The Solubilization of Tetrmeric Alkaline Phosphatase from Human Liver and Its Conversion into Various Forms by Phosphatidylinositol Phospholipase C or Proteolysis*

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When membrane-bound human liver alkaline phosphatase was treated with a phosphatidylinositol (PI) phospholipase C obtained from Bacillus cereus, or with the proteases ficin and bromelain, the enzyme released was dimeric. Butanol extraction of the plasma membranes at pH 7.6 yielded a water-soluble, aggregated form that PI phospholipase C could also convert to dimers. When the membrane-bound enzyme was solubilized with a non-ionic detergent (Nonidet P-40), it had the M₆₀ of a tetramer; this, too, was convertible to dimers with PI phospholipase C or a protease.

Butanol extraction of whole liver tissue at pH 6.6 and subsequent purification yielded a dimeric enzyme on electrophoresis under non-denaturing conditions, whereas butanol extraction at pH values of 7.6 or above and subsequent purification by immunooaffinity chromatography yielded an enzyme with a native M₆₀, twice that of the dimeric form. This high molecular weight form showed a single Coomassie-stained band (M₆₀ = 83,000) on electrophoresis under denaturing conditions in sodium dodecyl sulfate, as did its PI phospholipase C cleaved product; this M₆₀ was the same as that obtained with the enzyme purified from whole liver using butanol extraction at pH 6.6. These results are highly suggestive of the presence of a butanol-activated endogenous enzyme activity (possibly a phospholipase) that is optimally active at an acidic pH. Inhibition of this activity by maintaining an alkaline pH during extraction and purification results in a tetrameric enzyme.

Alkaline phosphatase, whether released by phosphatidylinositol (PI) phospholipase C or protease treatment of intact plasma membranes, or purified in a dimeric form, would not adsorb to a hydrophobic medium. PI phospholipase C treatment of alkaline phosphatase solubilized from plasma membranes by either detergent or butanol at pH 7.6 yielded a dimeric enzyme that did not adsorb to the hydrophobic medium, whereas the untreated preparations did. This adsorbed activity was readily released by detergent. Likewise, alkaline phosphatase solubilized from plasma membranes by butanol extraction at pH 7.6 would incorporate into phosphatidylinositol liposomes, whereas the enzyme released from the membranes by PI phospholipase C would not incorporate. The dimeric enzyme purified from a butanol extract of whole liver tissue carried out at pH 6.6 did not incorporate. We conclude that PI phospholipase C converts a hydrophobic tetramer of alkaline phosphatase into hydrophilic dimers through removal of the 1,2-diacylglycerol moiety of phosphatidylinositol. Based on these and others' findings, we devised a model of alkaline phosphatase's conversion into its various forms.

Alkaline phosphatase (orthophosphoric-monoester phosphohydrolase, alkaline optimum, EC 3.1.3.1) purified from human liver is a dimeric glycoprotein of M₆₀, 135,000–186,000 (1, 2). As the enzyme from this source can be released from the plasma membrane by a bacterial PI phospholipase C, it is most likely anchored to this membrane via a covalent attachment to phosphatidylinositol (PI) (3, 4). Membrane-bound alkaline phosphatases from other mammalian tissues can also be released by various bacterial PI phospholipase C enzymes (5–7). This type of anchorage may apply to other eukaryotic membrane-bound proteins (4, 6, 8–12).

Human liver alkaline phosphatase, when purified from a butanol extract of a liver homogenate carried out at pH 6.6, is dimeric in its quaternary structure (13). However, this enzyme may be tetrameric in its native, membrane-bound state (14), and in the present study, we have shown that when the enzyme is purified from a butanol extract of a liver homogenate carried out at pH 8.5, it is predominantly in a tetrameric state. We have used PI phospholipase C (15) and proteolytic enzymes as probes of alkaline phosphatase to study quaternary structure and the nature of its attachment to human liver plasma membranes, and employed these and others' data to construct a possible model of the enzyme in human liver plasma membranes.

EXPERIMENTAL PROCEDURES

Materials

Phospholipase C (Bacillus cereus) type III, ficin, bromelain, subtilisin Carlsberg, Nonidet P-40, Triton X-100, p-nitrophenyl phosphate, naphthol AS-MX phosphate, dipalmitoyl phosphatidylcholine, 2-amino-2-methyl-1-propanol, and phenylmethylsulfonyl fluoride (PMSF) were purchased from Sigma; ethylaminoethanol from Aldrich; molecular weight markers for gradient gel electrophoresis and octyl-Sepharose CL-4B from Pharmacia (Uppsala, Sweden); acrylamide, N,N'-methylenebisacrylamide, sodium dodecyl sulfate (SDS), and SDS molecular weight protein markers were from Bio-Rad; and

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1The abbreviations used are: PI, phosphatidylinositol; PMSF, phenylmethylsulfonyl fluoride; SDS, sodium dodecyl sulfate; l, liter.
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2.5-27% polyacrylamide gradient gels from Isolab (Akron, OH). All other chemicals were reagent grade (Fisher Scientific, Fairlawn, NJ).

Methods

Plasma Membrane and Enzyme Preparation, and Enzyme Assay—Healthy human liver removed within 12 h of death and cut into small pieces was cryopreserved at −70 °C until needed. The pieces were homogenized, sonicated, and centrifuged to yield purified liver plasma membranes (3). Dimeric alkaline phosphatase was purified from a butanol extract of a liver homogenate carried out at pH 6.6 as described previously (16). PI phospholipase C was purified from a commercial preparation of nonspecific phospholipase C (17). Alkaline phosphatase activity was assayed in 1 ml of medium containing 2 mg/ml bovine serum albumin, 0.5 mg/ml of MgCl₂, and 1 mg/ml of 1-ethylaminoethanol, pH 10.3, at 30 °C. The increase in absorbance at 404 nm was monitored with a spectrophotometer (Varian, model 2200), and enzyme activity was expressed as p-nitrophenol released (μmol/min/l).

Alkaline Phosphatase Release from Plasma Membranes with Proteases—Aliquots of isolated plasma membranes (alkaline phosphatase activity approximately 600 units/l) were suspended in a buffer containing 50 mmol/l Tris with 10% (v/v) glycerol, pH 7.6 (hereafter known as Tris buffer). Test samples were incubated with equal volumes of bromelain or ficin (final concentration, 5 mg/ml) in the same buffer containing 1 mmol/l of ZnCl₂, 1 mol/l of MgCl₂, and 0.1% (v/v) ethylaminoethanol, pH 10.3, at 30 °C. The increase in absorbance at 404 nm was monitored with a spectrophotometer (Varian, model 2200), and enzyme activity was expressed as a percentage of the activity in the corresponding uncentrifuged sample.

Gradient and SDS-Polyacrylamide Gel Electrophoresis—The nondenatured M₀ of alkaline phosphatase in each sample (100 μl, alkaline phosphatase activity approximately 250 units/l) was determined by electrophoresis in gradient gels (2.5-27% polyacrylamide) for 18 h at 150 V in a buffer containing (per liter) 0.08 mol of Tris and 0.08 mol of boric acid, pH 8.4 (2). No detergents were present in the electrophoresis buffer. The gels were stained using 3.0 mg/ml naphthol ASMX phosphorus, 1.5 mmol/l MgCl₂, and 1.0 mol/l ethylaminoethanol, pH 10.3 as substrate medium. Fluorescing bands (indicating alkaline phosphatase activity) were photographed under UV light (2), and the M₀ markers were stained with Coomassie Blue G-250 (18).

Alkaline phosphatase was resolved by polyacrylamide gel electrophoresis in the presence of SDS by the method of Laemmli (19). The support consisted of a stacking gel (3% (w/v) acrylamide) and a resolving gel (10% (w/v) acrylamide). Each sample of enzyme (3 μg of protein) was added as an equal amount of the stacking gel (2.5% (w/v) SDS, 5% (v/v) β-mercaptoethanol, and 10% (v/v) glycerol) in 62.5 mmol/l Tris-HCl, pH 6.8. The samples were then heated in boiling water for 3 min before tracking dye (0.1% (w/v) bromphenol blue, final concentration) was added. The samples (50-75 μl) were then applied to the gel, and electrophoresis was carried out at a constant current of 15 mA in a Bio-Rad Protein II apparatus. Proteins were stained with Coomassie Blue R-250 (20).

Treatment of Detergent-solubilized Tetrmeric Alkaline Phosphatase with Subtilisin Carlsberg—Nonidet P-40 was added to purified plasma membranes (alkaline phosphatase activity approximately 600 units/l) suspended in the Tris buffer, to give 1% (v/v) Nonidet P-40. This was mixed at 4 °C for 3 h. Then centrifuged at room temperature for 15 min at 90,000 × g. The supernatant fluid was divided into three aliquots; to one (control) was added an equal amount of the Tris buffer; to each of the other two aliquots was added an equal volume of subtilisin Carlsberg in the Tris buffer, to give final subtilisin concentrations of 0.5 and 5.0 mg/ml. Nonidet P-40 was added to all three, to a final concentration of 6% (v/v); the mixtures were incubated at 30 °C for 3 h, and then applied to gradient gels.

PI Phospholipase C Treatment of a Butanol Extract of Nonidet P-40-solubilized Alkaline Phosphatase—Nonidet P-40 solubilization was accomplished as described above. Following centrifugation, Nonidet P-40 was added to the supernatant fluid to give a final concentration of 6% (v/v). To an aliquot of this supernatant was added an equal volume of cold (−20 °C) 1-butanol. The mixture was put on a wrist-action shaker for 1 h at 4 °C, and then centrifuged at 10,000 × g for 10 min at room temperature. The bottom aqueous layer was recovered. PI phospholipase C was added, to a final concentration of 0.1 μmol/l, and the sample incubated at 30 °C for 3 h before application to gradient gels.

Purification of Tetrameric Alkaline Phosphatase—All procedures were carried out at 4 °C unless stated otherwise. Human liver (100 g) obtained at autopsy and stored as described above was thawed and homogenized in a Waring Blender in 100 ml of 50 mmol/l Tris-HCl, pH 8.5, 1 mmol/l MgCl₂, 0.1 mmol/l ZnCl₂, 0.1% PMSF (w/v), 100 μmol/l leupopin, 20 μmol/l pepstatin for 5 min at slow speed followed by 5 min at high speed. This mixture was spun at 90,000 × g for 10 min at room temperature. The supernatant was added and this mixture stirred for 1 h and then centrifuged at 9,000 × g for 30 min. The aqueous layer was recovered and centrifuged as above to sediment any remaining particulate matter. The aqueous layer was then reapplied to a monoclonal antibody column (specific for liver-type alkaline phosphatase) of 4 ml and washed with several column volumes of 50 mmol/l Tris, pH 8.5, 1 mmol/l MgCl₂, 0.1 mmol/l ZnCl₂, 0.1% (v/v) Triton X-100 at 0.5 ml/min. The enzyme was eluted with 0.8 ml of 2-amino-2-methyl-1-propanol, pH 10.3, pooled, and dialyzed extensively against 50 mmol/l Tris, pH 8.5, 1 mmol/l MgCl₂, 0.1 mmol/l ZnCl₂. To remove detergent, an equal volume of 1-butanol at −20 °C was added and a butanol extract was carried out as before. The enzyme was concentrated and small amounts of dimer removed by adsorption to octyl-Sepharose; the enzyme was added to 20 °C to 3.5 ml of packed beads suspended in 50 mmol/l Tris, pH 8.5, 1 mmol/l MgCl₂, 0.1 mmol/l ZnCl₂. The beads were gently mixed for 15 min at 20 °C and centrifuged at 12,000 × g for 20 min, 4 °C. The buffer, and the enzyme was eluted in this buffer plus 10% (v/v) Triton X-100.

Hydrophobic Chromatography—The PI phospholipase C-released form of the enzyme was prepared by incubating liver plasma membranes with 6 μg/ml PI phospholipase C at 30 °C for 2.5 h. The ficin-released form of the enzyme was prepared by incubating liver plasma membranes with 5 mg/ml of ficin for 3 h at 37 °C with (or without) 20 μg/ml PI phospholipase C. The enzyme was concentrated and small amounts of dimer removed by adsorption to octyl-Sepharose; the enzyme was added to 20 °C to 3.5 ml of packed beads suspended in 50 mmol/l Tris, pH 8.5, 1 mmol/l MgCl₂, 0.1 mmol/l ZnCl₂. The beads were gently mixed for 15 min at 20 °C and centrifuged at 12,000 × g for 20 min, 4 °C. The buffer, and the enzyme was eluted in this buffer plus 10% (v/v) Triton X-100.

Liposome Preparation and Alkaline Phosphatase Incorporation—Liposomes were prepared by dissolving 6 mg of dipalmitylphosphatidylycholine in 1 ml of chloroform and drying under nitrogen. A volume of 1 ml of 50 mmol/l Tris, pH 7.6, 10% (v/v) glycerol was then added and this was sonicated (under nitrogen) on a bath sonicator for 15 min. Forms of alkaline phosphatase as described above were added to an equal volume of liposomes and mixed gently for 18 h. The liposomes were separated by centrifugation at 20 °C (140,000 × g, 15 min) and the liposomes and supernatant were assayed for alkaline phosphatase activity.

RESULTS

Although both ficin and bromelain solubilized the liver alkaline phosphatase of purified plasma membranes, ficin was more proficient (Fig. 1). The released enzyme was dimeric, with an M₀ value of approximately 214,000—the same as that for the enzyme purified from a butanol extract done at pH 6.6 (Fig. 2).

Proteolysis by subtilisin Carlsberg converted the detergent-solubilized tetrameric enzyme to dimers; this process, which was concentration-dependent, was inhibited by PMSF.
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**FIG. 1.** Solubilization of membrane-bound alkaline phosphatase by proteolytic enzymes. O, control; ▲, bromelain, 5.0 mg/ml; and ■, ficin, 5.0 mg/ml.

**FIG. 2.** Gradient gel electrophoretogram of purified alkaline phosphatase and of the enzyme released from plasma membranes by proteases (5.0 mg/ml). a, markers; b, purified liver alkaline phosphatase; c, enzyme released by bromelain; d, enzyme released by ficin.

**FIG. 3.** Gradient gel electrophoretogram of Nonidet P-40-solubilized alkaline phosphatase. a, control; b, subtilisin, 0.5 mg/ml; c, subtilisin, 5.0 mg/ml; d, subtilisin, 5.0 mg/ml and 0.02% (w/v) PMSF.

**FIG. 4.** Gradient gel electrophoretogram of Nonidet P-40-solubilized alkaline phosphatase treated with butanol and PI phospholipase C. a, control; b, after butanol extraction; c, butanol extract treated with PI phospholipase C, 50.1 µg/ml.

**FIG. 5.** SDS gel electrophoretogram of purified human liver alkaline phosphatases. a, PI phospholipase C-treated tetrameric enzyme; b, molecular weight markers; c, tetrameric enzyme; d, dimeric enzyme purified from a butanol extract of liver homogenate carried out at pH 6.6.

(0.02%, w/v), an inhibitor of serine proteases (20) (Fig. 3). Ficin destroyed a significant amount of the activity of the detergent-solubilized alkaline phosphatase, with little or no conversion of tetrameric to dimeric form, whereas bromelain did not inactivate the alkaline phosphatase activity but was less competent than subtilisin Carlsberg in converting tetrameric to dimeric form (results not shown).

Butanol extraction of the Nonidet P-40-solubilized alkaline phosphatase produced a water-soluble form of enzyme that, on electrophoresis, clumped in aggregates of high Mr, and that PI phospholipase C converted to dimers (Fig. 4). The same is true of the enzyme directly extracted from membranes by butanol and subsequently treated with PI phospholipase C (results not shown). The tetrameric form of the enzyme purified by immunoadsorption was shown to have an Mr of 83,000 (Fig. 5). The purified tetrameric enzyme, subsequently treated with PI phospholipase C, and the enzyme purified from a butanol extract carried out at pH 6.6 were
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**FIG. 6.** Elution profiles of different forms of alkaline phosphatase chromatographed on octyl-Sepharose CL-4B. Arrow indicates the application of Triton X-100 (10% v/v). a, alkaline phosphatase released from plasma membranes by PI phospholipase C; b, alkaline phosphatase extracted from plasma membranes by butanol at pH 7.6 subsequently treated with (A) or without (O) PI phospholipase C; c, alkaline phosphatase released from plasma membranes by ficin.

**FIG. 7.** Elution profiles of different forms of alkaline phosphatase chromatographed on octyl-Sepharose CL-4B. Arrow indicates the application of Triton X-100 (10% v/v). a, alkaline phosphatase purified from a butanol extract of a homogenate of liver tissue carried out at pH 6.6; b, alkaline phosphatase solubilized from plasma membranes by Triton X-100 subsequently treated with (A) or without (O) PI phospholipase C.

also shown to have a subunit Mr of 83,000 (Fig. 5). Alkaline phosphatase released from plasma membranes by PI phospholipase C or ficin did not adsorb to the hydrophobic column (Fig. 6). However, the butanol-extracted form of the enzyme did, and could be eluted by the addition of a 10% v/v Triton solution; PI phospholipase C treatment of the butanol extract converted the alkaline phosphatase into a form that was unable to adsorb to the hydrophobic column (Fig. 6). Alkaline phosphatase purified from a butanol extract of a liver homogenate carried out at pH 6.6 was unable to adsorb to the hydrophobic column (Fig. 7). However, enzyme solubilized from plasma membranes by non-ionic detergent was able to adsorb, and could be eluted by the addition of a 10% v/v Triton solution; PI phospholipase C treatment of the Triton-solubilized enzyme converted the enzyme into a form that was unable to adsorb to the column.

Alkaline phosphatase extracted from plasma membranes by butanol was able to incorporate into phosphatidylcholine liposomes (Fig. 8). Maximum incorporation required 12 h or more. Enzyme released from plasma membranes by PI phospholipase C or purified from a butanol extract of liver homogenate carried out at pH 6.6 was unable to incorporate into the liposomes. Attempts to incorporate detergent-solubilized enzyme into liposomes were unsuccessful because the liposomes were destroyed by the concentration of detergent required to solubilize the enzyme.

**DISCUSSION**

Ficin and bromelain are relatively nonspecific in their choice of cleavage sites on the polypeptide chains of proteins (21), therefore they were chosen to solubilize the membrane-bound form of alkaline phosphatase. The ability of bromelain and ficin to solubilize membrane-bound alkaline phosphatase, and their lack of ability to efficiently or effectively convert Nonidet P-40-solubilized alkaline phosphatase to dimers is probably due to the detergent affecting the activity or specificity of these proteases. Subtilisin Carlsberg released the membrane-bound alkaline phosphatase poorly. This protease is also nonspecific in its choice of cleavage sites but is known to work well in the presence of detergent (22). It was used to convert Nonidet P-40-solubilized alkaline phosphatase to dimers. Subtilisin has been previously shown to cleave a small (2 kDa) portion of the carboxyl terminus from human placental alkaline phosphatase (23) and bromelain has also been shown to cleave a similar-sized segment from this enzyme (24).

We believe that conversion of the various forms of alkaline phosphatase from human liver occurs in a similar manner to
that proposed for the membrane-bound acetylcholinesterase of *Torpedo* electric organ (25). This enzyme, like the alkaline phosphatase from human liver, is released from the membrane by both PI phospholipase C or proteolysis; however, acetylcholinesterase is a dimer in the membrane and when solubilized by detergent. This dimeric structure is maintained after treatment of the membrane-bound or detergent-solubilized forms with PI phospholipase C or proteinase K. The native form of alkaline phosphatase from human liver, however, has the $M_r$ of a tetramer (441,000) but becomes 214,000 when treated with PI phospholipase C or proteases. This high $M_r$ species is unlikely to be due to a detergent micelle since gradient gel electrophoresis is essentially an equilibrium technique and after 18 h of applied potential there should be little detergent associated with the protein. The tetramer-like enzyme, when purified to homogeneity, was composed of a single polypeptide with a subunit $M_r$ of 83,000. Enzyme purified from a butanol extract of liver homogenate done at pH 6.6 or treatment of the purified tetrameric enzyme with PI phospholipase C resulted in a dimeric form of the enzyme that had this same subunit $M_r$. We believe the high $M_r$ species is a tetramer composed of subunits of the same molecular mass and that cleavage of the hydrophobic membrane anchor from the polypeptides, resulting in the formation of dimeric enzyme, does not significantly change the mass of the subunit.

Forms of the enzyme that lack the PI anchor (protease treated, PI phospholipase C-treated, or extracted by butanol at pH 6.6) were unable to adsorb to a hydrophobic column and/or incorporate into liposomes, whereas forms of the enzyme having the membrane anchor (detergent-solubilized or extracted by butanol at pH 8.5) were able to incorporate into liposomes and/or adsorb to a hydrophobic column. The hydrophobic and hydrophilic forms of the enzyme obtained through butanol extractions of liver tissue done at slightly alkaline and slightly acidic pH values, respectively, may be due to an endogenous, butanol-stimulated enzyme activity that could be a phospholipase (26-28).

In the structural model we propose (Fig. 9), both the membrane-bound tetrameric alkaline phosphatase and the PI molecules that are the attachment site for the enzyme are on the outer surface of the plasma membrane lipid bilayer. This location of the alkaline phosphatase and PI is based on the finding that alkaline phosphatase, 5'-nucleotidase, and acetylcholinesterase from certain mammalian sources are released from intact cells by bacterial PI phospholipase C without cell lysis (29-32). The attachment of the alkaline phosphatase to the PI is probably at the carboxyl terminus, in view of the almost equal ratio of myo-inositol with each subunit in *Torpedo* acetylcholinesterase or human placental alkaline phosphatase (35, 36).

The amino acid sequence of human liver alkaline phosphatase, by isolation and characterization of the cDNA corresponding to this enzyme, includes a stretch of hydrophobic amino acids at the carboxyl-terminal region of the enzyme precursor (37). This hydrophobic span could help to anchor the alkaline phosphatase to the membrane. If that were the case, however, PI phospholipase C treatment should not be able to solubilize the membrane-bound enzyme but proteolysis could, and the latter would leave the hydrophobic “tail” in the membrane. As the enzyme is solubilizable by both PI phospholipase C and proteolysis, the most likely thing to occur is post-translational processing in which the carboxyl terminus (containing a span of hydrophobic amino acids) of the enzyme precursor is proteolytically detached and the PI is attached, directly or indirectly, to an amino acid near or at the newly formed carboxyl terminus. The mature enzyme would be anchored to the membrane by PI and not by a membrane-spanning stretch of hydrophobic amino acids. This type of post-translational modification probably occurs in the trypanosome membrane variable surface glycoprotein (38); also, cDNA analysis of rat Thy-1 (39), another PI phospholipase C-releasable membrane-bound protein, has shown 32 extra amino acids at the carboxyl terminus (of which 20 are hydrophobic) that are absent from the mature protein (33).

As the human liver alkaline phosphatase is attached to the PI, solubilization by a detergent requires interaction of the hydrophobic portion of the detergent's molecules with the PI's fatty acyl chains. This treatment, however, with non-ionic detergent cannot disrupt the forces holding two pairs of dimers together, and the enzyme thus solubilized is tetrameric. When tetrameric alkaline phosphatase is treated with PI phospholipase C or proteolytic enzymes, conversion to dimers occurs. Since the interaction with PI is needed for membrane binding or detergent solubilization, the dimeric forms of the alkaline phosphatase would be water-soluble since they should not have the fatty acyl chains of the PI associated with them. The inability of alkaline phosphatase solubilized from membranes by either PI phospholipase C or protease to adsorb to a hydrophobic column is also evidence that the enzyme is in a hydrophilic form that lacks the fatty acyl chains of the PI membrane anchor. Although we do not have definitive structures of the various forms of alkaline phosphatase and their interconversions. PI anchors the enzyme (possibly tetrameric) to the plasma membrane (I), and its fatty acyl groups interact with non-ionic detergent (•) during solubilization with this detergent (II). PI phospholipase C (PI-PLC) cleaves both the membrane-bound and the detergent-solubilized enzyme (I and II) at a site on the PI molecule between the glycerol backbone and the inositol, leaving the hydrophobic diacylglycerol moiety in the membrane of (I) or cleaving it away (II), and thus allowing release of the alkaline phosphatase in a hydrophilic, dimeric form (III). The similar $M_r$ of the protease and the PI phospholipase C-solubilized enzyme indicate that the proteolytic enzymes cleave the polypeptide chain close to the amino acid responsible for the binding to the PI, creating an effect identical to that with PI phospholipase C (IV). In the diagram a small peptide tail is shown attached to the glycan structure, represented by a box that in turn is attached to the inositol headgroup of the phospholipid.

![FIG. 9. Structures of the various forms of alkaline phosphatase and their interconversions. PI anchors the enzyme (possibly tetrameric) to the plasma membrane (I), and its fatty acyl groups interact with non-ionic detergent (•) during solubilization with this detergent (II). PI phospholipase C (PI-PLC) cleaves both the membrane-bound and the detergent-solubilized enzyme (I and II) at a site on the PI molecule between the glycerol backbone and the inositol, leaving the hydrophobic diacylglycerol moiety in the membrane of (I) or cleaving it away (II), and thus allowing release of the alkaline phosphatase in a hydrophilic, dimeric form (III). The similar $M_r$ of the protease and the PI phospholipase C-solubilized enzyme indicate that the proteolytic enzymes cleave the polypeptide chain close to the amino acid responsible for the binding to the PI, creating an effect identical to that with PI phospholipase C (IV). In the diagram a small peptide tail is shown attached to the glycan structure, represented by a box that in turn is attached to the inositol headgroup of the phospholipid.](image-url)
Evidence that human liver alkaline phosphatase is present as a tetramer on the plasma membrane itself, it is a possibility, since we have shown that mouse osteosarcoma alkaline phosphatase has a tetrameric structure upon release from the membranes by PI phospholipase C. As yet we have been unable to cross-linking experiments to directly demonstrate the existence of a tetramer in the plasma membrane of human liver cells.

Treatment of the plasma membranes with butanol at a slightly alkaline pH will extract the lipids of the bilayer but leave the PI attached to the alkaline phosphatase (present in the aqueous phase of the extract). The high affinity of the enzyme for PI may be necessary in carboxylation of the enzyme from pig kidney, in that the porcine enzyme lost, as when the alkaline phosphatase is released from membranes by PI phospholipase C or proteolysis, the enzyme takes on a dimeric structure.

Physiologically, a tetrameric structure of human liver alkaline phosphatase and its binding to PI may be necessary in carboxylation of the enzyme from pig kidney, in that the porcine enzyme lost, as when the alkaline phosphatase is released from membranes by PI phospholipase C.

Thus it seems likely that the major force between pairs of dimers maintaining the tetrameric structure resides in the PI molecules that are attached to the enzyme: when the PI is lost, as when the alkaline phosphatase is released from membranes or from detergent-lipid complexes by PI phospholipase C or proteolysis, the enzyme takes on a dimeric structure. Physiologically, a tetrameric structure of human liver alkaline phosphatase and its binding to PI may be necessary in carrying out its function. Little is known of this function, although the membrane-bound form of the enzyme has been shown to dephosphorylate certain human liver plasma membrane proteins (41, 42).

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