiation (Fedarko *et al.* 1990, Owen *et al.* 1990 and Andracchi and Korte 1991), and a protein tyrosine phosphatase in some tissues (Swarup *et al.* 1981 and Lau *et al.* 1985). In liver plasma membranes, a 18KDa phosphoprotein has been demonstrated as a substrate for dephosphorylation by APase . It has also been shown that APase is involved in the regulation of phosphate transport in intestine, kidney and in calcium transport in the intestinal and kidney epithelial cells. In bone, TNAPase is thought to mediate phosphate assimilation (Narisawa *et al.* 1997 and Zurutuza *et al.* 1999). TNAPase may also play a role in the renal transport of phosphate (Petitclerc and Plante, 1981). In the intestine, IAPase has been proposed to participate in the absorption and transport of lipids and nucleotides (Young *et al.* 1981). IAPase may also function as a Pi binding protein under phyiological conditions in the brush border membrane vesicles.

Structure and Functions of Tissue Non specific Alkaline Phosphatase (TNAPase)

Structure of the human PLAPase provides a complete view of an APase from higher organisms (Le Du *et al.* 2001). Based on the structural evidence and functional divergence of APases conservation in mammals has been proposed (Mornet *et al.* 2001 and Le Du and Millan, 2002). The molecular and structural data suggests that APase acquired specialized functions progressively during evolution. Five functional regions have been identified in TNAPase. The active site is the centre of the catalytic activity. The N-terminal arm is essential for stability and allosteric properties of the enzyme (Hoylaerts, 2006). The crown domain is a key factor of uncompetitive inhibition heat stability and allosteric behaviour (Bossi *et al.* 1993, Kozlenkov *et al.* 2004). The crown domain may also be involved in the binding of TNAP to collagen (Bossi *et al.*1993) corroborating previous studies that suggested this property of TNAP .

TNAPase cleaves the extracellular substrates - inorganic pyrophosphate (PPi), Pyridoxal-5'-phosphate (PLP) and phosphoethanolamine (PEA) (Millan, 2006). Function of TNAPase in bone and dental mineralisation is likely to involve hydrolysis of PPi, to maintain a proper concentration of this inhibitor to ensure normal bone mineralization and collagen and calcium binding (Hoylaerts *et al.* 1997, Whyte, 2001, Mornet *et al.* 2001, Hessle *et al.* 2002). Significant physiological function of APase in mammals is provided by studies of human hypophosphatasia where a deficiency in the TNAPase is caused by deactivating mutations in its gene (Henthorn *et al.* 1992, Mornet, 2000). This is associated with defective bone mineralisation in the form of rickets and osteomalacia (Whyte, 2001). Pyridoxal phosphate (PLP) is the phosphorylated form of pyridoxine. TNAPase hydrolyses PLP, and the unphosphorylated pyridoxal crosses the blood brain barrier to be regenerated as PLP in the cell (Whyte, 2001). Consequently, in patients with hypophosphatasia, inability to cleave PLP has been shown to result in a localized vitamin B6 deficiency in the central nervous system causing seizures (Waymire *et al.* 1995). TNAPase could be also involved in the intra neuronal balance between pyridoxal and PLP. In addition, studies of TNAPase activity in primate brain proposes an important role for this enzyme in neurotransmission.

Structure and Functions of Intestinal Alkaline Phosphatase (IAPase)

It is expressed in the small intestine of many species. Foetal intestinal alkaline phosphatase contains two distinct subunits, one with isoelectric point (pI) 6.0 and apparent molecular weight (Mi) approx. 64,000, the other demonstrating pI 5.5 and Mr 72000. Foetal intestinal enzyme subunit with pI 6.0 and Mr 64,000 corres-

ponded precisely to the subunits of the placental APase enzyme. The adult intestinal isoenzyme was found to contain a single subunit with pI5.9 and Mr 70,000. The larger, more-acidic, component of the foetal intestinal enzyme represents a glycosylated variant of the adult intestinal alkaline phosphatase. Lymph and serum levels of IAPase increase after a fatty meal (Mc Comb *et al.* 1979). IAPase is found associated with the brush border of the intestinal epithelium and enriched in Surfactant-Like Particles (SLP). IAPase isozyme is likely to be involved in the intestinal absorption of lipids/nutrients across the cell membranes via its association with SLPs (Narisawa *et al.* 2003).

Structure and Functions of Placental Alkaline phosphatase (PLAPase)

The human placental alkaline phosphatase enzyme is a dimer. It has a mol. wt. of 116, 000 and is composed of monomer subunits having an identical mol. wt. of 58,000. PLAPase has been proposed to be involved in the transfer of maternal IgG to the foetus during gestation. It acts as a Fc receptor and has been shown to be involved in the internalization of IgG in HepG2 cell line (Makiya and Stigbrand, 1992 and Stefaner *et al.* 1997). Other studies indicate a role of PLAP in regulation of cell division (Telfer and Green, 1993 and She *et al.* 2000a). PLAPase stimulates DNA synthesis and cell proliferation in synergism with insulin, zinc and calcium and that it also acts as a survival factor in combination with ATP in serum-starved mouse embryonic and human fetal fibroblast cultures (She *et al.* 2000b). Since PLAP is synthesized in the placenta, its effects on the growth and survival of fetal cells strongly suggest that it may have a key role regulating the growth of the fetus.

Functions of Germ Cell Alkaline Phosphatase (GCAPase)

It had been postulated that germ cell APase (GCAP) may be able to interact with extracellular matrix proteins and therefore serves as the cell guidance molecule during the migration of germ cells (Millan, 1990). Ligands involved in directing the cell migration via APase binding might be phosphoprotiens representing its natural substrates. The enzyme activity expression has been shown to be stage specific during embryonic development.

B-Lymphocyte APase

sAPase activity has been shown to be expressed specifically by mitogen-stimulated B-lymphocytes (Greaves and Janossy, 1972). It has also been used as a marker of B cell activation (Burg and Feldbush, 1989 and Marquez *et al.* 1989). Resting B-lymphocytes and resting and activated T lymphocytes do not express APase activity (Garcia-Rozas *et al.* 1982). Expression of Apase activity occurs in early G1 phase (around eight hrs) after mitogenic stimulation and continues till differentiation (120 hr). It has also been shown that the enhancement of APase activity correlates with proliferation and differentiation. It precedes maximal immunoglobulin secretion (Kasyapa and Ramanadham 1992 and Souvannavong *et al.* 1994). APase has also been proposed to be involved in the Ig transport in B-lymphocytes as it was shown to be complexed and secreted out along with IgM. A role for APase in phosphorylation/dephosphorylation reactions in early stages of signalling in mitogen activated B lymphocytes has also been suggested (Souvannavong *et al.* 1992). It has also been shown that APase activity is not expressed in B-lymphocytes stimulated with incomplete mitogens . cAMP has been reported as a positive regulator of enhancement of APase activity in mitogen stimulated B-cells (Kasyapa and Ramanadham, 1995). Treatment of mitogen stimulated cells with antibody to APase resulted in the inhibition of proliferation and APase activity. It has been shown that the level of APase mRNA increased in mitogen stimulated cells and probably plays a crucial role in cell cycle progression. Normal B cells do not spontaneously show APase activity but cell activation induces APase expression (Burg and Feldbush, 1989, Marquez *et al.* 1989, Souvannavong and Adam, 1990). Importance of APase is indicated by the capacity of antibodies to various glycosyl phosphatidylinositol anchored molecules to transduce activation signals (Low, 1989). Recently it has been demonstrated that alkaline phosphatase activity is expressed in a subset of $CD19^{+}$ BAP^{+} normal human B cells (Hossain and Jung, 2008). It has also been shown that upon PWM stimulation of human PBMC, a fraction of proliferating B cells expressed alkaline phosphatase immunologically similar to bone cell APase.

It has been proposed that Apase may have a physiological role in the activated B cell in terms of-

a) As a phosphotyrosine phosphatase in the early stages of signaling,

b) Involvement in the proliferation and differentiation,

c) Transport of Ig molecules from the plasma cells.

Inhibitors and enhancers of Alkaline Phosphatase

Mammalian APases can be inhibited uncompetitively by a wide range of compounds, which include amino acids, tetramisole, theophylline and NADH. Inhibition by amino acids is stereospecific as L- isomers only display inhibitory activity. Similarly, only the L-isomer of tetramisole (Levamisole) is a good inhibitor of certain APases (Van Belle, 1976). L-Phenylalanine and L-Tryptophan, amino acids with hydrophobic side chains, inhibit mammalian IAPases and human PLAPases with Ki values of about 1mM (Ghosh and Fishman, 1966). They are not active towards TNAPase. On the other hand, amino acids with positively charged side chain, such as L-lysine, L-arginine, and especially L-homoarginine, are active as inhibitors with similar Ki values towards TNAPase but not towards other iso-

zymes (Fishman and Sie, 1970). Levamisole is also a good selective inhibitor of TNAPases, with Ki values lower than 100µM. Several drugs like puromycin, actinomycin D, Colchicine enhance APase activity in culture. Levamisole, an uncompetitive inhibitor of TNAPase has been shown to inhibit APase activity of liver, bone and kidney. 5-'Bromdeoxyuridine, a thymidine analogue, dbtc AMP, an analogue of cAMP, sodium butyrate and prednisolone, an analogue of hydrocortisone are some of the potent inducers of APase activity. APase substrates like phenyl phosphate have also been shown to enhance APase activity.

Levamisole and its derivatives have also been used as potent inhibitors of alkaline phosphatase, thymidylate synthase and tyrosine phosphatase (Van belle 1972 and 1976). It is also a good selective inhibitor of TNAPs, with Ki values lower than 100 uM in vitro. Mammalian APase activity of Liver/bone/kidney type has been demonstrated to be inhibited uncompetetively by Levamisole. Levamisole at 2mM concentration has been shown to inhibit the proliferation of cultured bone cells with concomitant inhibition in bone APase activity. Also, levamisole was found to bring significant clinical improvement in patients with chronic infections and inflammatory diseases such as Herpes and Rheumatoid arthritis. Further, it has been demonstrated that levamisole exerts anti-metastatic effects, particularly when it is used as an adjuvant to conventional anti-neoplastic therapy.

Alkaline Phosphatase in Diseases

An tumor marker would be one that is produced solely by the tumor and is secreted in measurable amounts in body fluids. While at present no such ideal marker exists, amongst the APase isozymes, PLAPase and GCAPase come closest to the definition given above and therefore have been evaluated extensively in various malignant conditions. TNAPase due to its ubiquitous nature fails to serve as a marker of primary malignancy and its increased level is often taken as a confirmatory finding. Human APases are abundantly expressed in tumor cells, and serum levels of APase isozymes are often used as tumor markers (Millan and Fishman, 1995). Many different isozyme patterns have been reported in malignancies and renal diseases (De Broe and Van Hoof, 1991). APase activity provides the clinician valuable information for diagnosis and follow up of patients during treatment.

The different mechanisms that have been suggested for the enhanced APase expression in tumor cells are:

a) Functional involvement of APase isozymes in tumorgenesis,
b) Representing one crucial factor in a multifactorial etiology.
c) A close linkage of APase gene with disease susceptibility.
d) Simultaneous deregulation with disease susceptibility gene.
e) Result of random chromosomal aberrations.

APase activity has been reported to be enhanced in cancer patients . Various tumor cell lines like teratocarcinomas and osteaosarcomas also show elevated APase activity (Bacci *et al.* 1993). Hemopoietic tumor cell lines have been shown to express high APase activity (Neumann *et al.* 1976). Especially in B lymphoid cell lines, APase activity has been reported to be high . Thus expression of APase is considered as one of the important identifiable markers of malignancy (Ruddon 1987, Millan and Fishman, 1995). Deficiency of TNAPase is associated with hypophosphatasia which manifests as a rare form of rickets and osteomalacia (Henthorn *et al.* 1992, Whyte 1995). Additionally, hypophosphatsia abnormalities in the metabolism of pyridoxal-5'-phosphate (PLP), 'putative' natural substrate of TNAPase, leads to epileptic seizures, apnea and perinatal death (Narisawa *et al.* 2001). Elevated plasma TNAPase levels have been reported in Paget's disease and osteoblastic bone metastases (Farley *et al.* 1991 and Demers *et al.* 1995). Plasma TNAPase level (particularly heat labile bone derived fraction) has long been recognized as an indicator of osteoblastic activity (Leunge *et al.* 1993). Leukocyte TNAPase has been shown to serve as a useful marker in cases of advanced lung cancer (Walach and Gur, 1993). GCAPase is a useful immunohistochemical marker of carcinoma-in-situ of the testis and IAPase is a marker of hepatocellular carcinoma (Roelofs *et al.* 1999). While APases are homodimeric molecules, there is re-expression in cancer cells of more than one APase isozyme in human cancer cell lines and cancer sera. The human postnatal intestine also contains heterodimers of IAP and PLAP (Behrens *et al.* 1983). Ovarian cancer cells often express both PLAPase and GCAPase (Smans *et al.* 1999) and cell lines derived from these tumors have been shown to express PLAPase/GCAPase heterodimers (Watanabe *et al.* 1989 and Hendrix *et al.* 1990). Increased PLAPase activity has frequently been found in serum samples from ovarian cancer patients and testicular cancer patients (Vergote *et al.* 1987, De Broe and Pollet 1988). PLAPase is a marker of cancer of the ovary, testis, lung, and the gastrointestinal tract (Loose *et al.* 1984).

Increased level of APase activity has been reported in neutrophlis in chronic myeloproliferative diseases (Rosenblum and Petzold, 1975). Hemopoietic tumor cell lines have been shown to express high APase activity . Especially in B lymphoid cell lines APase activity has been reported to be high. APase activity has been demonstrated in several murine tumor cell lines of B lymphoid lineage and in few human lymphoid cell lines (Harb *et al.* 1991). In humans, the role of APases *in vivo* has been demonstrated by studies on the deficiency of TNAPase resulting in rickets and osteomalacia. Reduction in TNAPase activity has been implicated in the defective mineralization of bone (Whyte 1994). The role of TNAPase seems to be in the generation of Pi needed for hydroxyapatite crystallization (Fallon *et al.* 1980).

TNAPase also hydrolyzes the mineralization inhibitor, pyrophosphate (PPi) (Meyer, 1984). Hence, deficiency of TNAPase also leads to elevated levels of extracellular PPi . The role of TNAPase in other organs e.g. liver, kidney and skin, are much less understood. Some recent reports have suggested a role for TNAPase in the regulation of secretory activity of intrahepatic biliary epithelium. Apart from PPi, pyridoxal phosphate has been shown to be a physiological substrate for TNAPase. A role for TNAPase in phosphate resorption through the brush border of proximal convoluted kidney tubules has been proposed (McComb *et al.* 1979). TNAPase activity on endothelial cell surfaces appears to protect tissues from ischemic damage that results from injury. Also TNAPase has been postulated to play a major role in exrtacellular nucleotide catalysis (Gijsbers *et al.* 2001 and 2003).

APase isozymes have also been used as disease markers**.** PLAPase was one of the first enzymes to be recognized as an onco-fetal protein in a patient with a squamous cell carcinoma of the lung. A rare variant of PLAPase was detected in serum of a patient with pleuritis carcinomatosa (Nakayama *et al.* 1970). Many samples derived from sera of patients with seminoma of the testis showed the presence of PLAPase isoenzyme. Numerous associations have been reported between the expression of GCAPase and PLAPase and malignancy. Some human tumor cell lines (HeLa) showed an enhanced expression of PLAPase. Also, high TNAPase activity was detected in cell lines derived from human testicular germ cell tumors. In some choriocarcinoma cell lines the transformation of normal to malignant trophoblast is associated with a switch in expression from PLAPase to GCAPase. The ectopic expression of PLAPase has been observed in cancer of the lung, ovary, uterus and gastrointestinal tract. Germ cell neoplasms and somatic tumors were found to express PLAPase / GCAPase. Seminomas, embryonal carcinomas and yolk sac carcinoma, gonadoblastomas were positive for PLAPase/GCAPase. All seminomas and malignant teratomas were positive for PLAPase (Epenetos *et al.* 1984). GCAPase is particularly good marker to diagnose carcinoma in situ of testis (Wahren *et al.* 1979, Paiva *et al.* 1983). PLAPase / GCAPase can serve as useful tumor markers. PLAPase also showed rising levels during progression of colorectal carcinoma. IAPase was found in teratocarcinomas and trophoblastic giant cells of two seminomas (Paiva *et al.* 1983). Intestinal-like APase variant was found in patients with hepatocellular carcinoma. It was also detected in renal cell carcinoma. The loss of a tumor suppressor on chromosome 11 was closely linked to the expression IAP (Lantham and Stanbridge, 1990). The significance of IAP as tumor marker is evident from its apparent strong association with a tumor suppressor locus (Lantham and Stanbridge, 1992).

TNAPase has been used for many years as a biochemical marker of bone turnover, specifically bone formation, and for monitoring the treatment of patients with metabolic bone disease. Bone APase has been used to identify renal bone disease common in chronic renal failure. Osteosarcomas display high serum TNAPase levels and these levels are significantly higher in metastatic disease than in patients with localized disease. Patients with cholestasis have greatly elevated tissue and serum levels of liver- derived TNAPase. TNAPase levels can also be informative in other pathologies like in patients with hyperthyroid Graves disease, patients with rheumatoid arthritis and Paget's disease. TNAPase is also expressed in cells of the hematopoietic lineage. In the normal hematopoietic system, the post mitotic neutrophilic granulocyte is the only cell type that expressed leukocyte APase under basal conditions. Significant APase activity is expressed in cell lines of B lymphoid lineage, including Abelson pre-B, B lymphoma and plasma cell tumor lines. In contrast, T lymphoid and non lymphoid hematopoietic lines had very low activity. Hence, TNAPase seems to be a useful marker of B lymphoid lineage.

CONCLUSIONS

Although APase has been studied for many years, its role has remained largely enigmatic and is still under intensive investigation. Presence of APase activity specifically in malignant human B lymphocytes, but not in normal human B cells provides a experimental model to analyse the role of APase in the proliferative and differentiative events of these cells. Expression of APase activity preferentially in malignant cells could constitute a relevant target for drug therapy**.** APase activity is expressed in cell lines of B lymphoid lineage, including Abelson pre-B, B lymphoma and plasma cell tumor lines. Hence, TNAPase can be a useful marker of B lymphoid lineage. The role of TNAPase in other organs e.g. liver, kidney and skin, are much less understood. Some recent reports have suggested a role for TNAPase in the regulation of secretory activity of intrahepatic biliary epithelium.

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