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# Tetrameric alkaline phosphatase from human liver is converted to dimers by phosphatidylinositol phospholipase C

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Membrane-bound human liver alkaline phosphatase solubilized by a non-ionic detergent, Nonidet P-40 (NP-40), has the molecular mass of a tetramer. It can be converted to a dimeric form by treatment with a phosphatidylinositol phospholipase C (PI-PLC) obtained from *Bacillus cereus*. When human liver plasma membranes were directly treated with PI-PLC, the released alkaline phosphatase was dimeric. Thus, phosphatidylinositol may help maintain the tetrameric quaternary structure of alkaline phosphatase and aid its binding to human liver plasma membranes.

Alkaline phosphatase; Liver (human); Plasma membrane; Phosphatidylinositol phospholipase C; Detergent -solubilized alkaline phosphatase

## 1. INTRODUCTION

Alkaline phosphatase (orthophosphoric-monoester phosphohydrolase [alkaline optimum, EC 3.1.3.1]) purified from human liver is a dimeric glycoprotein of $M_r$ 135000–186000 [1,2]. However, recent work in this laboratory has revealed alkaline phosphatase in a tetrameric form in its native, membrane-bound state [3]. Also, others have found the enzyme associated with PI in the membranes of various mammalian tissues, having released the alkaline phosphatase from the membranes with purified PI-PLCs from bacterial sources [4,5]. The attachment of alkaline phosphatase to the membrane may be due to a strong interaction, covalent or non-covalent, with the membrane's PI [6,7], an attachment thought to apply to several other mammalian membrane-bound enzymes that can be released with bacterial PI-PLCs [5,8,9]. We now report the use of PI-PLC as a probe to study the nature of attachment of alkaline phosphatase to human liver plasma membranes and to study the relationship between the enzyme's tetrameric and dimeric forms.

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*Abbreviations:* PI-PLC, phosphatidylinositol phospholipase C; NP-40, Nonidet P-40; PI, phosphatidylinositol; PMSF, phenylmethylsulfonyl fluoride

## 2. EXPERIMENTAL

Phospholipase C (*Bacillus cereus*) type III, NP-40, *p*-nitrophenylphosphate, and naphthol AS-MX phosphate were purchased from Sigma (St Louis, MO), ethylaminoethanol from Aldrich (Milwaukee, WI), molecular-mass markers for electrophoresis from Pharmacia (Uppsala, Sweden), and 2.5–27% polyacrylamide gradient gels from Isolab (Akron, OH). All other chemicals were reagent grade (Fisher Scientific, Fairlawn, NJ, USA).

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Plasma membranes were isolated [3], and alkaline phosphatase was purified to homogeneity as judged by gel electrophoresis in SDS [10]. PI-PLC was purified from a commercial preparation of nonspecific phospholipase C [11]. Alkaline phosphatase was assayed in 1 ml of medium containing *p*-nitrophenylphosphate (10 mmol/l) and ethylaminoethanol (1.0 mol/l), pH 10.3, at 30°C; the increase in absorbance at 404 nm was monitored with a spectrophotometer (Varian, model 2200). Enzyme activity (U) was expressed as $\mu$mol of *p*-nitrophenol released/min per l. Protein concentrations were determined using a BCA protein assay reagent (no.23225; Pierce, Rockfold, IL, USA).

Aliquots of isolated plasma membranes (alkaline phosphatase activity = 600 U/l) suspended in a buffer containing 50 mmol/l Tris, 10% (v/v) glycerol, pH 7.6, were incubated with equal volumes of PI-PLC in the same buffer at 30°C. Final PI-PLC concentrations were from 0 $\mu$g/ml (control) to 5.0 $\mu$g/ml and incubation times were from 15 to 75 min. At each incubation time, an aliquot of each sample was centrifuged at 95000 × *g* for 8 min; enzyme activity recovered from the supernatant fluid was expressed as a percentage of the activity in the uncentrifuged control.

The molecular mass of alkaline phosphatase in each sample (250 U/l in 100 $\mu$l) was determined by electrophoresis in gradient gels (2.5–27% polyacrylamide gradient) for 18 h at 150 V in a buffer containing (per liter) 0.09 mol Tris and 0.08 mol borate, pH 8.4 [2]. The gels were stained in naphthol AS-MX phosphate (3 mg/ml) dissolved in ethylaminoethanol 1.0 mol/l, pH 10.3. They were viewed under UV light until fluorescing band(s) of alkaline phosphatase became visible [2], then the markers were stained with Coomassie blue G-250 [3].

NP-40 was added to purified plasma membranes (600 U activity/l) suspended in 50 mmol/l Tris, 10% (v/v) glycerol, pH 7.6, to give 1% (v/v) NP-40. Samples were mixed at 4°C for 1 h and then centrifuged for 15 min at 95000 × *g*. The supernatant fluid (containing the detergent-solubilized enzyme) was divided into three aliquots: to one aliquot (control) was added an equal amount of buffer (50 mmol/l Tris, 10% (v/v) glycerol, pH 7.6) containing no PI-PLC; to the other two was added an equal volume of PI-PLC (in 50 mmol/l Tris, and 10% (v/v) glycerol, pH 7.6) to give final PI-PLC concentrations of 5.0 $\mu$g and 57.4 $\mu$g/ml, respectively. NP-40 was added to all 3 incubation mixtures, to a final concentration of 6% (v/v). The samples were incubated at 30°C for 1 h and then applied to gradient gels.

## 3. RESULTS AND DISCUSSION

The amount of alkaline phosphatase released from the liver plasma membranes was proportional to the PI-PLC concentration and the duration of treatment at a fixed PI-PLC concentration (fig.1). Inhibitors of proteolytic enzymes (PMSF, 0.02% (w/v); leupeptin, 1 $\mu$mol/l; bestatin, 0.23 mmol/l; and pepstatin A, 0.10 mmol/l) did not inhibit the PI-PLC-mediated release of the enzyme (not shown). The released enzyme had a molecular mass of 214 kDa, and thus was indistinguishable from the 'soluble' alkaline phosphatase purified by our standard protocol. Also, PI-PLC converted detergent-solubilized tetrameric enzyme to dimers (fig.2), to a degree proportionate to the PI-PLC concentration.

Bacterial PI-PLCs can solubilize membrane-bound ALP from various non-human mammalian tissues. This indicates that PI contributes to the enzyme's attachment on the plasma membrane [4,5]; a role applicable to the human liver enzyme, which can also be solubilized by a bacterial PI-PLC [12]. Alkaline phosphatase released from the human liver plasma membranes by PI-PLC was in the

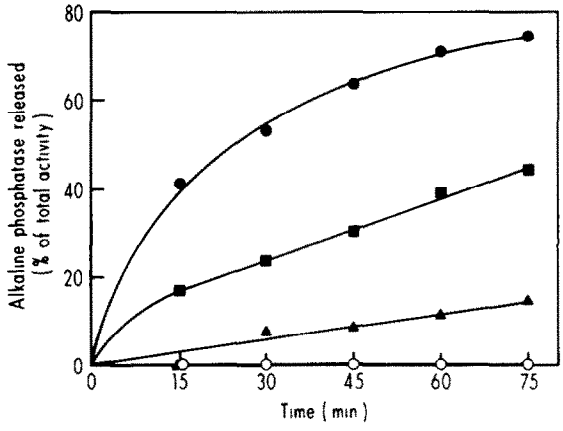


Fig.1. Solubilization of membrane-bound alkaline phosphatase by PI-PLC applied in the following concentrations ($\mu$g/ml): ○, 0; ▲, 0.2; ■, 1.0; ●, 5.0.

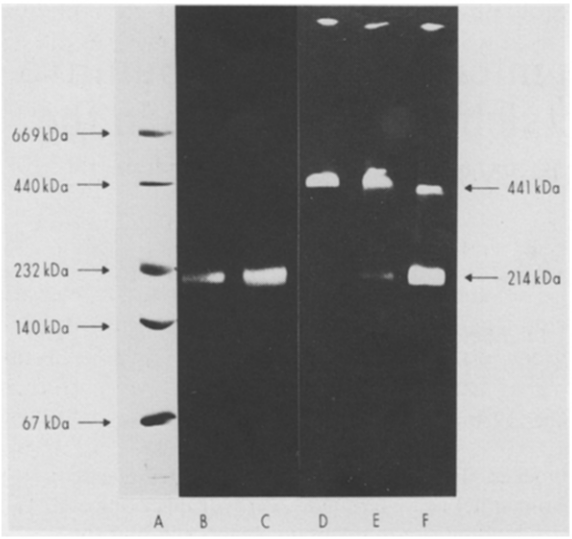


Fig.2. Gradient gel electrophoresis of alkaline phosphatase released from plasma membranes by PI-PLC and the detergent-solubilized enzyme treated with PI-PLC. Lanes: A, protein markers; B, purified liver alkaline phosphatase; C, alkaline phosphatase released from membranes by PI-PLC. Lanes D–F, NP-40-solubilized alkaline phosphatase; D, control, incubated without PI-PLC; E, incubated with PI-PLC (5.0 $\mu$g/ml); F, incubated with PI-PLC (57.4 $\mu$g/ml).

dimeric, water-soluble form. It is highly unlikely that proteolytic activity was a cause of this release, in view of the lack of inhibition of this release by protease inhibitors. Conversion of the detergent-soluble tetrameric alkaline phosphatase to dimers by PI-PLC shows that PI may also have a role in maintaining the quaternary structure of this enzyme.

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