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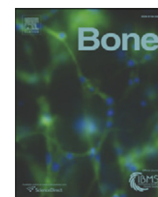

Original Full Length Article

Isozyme profile and tissue-origin of alkaline phosphatases in mouse serum

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ABSTRACT

Mouse serum alkaline phosphatase (ALP) is frequently measured and interpreted in mammalian bone research. However, little is known about the circulating ALPs in mice and their relation to human ALP isozymes and isoforms. Mouse ALP was extracted from liver, kidney, intestine, and bone from vertebra, femur and calvaria tissues. Serum from mixed strains of wild-type (WT) mice and from individual ALP knockout strains were investigated, i.e., *Alpl*$^{-/-}$ (a.k.a. *Akp2* encoding tissue-nonspecific ALP or TNALP), *Akp3*$^{-/-}$ (encoding duodenum-specific intestinal ALP or dIALP), and *Alpi*$^{-/-}$ (a.k.a. *Akp6* encoding global intestinal ALP or gIALP). The ALP isozymes and isoforms were identified by various techniques and quantified by high-performance liquid chromatography. Results from the WT and knockout mouse models revealed identical bone-specific ALP isoforms (B/I, B1, and B2) as found in human serum, but in addition mouse serum contains the B1x isoform only detected earlier in patients with chronic kidney disease and in human bone tissue. The two murine intestinal isozymes, dIALP and gIALP, were also identified in mouse serum. All four bone-specific ALP isoforms (B/I, B1x, B1, and B2) were identified in mouse bones, in good correspondence with those found in human bones. All mouse tissues, except liver and colon, contained significant ALP activities. This is a notable difference as human liver contains vast amounts of ALP. Histochemical staining, Northern and Western blot analyses confirmed undetectable ALP expression in liver tissue. ALP activity staining showed some positive staining in the bile canaliculi for BALB/c and FVB/N WT mice, but not in C57Bl/6 and ICR mice. Taken together, while the main source of ALP in human serum originates from bone and liver, and a small fraction from intestine (<5%), mouse serum consists mostly of bone ALP, including all four isoforms, B/I, B1x, B1, and B2, and two intestinal ALP isozymes dIALP and gIALP. We suggest that the genetic nomenclature for the *Alpl* gene in mice (i.e., ALP liver) should be reconsidered since murine liver has undetectable amounts of ALP activity. These findings should pave the way for the development of user-friendly assays measuring circulating bone-specific ALP in mouse models used in bone and mineral research.

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Introduction

Alkaline phosphatase (EC 3.1.3.1, ALP) is a family of enzymes that is present in most species from bacteria to man [1]. ALP catalyses the hydrolysis of a wide range of phosphomonoesters, in vitro at an alkaline pH, and is present in practically all tissues in the human body, anchored to the cell membrane lipid bilayer via a glycosylphosphatidylinositol (GPI) moiety [2].

Mice are often used as animal models to study genetic and molecular control of bone and mineral metabolism, gastrointestinal physiology and other cellular and hormonal mechanisms in order to elucidate biological functions. Less attention has been given to markers of bone remodeling (turnover) suitable for skeletal research on rodents. Some assays have, however, been developed for determination of tartrate-resistant acid phosphatase (TRACP5b) [3], carboxy-terminal cross-linked telopeptide of type I collagen (CTX) [4,5], amino-terminal procollagen type I propeptide (PINP) [6], and osteocalcin [7] in mouse and rat serum. However, no commercial assay has been successfully developed for determination of mouse serum bone ALP [8], and most "in-house" methods assume that mice have approximately the same constitution of ALP isozymes and isoforms as humans [9].

In humans, there are four gene loci encoding ALP isozymes, i.e., intestinal ALP (IALP) encoded by the *ALPI* gene, placental ALP (PALP) encoded by the *ALPP* gene, germ cell ALP (GCALP) encoded by the *ALPPL2* gene, and tissue-nonspecific ALP (TNALP) encoded by the *ALPL* gene (Table 1). The tissue-specific ALPs (i.e., IALP, PALP, and GCALP) are clustered on chromosome 2, bands q34.2–q37, and are 87–98% homologous to each other, but only about 50% identical to TNALP, which is located on chromosome 1, bands p36.1–p34 q37 [10–12]. The highest levels of human TNALP are expressed in bone and liver, and account for about 95% of the total ALP activity in serum, with a ratio of approximately 1:1 in healthy adults [13]. While the TNALP gene is not highly polymorphic, the TNALP isozyme

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Table 1
Nomenclature and accession numbers of the human and mouse ALP isozymes and genes.

Gene	Protein name (abbreviation)	Tissue distribution	Function	Accession number
Human genes				
ALPL	Tissue-nonspecific alkaline phosphatase; "liver-bone-kidney type" ALP (TNALP)	Developing nervous system, skeletal tissue, liver and kidney	Skeletal mineralization	NM_000478
ALPP	Placental alkaline phosphatase (PALP)	Syncytiotrophoblast, a variety of tumors	Unknown	NM_001632
ALPPL2	Germ cell alkaline phosphatase (GCALP)	Testis, malignant trophoblasts, testicular cancer	Unknown	NM_031313
ALPI	Intestinal alkaline phosphatase (IALP)	Gut, influenced by feeding and ABO blood group status	Fat absorption Detoxification of lipopolysaccharide	NM_001631
Mouse genes				
Alpl (*Akp2*)	Tissue-nonspecific alkaline phosphatase; "liver-bone-kidney type" ALP (TNALP)	Developing nervous system, skeletal tissues and kidney	Skeletal mineralization	NM_007431
Akp3	Duodenum-specific intestinal alkaline phosphatase (dIALP)	Gut	Fat absorption Detoxification of lipopolysaccharide	NM_007432
Alppl2 (*Akp5*)	Embryonic alkaline phosphatase (EALP)	Preimplantation embryo, testis, gut	Early embryogenesis	NM_007433
Akp-ps1	ALP pseudogene, pseudoALP	Not transcribed		NG_001340
Alpi (*Akp6*)	Global intestinal alkaline phosphatase (gIALP)	Gut	Under investigation	AK008000

exists as numerous isoforms in biological fluids differing primarily in the extent and type of glycosylation [14–16]. At least six different TNALP isoforms can be separated and quantified by weak anion-exchange high-performance liquid chromatography (HPLC), in serum from healthy individuals: one bone/intestinal (B/I), two bone (B1 and B2), and three liver ALP isoforms (L1, L2, and L3). A fourth bone-specific ALP isoform, identified as B1x, has also been demonstrated in serum from patients with chronic kidney disease (CKD) [17,18], and in extracts of human bone tissue [19].

Fifty-two amino acids, out of 524 amino acids for the entire protein, are different between mouse and human TNALP on the protein level, and they contain the same putative N-linked residues. The mouse ALP genome is very similar to the human in organization, but there are some differences in the expression of the genes (Table 1). Five ALP loci have been described in the mouse genome: TNALP (*Alpl*, a.k.a *Akp2*), embryonic ALP (EALP, *Alppl2*, a.k.a. *Akp5*), two different IALPs, i.e., the duodenum-specific IALP (dIALP) (*Akp3* gene) and a global IALP (gIALP) (*Alpi*, a.k.a. *Akp6* gene), and a putative pseudo-ALP. In humans, the TNALP isozyme is mostly expressed in liver, kidney and bone, but TNALP is also expressed in the placenta during the first trimester of pregnancy and in the neural tube during development [20,21]. In mice, the TNALP gene *Alpl* is also expressed in the placenta and primordial germ cells [22,23]. IALPs are encoded by the mouse *Akp3* and *Alpi* genes and the *Alppl2* gene encodes for EALP, all three located on chromosome 1 [24]. EALP appears to be related to the human PALP and GCALP isozymes and is expressed under the early embryonic period, but is not detectable after embryonic day seven. IALP expression is not only restricted to the intestine, but can also be expressed in thymus and embryonic stem cells. The ALP pseudogene (*Akp-ps1*) has high homology to EALP and IALP, but the gene is not transcribed [23–25].

This study was primarily designed to characterize the circulating and tissue-derived mouse ALP isozymes and isoforms. Here we report that no ALP activity is detectable in mouse liver, and nor did we detect any liver ALP in the circulation. Furthermore, mouse serum consists mostly of bone ALP (including all four bone isoforms B/I, B1x, B1, and B2) and both IALP isozymes, i.e., dIALP (*Akp3*) and gIALP (*Alpi*). We used tissues and serum from wild-type (WT) mice and ALP knockout mice models to investigate the localization and properties of the murine isozymes in relation to the known composition of circulating human ALP isozymes and isoforms.

Materials and methods

Tissue sources and serum from WT and ALP knockout mice

Liver, kidney, intestine, and bone from vertebra, femur and calvaria were obtained from 20 BALB/c WT female mice, 7 to 9 weeks old. The different organs/tissues were divided into four groups (one group contained the same organ from five mice). A separate group of mouse intestines, isolated in 7 segments in a total from 8 WT mice, were also collected and ingesta/feces were washed out with phosphate-buffered saline (PBS). Additional mouse tissues and serum were also collected from four different WT mice strains, 8-week-old males (5 in each group): C57Bl/6J and BALB/c male mice (Jackson laboratory, Sacramento, CA, USA) and FVB/N and ICR male mice (Taconic Farm Inc., Oxnard, CA, USA). The ApoE-TNALP transgenic (Tg) (+) mouse line (used as positive control for Western blot analysis and ALP histochemical staining) was reported previously [26]. Sera from ALP knockout mouse strains (corresponding protein name) were also assessed: i.e., $Alpl^{+/+}$, $Alpl^{+/-}$ and $Alpl^{-/-}$ (TNALP); $Akp3^{+/+}$ and $Akp3^{-/-}$ (dIALP); and $Alpi^{+/+}$ and $Alpi^{-/-}$ (gIALP). The genetic background of the *Alpl* knockout line is hybrids of C57Bl/6 and 129/Sv +/+, and both the *Akp3* knockout and *Alpi* knockout lines have C57Bl/6 backgrounds. Mouse blood was collected by cardiac acupuncture from mice anesthetized with Avertin (0.017 mL/g body weight), and the serum samples were stored at −70 °C until analysis. Commercially available preparations, pooled from mixed strains of WT mice (Sigma, St Louis, MO, USA), were also assessed.

Separation and quantitation by HPLC

Serum and extracts from the different tissue samples were determined by a previously described HPLC method for separation and quantitation of ALP isozymes and isoforms [27,28]. In brief, the ALP samples were separated using a gradient of 0.6 M sodium acetate on a weak anion-exchange column, SynChropak AX300 (250 × 4.6 mm I.D.) (Eprogen, Inc., Downers Grove, IL, USA). The effluent was mixed on-line with the substrate solution [1.8 mM p-nitrophenylphosphate (pNPP) in a 0.25 M diethanolamine (DEA) buffer at pH 10.1] and the ensuing reaction took place in a packed-bed post-column reactor at 37 °C. The formed product (p-nitrophenol) was then directed on-line through the detector set at 405 nm. The areas under each peak

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