Characterization of Alkaline Phosphatases from Human First Trimester Placentas*

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Alkaline phosphatase from human first trimester placentas was purified, characterized, and compared with alkaline phosphatases from term placenta and liver. Three forms of first trimester placental alkaline phosphatase (I, IIa, and IIb) were isolated; their relative amounts were 35%, 39%, and 26%, respectively. Phosphatases I and IIa were found to be dimers, whereas phosphatase IIb appeared to be a tetramer consisting of two dimers of phosphatase I or IIa. Phosphatase I was indistinguishable from liver phosphatase by several criteria including apparent molecular weight (Mr = 165,000), size of the monomeric subunit (Mr = 77,000), heat lability, insensitivity to inactivation by antiserum against term placental alkaline phosphatase, and sensitivity to inactivation by antisera against liver alkaline phosphatase. In addition, phosphatase I and liver phosphatase were equally sensitive to inhibition by amino acids, levamisole, I-p-bromotetramisole, and EDTA. Phosphatase IIa, in contrast, was indistinguishable from term placental alkaline phosphatase by the same criteria: apparent molecular weight (Mr = 115,000), size of the monomeric subunit (Mr = 63,000), heat stability, inactivation by antiserum against term placental alkaline phosphatase, and sensitivity to inhibition by various compounds. These findings clearly demonstrate the existence of two distinct placental alkaline phosphatases, one (phosphatase I) specific for the first trimester placentas and the other (phosphatase IIa) occurring in both first trimester and term placentas.

The appearance of this heat-stable enzyme is not, however, universal in human tumors. Many tumor alkaline phosphatases are heat-labile and do not react with antiserum against term placental alkaline phosphatase. Because induction of placental enzymes is characteristic of neoplasia, we thought that the heat-labile alkaline phosphatase found in some tumors might correspond to the alkaline phosphatase specific for first trimester placentas. It is possible that derepression of one of the two forms of placental alkaline phosphatase always follows malignant transformation.

This paper presents the purification and characterization of the alkaline phosphatase from human first trimester placentas. This first trimester enzyme has been found to differ from the alkaline phosphatase of term placenta in immunological and physicochemical properties.

**EXPERIMENTAL PROCEDURES**

Enzyme Assay and Immunoassay—Alkaline phosphatase activity was measured by the release of p-nitrophenol from p-nitrophenyl phosphate at pH 10.5 and 37°C (20). By definition, 1 unit of enzyme releases 1 µmol of p-nitrophenol/min.

A double antibody technique was used in the immunochemical assay for alkaline phosphatase. The enzyme in 10 mM Tris-HCl, pH 7.4, containing 0.9% NaCl and 0.1% bovine serum albumin, was first allowed to react with rabbit antiserum against term placental or liver alkaline phosphatase. After incubation at 37°C for 2 h and at 4°C for 16 h, the enzyme-antibody complex was quantitatively precipitated with sheep anti-rabbit IgG globulin serum. Any residual enzyme activity in the supernatant fraction was enzyme that was unable to react with the specific antiserum.

Polyacylamide Gel Electrophoresis—Analytical gel electrophoresis was carried out in 7.5% acrylamide gels by the method of Davis (21). Phosphatase activity was determined by incubating the gels in 50 mM Tris·HCl, pH 9.0, containing o-naphthyl acid phosphate, polyvinylpyrrolidone iodide, and Fast Blue 2B (11). Polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate was carried out according to the method of Laemmli (22). β Galactosidase, phosphatase α, fumarase, γ-globulin, lactic dehydrogenase, bovine serum albumin, ovalbumin, α-chymotrypsinogen (all from Sigma Chemical Co.), catalase (Worthington Biochemical Co.), apoferritin (Mann Research Laboratories), and glucose-6-phosphate dehydrogenase (Boehringer Mannheim Corp.) were used as molecular weight standards.

The size of native alkaline phosphatase was estimated in nondenaturing polyacrylamide electrophoretic gels according to the method of Hedrick and Smith (23). The mobilities of alkaline phosphatases and standard proteins were determined using seven different gel concentrations between 4 and 10%. The ratio of bis to acrylamide (1:38) was maintained constant. A plot of log RF (ratio of protein migration to dye migration) versus gel concentration resulted in straight lines for each of the proteins used. A graph of the slopes of these lines versus the molecular weights of the protein standards was used to estimate the molecular weights of the phosphatases.

Neuraminidase Treatment—Alkaline phosphatase preparations were treated with neuraminidase (Worthington NEUP, 1.6 IU/ml) for 16 h at 37°C (24). The resulting mixtures were analyzed by polyacylamide gel electrophoresis.

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The placental form of human alkaline phosphatase (EC 3.1.3.1) has attracted interest because it is tissue-specific (1-4) and appears in many nontrophoblastic tumors (5-13). Although the alkaline phosphatase from term placenta has been extensively studied (2, 14-17), little is known about this enzyme in human placenta at earlier stages of gestation. The existence of an alkaline phosphatase that is specific for first trimester placentas (less than 10 weeks old) has been demonstrated by Fishman and co-workers (18). This enzyme had not, however, been purified or characterized. In summary, it appears that two forms of alkaline phosphatase are characteristic of human placenta.

The term placental alkaline phosphatase has been considered to be a marker for malignant transformation, because it occurs frequently in human nontrophoblastic tumors (19).
**RESULTS**

**Purification of Alkaline Phosphatase from First Trimester Placenta—** Alkaline phosphatases from first trimester placentas were extracted and purified by a modification of the method of Sussman et al. (2). The placentas were homogenized in 10 mM Tris-HCl, pH 7.4, containing 1-butanol (30%, v/v). The enzyme, in the aqueous phase, was precipitated with cold acetone. Human livers were initially processed by Dr. G. Edwards (George Washington University, Washington, D.C.), who kindly provided the acetone powder of the enzyme. The acetone precipitate was dissolved in 10 mM Tris-HCl, pH 7.4, dialyzed against the same buffer and chromatographed on DEAE-cellulose (Whatman DE52) previously equilibrated with the same buffer. The column was eluted at 4°C with the same buffer containing two successive linear NaCl gradients. Alkaline phosphatase activities appeared in two peaks, I and II (Fig. 1).

The most active fractions of each peak of alkaline phosphatase were pooled and concentrated by ultrafiltration using a UM-10 membrane in an Amicon cell. They were purified further on a Bio-Gel A-0.5m column, a DEAE-cellulose column, and finally a Sephacryl S-200 column.

Phosphatase I appeared as a single peak throughout purification. The apparent loss of specific activity of phosphatase I in the second DEAE-cellulose column was due to the separation of phosphatases I and II by this column. Phosphatase II was separated into two peaks (IIa and IIb) on the second DEAE-cellulose column (Fig. 1, inset). The relative amounts of I, IIa, and IIb were 35%, 39%, and 26%, respectively. A summary of the purification is presented in Table I.

**Polyacrylamide Gel Electrophoresis—** In a nondenaturing 7.5% polyacrylamide gel system, phosphatases I, IIa, and IIb migrated into the gel (Fig. 2A). Phosphatase IIa had the same electrophoretic mobility as the term placental alkaline phosphatase ($R_f = 0.31$), whereas phosphatases I and IIb migrated more slowly ($R_f = 0.21$ and 0.08, respectively). Liver alkaline phosphatase showed an intermediate mobility ($R_f = 0.29$). All three first trimester placental alkaline phosphatases reacted with neuraminidase, as do the liver and the term placental enzymes, indicating that these enzymes contain sialic acid residues. After neuraminidase treatment, the first trimester placental phosphatase I co-migrated with the liver phosphatase ($R_f = 0.16$). It is possible that phosphatase I differed from the liver alkaline phosphatase only in its sialic acid content.

**Molecular Weight Estimation—** Inorganic $^{32}$P phosphate has been shown to bind tightly to the active site of alkaline phosphatase (25). Liver, first trimester, and term placental...
alkaline phosphatases were labeled with $\text{H}_3\text{PO}_4$ at pH 5.0 and used for electrophoresis in a polyacrylamide gel containing SDS (Fig. 2B). The mobility of the subunit of phosphatase I was the same as that of the subunit of liver alkaline phosphatase ($M_r = 77,000$). The subunit of phosphatase IIa migrated faster than the subunit of liver enzyme. The mobility of the subunit of phosphatase IIa, however, was the same as that of the subunit of term placental alkaline phosphatase ($M_r = 63,000$). Phosphatase IIb contained both types of subunit, indicating that phosphatase IIb was probably a mixture of aggregated form of phosphatases I and IIa.

The molecular weights of native first trimester placental alkaline phosphatases were estimated both by gel filtration (Sephacryl S-200 column) and by polyacrylamide gel electrophoresis according to the method of Hedrick and Smith (23). Both methods gave similar results. The apparent molecular weight for phosphatase I was 165,000, that for phosphatase IIa was 115,000, and that for phosphatase IIb was 240,000. The apparent molecular weights for term placental and liver alkaline phosphatases were 115,000 and 165,000, respectively.

In summary, there are two types of alkaline phosphatases in first trimester placentas: the term placental type and the liver type. Each of these first trimester phosphatases occurred in a dimeric form (I and IIa) and a tetrameric form (IIb, a mixture of phosphatases I and IIa).

Characterization of First Trimester Placental Alkaline Phosphatase—Phosphatase I from first trimester placenta was precipitated with the specific antiserum against liver alkaline phosphatase (Fig. 3), whereas phosphatase IIa and term placental phosphatase were not. In contrast, only phosphatase IIa and term placental phosphatase were precipitated with the specific antiserum against term placental alkaline phosphatase. Phosphatase IIb was partially precipitated by both antisera; this suggests that phosphatase IIb was a mixture of phosphatases I and IIa.

Phosphatase I and the liver alkaline phosphatase were precipitated in the immunoprecipitation of alkaline phosphatases by specific antisera against term placental and liver alkaline phosphatase. The double antibody immunoassay for alkaline phosphatase was performed as described under “Experimental Procedures.” Residual activity in the supernatant fraction represents the excess of enzyme for each amount of the specific antiserum added. The amount of phosphatase activity present in the immunoassay was 9 microunits for all phosphatases used. The background activity in the absence of phosphatase was 3.4 microunits and was subtracted from all samples. ○—○, term placental alkaline phosphatase; ●—●, phosphatase IIa; △—△, phosphatase I; ▲—▲, liver alkaline phosphatase; and □—□, phosphatase IIb.

Heat stabilities of alkaline phosphatase—Alkaline phosphatases were heated at 56°C in 0.3 M 2-amino-2-methyl-1-propanol (pH 11) or in Tris-HCl (pH 7.4); both buffers contained bovine serum albumin (1 mg/ml). Approximately the same enzyme activity (8 microunits) was used for each phosphatase. ○—○, term placental alkaline phosphatase; ●—●, phosphatase IIa; △—△, phosphatase I; ▲—▲, liver alkaline phosphatase; and □—□, phosphatase IIb.

Effect of L-phenylalanine, L-homoarginine, L-p-bromotetramisole, imidazole, EDTA, and levamisole on alkaline phosphatase activities. Approximately the same enzyme activity (4.5 microunits) was used for each phosphatase. ○—○, term placental alkaline phosphatase; ●—●, phosphatase IIa; △—△, phosphatase I; and ▲—▲, liver alkaline phosphatase.
intermediate stability of phosphatase IIb suggested the presence of more than one type of phosphatase.

The various phosphatases were tested for inhibition by each of several compounds (Fig. 5). Although phosphatase IIa and the term enzyme were more sensitive to inhibition by L-phenylalanine, phosphatase I, and the liver phosphatase were more sensitive to inhibition by L-homoarginine, L-p-bromotetramisole, imidazole, EDTA, and levamisole.

**DISCUSSION**

The appearance in nontrophoblastic tumors of a heat-stable isoenzyme of alkaline phosphatase similar to that in term placenta indicates a radical change in the control mechanism for the synthesis of this protein after transformation (5–13). Indeed, the presence of this term placental protein can provide a potential marker for neoplasia (19). Many tumors, however, produce alkaline phosphatase with properties that are unlike the isoenzyme in term placenta. These tumor phosphatases are heat-labile and do not react with antisera against term placental alkaline phosphatase (96). The identification of two distinct placental alkaline phosphatases, one found in the early placenta (heat-labile, liver type) and the other in both the term and early placenta (heat-stable) led us to believe that one of the two forms of placental alkaline phosphatase might be depressed in most tumors during transformation.

Three forms of first trimester placental alkaline phosphatase (I, IIa, and IIb) were isolated. The apparent molecular weights for phosphatases I, IIa, and IIb estimated by gel filtration and polyacrylamide gel electrophoresis were 165,000, 115,000, and 240,000, respectively. The apparent molecular weights of the monomeric subunits of phosphatases I and IIa were 77,000 and 63,000, respectively. Phosphatases I and IIa were therefore dimers, each containing two apparently identical subunits. Two types of monomeric subunits were found in phosphatase IIb: one co-migrated with the monomer of phosphatase I, and the other co-migrated with the monomer of phosphatase IIa. Phosphatase IIb could be a mixture of tetramers made only from phosphatase I and tetramers made only from phosphatase IIa; likewise, phosphatase IIb might include mixed tetramers of phosphatases I and IIa. The immunological and heat denaturation properties of phosphatase IIb suggest that phosphatase IIb probably exists as a mixture of tetramers derived from phosphatase I and tetramers derived from phosphatase IIa. Less than 10% of the alkaline phosphatase in term placenta was in the tetrameric form: in contrast, tetramers accounted for 26% of the phosphatase in first trimester placenta. Aggregated forms of alkaline phosphatase, including tetramers and more highly aggregated forms, have been found in trophoblastic and nontrophoblastic tumors (17, 27, 28); the significance of these aggregated enzymes is unknown.

Evidence presented in this report demonstrates the existence of two distinct types of placental alkaline phosphatase. Phosphatase I resembled the liver alkaline phosphatase rather than the term placental enzyme. First, the two enzymes co-migrated on polyacrylamide electrophoretic gels after the sialic acid residues were removed from both enzymes. The apparent molecular weight of phosphatase I and of the liver phosphatase was the same (Mr = 165,000), and the monomeric subunits appeared to be the same (Mr = 77,000). Secondly, both phosphatase I and liver alkaline phosphatase were heat-labile and had similar heat denaturation curves. Thirdly, phosphatase I and liver phosphatase were inhibited to the same extent by amino acids, levamisole, L-p-bromotetramisole, and EDTA. Finally, both enzymes were precipitated with antisera against liver alkaline phosphatase, but neither enzyme reacted with antisera against term placental alkaline phosphatase.

Phosphatase IIa was indistinguishable from the term placental alkaline phosphatase. First, phosphatase IIa co-migrated with the term enzyme in polyacrylamide electrophoretic gels. The apparent molecular weight of the two enzymes was the same (Mr = 115,000) and the monomeric subunits were the same size (Mr = 63,000). Secondly, both phosphatase IIa and the term enzyme were heat-stable and were inhibited to a similar extent by amino acids, levamisole, L-p-bromotetramisole, and EDTA. Thirdly, both phosphatase IIa and the term enzyme were inactivated by antisera against term placental alkaline phosphatase but not by antisera against liver alkaline phosphatase.

**Acknowledgments—**We thank Dr. C. Edwards for helpful suggestions and Dr. G. Edwards for the gift of acetone powder of the liver alkaline phosphatase.

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