

Serum Alkaline Phosphatase Isoenzymes as Markers of Liver Disease

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ABSTRACT

A perspective on serum alkaline phosphatase isoenzymes in liver disease is provided with a brief discussion of the location of the enzyme in liver and its presumed function. Mechanisms of entry of alkaline phosphatase into serum in liver disease are discussed. Characterization of high molecular weight alkaline phosphatase in obstructive jaundice is reviewed. The relationship between blood group O and the appearance of the intestinal enzyme in sera of such subjects with cirrhosis of liver is discussed. Properties of hepatoma alkaline phosphatase and the genesis of liver alkaline phosphatase in diseases not related to the liver are explored. Methods for detection of serum alkaline phosphatase isoenzymes in liver disease are discussed from the standpoint of the limitations of electrophoretic procedures, and the promise of procedures such as isoelectric focusing and high performance liquid chromatography that are currently non-routine.

Introduction

Alkaline phosphatase originating from liver and bone resemble each other very closely. Indeed, in terms of properties such as sensitivity to urea, inhibition by L-phenylalanine, and precipitation with antibody to bone alkaline phosphatase, the differences between the bone and liver enzymes are very slight. The bone enzyme is more heat-labile when compared to the liver enzyme. Furthermore, differences in stability and catalytic properties between alkaline phosphatase originating in bone, liver and kidney are slight, and their similarities are highlighted by their lack of antigenic specificity. As such, it can be inferred that a

single gene locus codes for liver, bone, and kidney alkaline phosphatases.¹¹ Indeed, the sequencing of nucleotides and the cloning of specific genes have established distinct genetic loci for placental, germ cell, intestinal and tissue unspecific alkaline phosphatase, the latter encoding for liver, bone, and kidney alkaline phosphatases.

Our current knowledge is that the genes for placental, germ cell, and intestinal alkaline phosphatase are closely associated on chromosome 2 and exhibit a striking sequence similarity. The gene coding for liver, bone, and kidney alkaline phosphatases, the so-called tissue unspecific alkaline phosphatase, is, in contrast located on chromosome 1.⁴

Since alkaline phosphatase from placenta and intestine are coded by two distinct genetic loci, it would be proper to refer to enzymes from these two sources as isoenzymes. However, although conventionally bone, liver and kidney alkaline phosphatases are referred to as isoenzymes, they are really isoforms resulting from modification of a single gene product. Differential mobility on electrophoresis of these isoforms are due to the differences in their sialic acid content.¹¹ To be consistent with conventional usage, even though strictly speaking it is a misnomer, the term isoenzyme will be used in this paper to describe serum alkaline phosphatase originating from the liver.

Location of Alkaline Phosphatase in Liver and Its Presumed Function

The genesis of serum alkaline phosphatase in liver disease can be understood better by knowing the location of this enzyme in the liver and its role. Alkaline phosphatase is located on the exterior surface of the bile canalicular portion of the plasma membrane. Because of this location, it can be presumed that the enzyme is involved in the transport of substances into the bile. Indeed, it may mediate bile acid transport into the bile.¹⁰

Entry into Serum of Alkaline Phosphatase in Liver Disease

Two mechanisms can be postulated. It is possible that bile acids accumulating in cholestasis solubilize alkaline phosphatase bound to bile canalicular membrane. The enzyme then enters the bile canaliculus and regurgitates into serum. Alternatively, it is also plausible that the distribution of alkaline phosphatase normally found on the exterior surface of the bile canalicular membrane changes upon bile duct obstruction. The enzyme then appears intracellularly and distributes

itself throughout the hepatocyte plasma membrane. This may explain why in some patients with cholestasis there are fragments of cell membrane from biliary tract cells containing alkaline phosphatase activity. Presumably, under these conditions, liver alkaline phosphatase can enter into serum directly.¹⁰

A clearer picture of the entry of alkaline phosphatase in circulation is bound to emerge from recent studies that suggest that alkaline phosphatase is anchored to the cell membrane by a phosphatidyl-inositol glycan tail.⁴ The attraction of long chain triglycerides of chylomicrons to the phosphatidyl-inositol glycan tail of intestinal alkaline phosphatase during fat absorption would presumably explain the coating of alkaline phosphatase by chylomicrons during fat absorption. Our future perspective on the mechanism of entry of liver alkaline phosphatase into circulation would include other hypothesis in addition to the possible damage to the microvilli which coat the lumen of the liver and other alkaline phosphatase rich tissues.

Serum Alkaline Phosphatase Isoenzymes in Liver Disease

OBSTRUCTIVE JAUNDICE

Electrophoresis of sera from patients with obstructive jaundice shows two bands of alkaline phosphatase activity. In media such as polyacrylamide, one of the bands stays at the origin which is suggestive of its high molecular weight.¹¹ In non-sieving media, such as cellulose acetate or agarose, this band moves faster towards the anode than the principal liver alkaline phosphatase band, indicating its greater negative charge, and is referred to as the fast liver or α -1 liver band. In addition to alkaline phosphatase, other membrane bound enzymes such as 5'-nucleotidase and γ -glutamyl

transferase are associated with this fraction. Since this fraction contains fragments of cell membrane from biliary tract cells, presumably released into serum owing to hepatic cell injury, they are also referred to as koinozymes owing to association of enzyme activity with these fractions.³

In some cases of obstructive jaundice, lipoprotein—X (LP-X) is also associated with the "high molecular weight" alkaline phosphatase fraction.¹² Indeed, it has been demonstrated that a fraction of liver alkaline phosphatase aggregates with LP-X in obstructive jaundice, thus increasing its apparent molecular size. Thus, although two fractions of alkaline phosphatase activity were recovered from gel permeation chromatography on Sephadex G-200 of sera containing LP-X, the fraction eluting with the void volume and the other eluting in the fraction where liver alkaline phosphatase usually elutes, the "high molecular weight" alkaline phosphatase fraction was absent in sera treated with *n*-butanol. Apparently butanol dissociated alkaline phosphatase bound to LP-X which then eluted with the principal liver alkaline phosphatase fraction as one band. Further proof for identity of alkaline phosphatase found in the two fractions recovered from gel permeation chromatography of untreated sera was demonstrated by similar behavior of these two fractions towards heat, urea, and L-phenylalanine treatment.²⁴

The migration of lipoprotein X—alkaline phosphatase complex as the fast liver alkaline phosphatase band, observed in sera of patients with intra- and extra-hepatic cholestasis and hepatic malignancy, is abolished by prior treatment of such sera with cetavon-diethyl ether, which dissociates alkaline phosphatase aggregated with lipoprotein-X.²³

The appearance of alkaline phosphatase LP-X complex in sera of patients

with liver disease is a reliable indicator of cholestasis. However, it is of limited value in distinguishing between intra-hepatic and extra-hepatic cholestasis.²²

CIRRHOSIS

Sera of patients with cirrhosis of the liver sometimes show evidence of two electrophoretic bands of intestinal alkaline phosphatase. The likelihood of these bands being present in the sera of patients with cirrhosis is greater in those whose blood type is O.²⁰ Proof that these two electrophoretic bands separated on agar gel containing Triton-X-100 were intestinal in origin were provided by characterization of fractions of such sera obtained on gel permeation chromatography. Thus, on Sephadex G-200 chromatography, two fractions containing alkaline phosphatase activity were isolated. Treatment of these two fractions, the high and the low molecular weight fractions with neuraminidase, did not alter their electrophoretic mobility, thus providing convincing proof that they were intestinal in origin, since the intestinal enzyme lacks sialic acid. Further proof that the two fractions were intestinal in origin was provided by their similar sensitivity to L-phenylalanine, as would be expected from intestinal alkaline phosphatase. Both fractions were also insensitive to inhibition by L-homoarginine and levimasole which inhibit liver alkaline phosphatase.²⁵ That the high molecular weight intestinal band was not due to aggregation with lipoproteins was demonstrated by the unalteration of electrophoretic mobility of this fraction upon treatment with *n*-butanol.²⁵

Therefore, in patients with cirrhosis, particularly in subjects with blood group O, the appearance of electrophoretic bands of intestinal alkaline phosphatase can be expected.

HEPATOMA

Alkaline phosphatase found in sera of patients with hepatoma is heat sensitive. This is in contrast to the heat stability of other tumor alkaline phosphatases, such as the Regan isoenzyme in branchogenic carcinoma and the Nagao isoenzyme appearing in sera of patients with pleuritis carcinomatosa. After incubation at 65° for 10 minutes, as much as 79 percent of the hepatoma alkaline phosphatase activity in sera is inhibited.¹³ Hepatoma alkaline phosphatase resembles more the Regan isoenzyme in terms of its sensitivity to 1 MM L-phenylalanine and 0.5 MM L-leucine as compared to the Nagao isoenzyme, 50 percent of whose activity is abolished with L-leucine. However, the hepatoma alkaline phosphatase does resemble the Nagao isoenzyme in that both are highly sensitive to ethylenediamine tetraacetic acid (EDTA). That the hepatoma alkaline phosphatase is different from liver alkaline phosphatase is evidenced by the lack of sensitivity of the hepatoma enzyme to L-homoarginine, an inhibitor of liver alkaline phosphatase.¹³

Electrophoresis is of limited value in the detection of hepatoma alkaline phosphatase because of the overlap of other alkaline phosphatase zones and the occasional altered mobility of the fractions complexed with either LP-X or immunoglobulins.¹³

Elevation of Liver Alkaline Phosphatase in Other Diseases

Sera of patients with either congestive heart failure, infections not affecting the liver, or Stage I and II Hodgkins disease have been reported to contain increased levels of liver alkaline phosphatase.¹⁰

It would be well to remember that liver contains, in addition to biliary tract, sinusoids (Vascular bed) and reticuloen-

dothelial tissue. The appearance of liver alkaline phosphatase in the sera of patients with diseases other than the liver can be related to the presence in capillaries and arterioles within portal triads, and, perhaps, in other blood vessels of an enzyme similar to the hepatic enzyme.¹⁰

Methods for Separation of liver Alkaline Phosphatase

HEAT TREATMENT

Perhaps the most practical method for distinguishing liver from bone alkaline phosphatase is heat treatment of sera at 56°C for 10 minutes. Bone alkaline phosphatase is rapidly inactivated under these conditions as compared to the liver. In general, if the activity of the serum sample drops below 20 percent upon incubation at 56° for 10 minutes, it can be presumed that the major fraction of isoenzyme in the sample originated from bone.¹¹

ELECTROPHORESIS

Various zone media have been used for the separation of serum alkaline phosphatase isoenzymes. While sieving media, such as polyacrylamide, have an advantage in terms of reducing zone diffusion because of the restricted size of the gel, they do not permit separation of "high-molecular weight" alkaline phosphatase, which because of its size is unable to penetrate the gel, and, as such, stays at the origin.¹¹

Non-sieving media, such as cellulose acetate and agarose, have been widely used for the separation of serum alkaline phosphatase isoenzymes.^{11,21} Separation of bone from the liver enzyme is facilitated by soaking cellulose acetate electrophoresis membranes in buffer to which wheat-germ lectin has been

added. Since the bone enzyme is precipitated by wheat-germ lectin, the mobility of this enzyme on electrophoresis is retarded, thus providing a good separation of the liver isoenzyme from the bone component.¹⁵

Electrophoresis, in general, has its limitations in terms of clear-cut separation of serum alkaline phosphatase isoenzymes, since overlapping of bands occurs in sera containing either alkaline phosphatase immunoglobulin complexes or alkaline phosphatase LP-X complexes. Resolution of electrophoretic fractions is further complicated when tumor alkaline phosphatase is present in sera.¹³

ISOELECTRIC FOCUSING

Perhaps, the best resolution of alkaline phosphatase isoenzymes is provided by isoelectric focusing. As many as 10 to 12 bands of alkaline phosphatase activity focusing in the isoelectric pH range (pI) of 3.0 to 4.9 are obtained with normal sera.⁹ Isoelectric focusing may be performed on polyacrylamide¹⁹ or thin-layer agarose gels.⁹ Since the high-molecular weight fast-liver fraction does not penetrate the polyacrylamide gel, serum containing such fractions should be disaggregated by zwitterionic detergents prior to isoelectric focusing on polyacrylamide gel.¹⁹ The pI range in which the disaggregated enzyme focuses in the polyacrylamide gel (pI range of 5 to 6) is different from the pI range at which the fast-liver fraction focuses on agarose gel. This would suggest that the fast-liver fraction complexes with carrier ampholytes used in agarose gel isoelectric focusing and behaves as a strongly acidic component.¹⁹ Bands focusing on thin-layer agarose gels have been identified as originating from biliary canaliculus (pI 3.01), hepatocyte (pI 3.65), hepatic reticuloendothelial tissue cells (pI 3.88), and the sinusoid (vascular bed) with pI

4.10.⁸ Refinement of isoelectric focusing procedures by including separators such as aspartic acid and glutamic acid to focus between two closely focussing bands improves resolution. These separator aminoacids have a pI-pK value of less than 1.5 pH units and permit the flattening of the pH gradient in the appropriate pI range of overlapping isoenzyme bands.⁹

Although isoelectric focusing is currently limited to a few clinical laboratories on a routine basis, the procedure offers promise for the optimum resolution of serum alkaline phosphatase isoenzymes in liver disease.

LIQUID CHROMATOGRAPHY

High performance liquid chromatography (HPLC) has been used to resolve serum isoenzymes of alkaline phosphatase in liver disease.^{1,14,16,17} Thus, HPLC anion-exchange chromatographic procedures have been used to resolve liver isoenzymes from other isoenzymes of alkaline phosphatase.^{14,16,17} Using a strong anion exchange column, high-molecular-mass alkaline phosphatase isoenzyme found in serum of patients with cholestasis was resolved.^{16,17} However, there have been conflicting reports in literature on the ability of the previous method to resolve completely the bone and liver isoenzymes of alkaline phosphatase in serum.^{2,6,18} A weak anion-exchange HPLC column has been reported to resolve efficiently the serum alkaline phosphatase isoenzymes.¹⁴

While wheat-germ-lectin affinity chromatography on a sepharose 4B column incompletely resolved bone and liver isoenzymes of serum alkaline phosphatase,⁵ an HPLC affinity chromatography procedure using wheat-germ-lectin conjugated to 7- μ m-diameter silica particles resulted in an improvement with only a 10 percent overlap between the bone and liver fractions.¹

The HPLC procedures, however, although useful as a research tool, have currently limited applications in the routine clinical laboratory for the separation of serum alkaline phosphatase isoenzymes.

Conclusion

The appearance of fast liver or α -1 liver high-molecular weight alkaline phosphatase band on electrophoresis in non-sieving media is associated with obstructive jaundice. The appearance of intestinal alkaline phosphatase bands in sera of blood group O subjects with liver disease is likewise associated with cirrhosis. Hepatoma alkaline phosphatase has characteristic properties permitting its differentiation from other tumor alkaline phosphatases. Finally, the appearance of liver alkaline phosphatase in the sera of patients with diseases other than of the liver is a reminder that the liver is made up of a heterogeneous mixture of cells, and an enzyme similar to the hepatic alkaline phosphatase is present in capillaries and arterioles within portal triads, and, perhaps, in other blood vessels. Optimized isoelectric focussing and HPLC techniques offer promise in terms of improved resolution of serum alkaline phosphatase isoenzymes in liver disease.

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