Alkaline phosphatase of the calf intestine hydrolyzes phospholipids

Heidrun Ehle, Elke Müller and Anton Horn

Institute of Physiological Chemistry, Friedrich-Schiller University, Löbderstraße 3, 6900 Jena, GDR

Received 9 February 1985

Pure alkaline phosphatase of the calf intestine is able to hydrolyze phosphatidylinositol 4,5-diphosphate (TPI) to phosphatidylinositol and P_i and to dephosphorylate phosphatidic acid. This phosphomonoesterase activity shows a considerably high specific activity when an incubation medium at neutral pH containing 3 mM deoxycholate is used. The activity is inhibited by low concentrations of Ca^{2+}. The enzyme has no detectable phosphodiesterase activity under the conditions tested.

Intestinal alkaline phosphatase *Phosphatidic acid* *Phosphatidylinositol 4,5-diphosphate*

1. INTRODUCTION

Alkaline phosphatases (EC 3.1.3.1) of the intestine and placenta are nonspecific phosphomonoesterases since they hydrolyze various phosphomonoesters at similar rates. Phosphomonoesters of hydrophilic aliphatic and cyclic hydrocarbons, amino acids, sugars, nucleotides, vitamins and proteins are known as substrates [1–4]. The functional importance of this activity is not yet known. To our knowledge the complete dephosphorylation of phospholipid monophosphate esters by alkaline phosphatase with high specific activity is described here for the first time.

2. MATERIALS AND METHODS

Alkaline phosphatase purified from calf intestinal mucosa according to [5] shows a single band in polyacrylamide gel electrophoresis (PAGE) and a specific activity of 3000 IU/mg measured under standard reaction conditions: 5.32 mM *p*-nitrophenyl phosphate, 1.0 M diethanolamine-HCl, pH 9.8, 25°C. Protein determination was performed according to [6] using bovine serum albumin (Serva) as standard. Enzyme activity with 10 mM Tris-HCl, pH 7.4, 140 mM NaCl, 3 mM deoxycholate, 5.32 mM *p*-nitrophenyl phosphate, 37°C, was measured discontinuously. The enzyme activity was stopped at various times by addition of 0.5 ml of 2 N HCl per 1.0 ml test. The concentration of *p*-nitrophenol released was determined after neutralization with 2 N NaOH in 1.0 M diethanolamine-HCl, pH 9.8, at 405 nm.

Phosphatidylinositol 4,5-diphosphate (TPI) was purified from bovine brain according to [7] and showed small impurities of phosphate-free lipids in TLC. TPI was stored in benzol/ethanol (4:1, v/v) under nitrogen at −21°C and dried under nitrogen at 25°C before use. Phosphatidic acid prepared from egg yolk lecithin was 98% pure. Lyophilized phosphatidic acid was stored under nitrogen at −21°C.

Reaction conditions: unless stated otherwise, phospholipids were dissolved in the following incubation medium: 10 mM Tris-HCl, pH 7.4, 140 mM NaCl, 3 mM deoxycholate (DOC). Samples of 2.0 ml of 87 μM TPI or 37 μM phosphatidic acid were preincubated for 15 min at 37°C. Thereafter the reaction was started by addition of 5 μl alkaline phosphatase solution (3 mIU to 50 IU, measured under standard reaction conditions). After incubation for 30 min at 37°C the en-

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/85/$3.30

zyme activity was terminated by addition of 2.5 ml of 0.2 N HCl. Samples were placed in an ice/NaCl mixture. Products soluble in water or organic solutions were extracted by addition of 10.0 ml distilled chloroform/methanol (1:1, v/v), shaking and centrifugation at 3000 × *g* for 15 min. The resulting phases were neutralized with 1 N $NaHCO_3$ or ammonia and the phosphate content of each phase determined. Aliquots of organic phases were dried under nitrogen, reconstituted in benzol/ethanol (4:1, v/v) and spotted onto TLC plates.

One-dimensional TLC of phospholipids was performed on glass plates (20 × 20 cm) coated with 6 g silica gel (60 HR, reinst, Merck) with 15 mg potassium oxalate running with chloroform/methanol/25% ammonia/H_2O (90:70:12.5:9.5, v/v). Phosphatidylinositol was identified by comparison with reference standard (Serva).

Phosphate determination was performed according to Bartlett [8] or Martin and Doty [9].

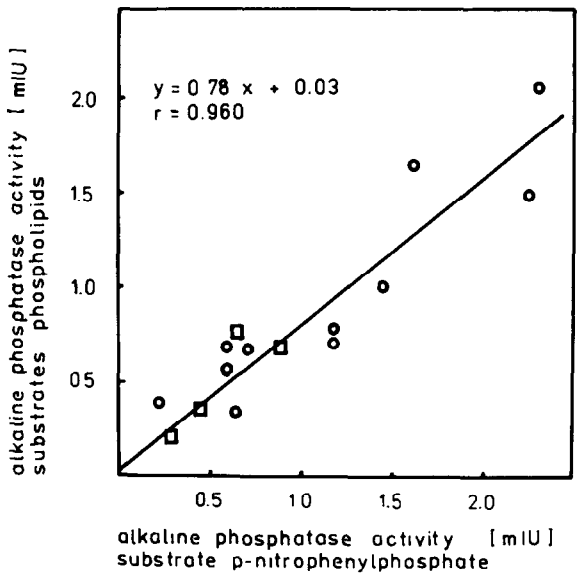

Fig.1. Hydrolysis of different substrates by alkaline phosphatase: correlation analysis. For reaction conditions see section 2. Substrate concentrations: TPI, 87 μM; phosphatidic acid, 37 μM; *p*-nitrophenyl phosphate, 5.32 mM. (○) TPI, (□) phosphatidic acid.

3. RESULTS

The results shown in table 1 indicate that two of the three phosphate groups of TPI are converted to water-soluble P_i after incubation with 0.5 IU alkaline phosphatase for 30 min. This ratio was not increased by the use of 50 IU enzyme. Phosphatidic acid is completely dephosphorylated by alkaline phosphatase. The ability of the enzyme to dephosphorylate completely phosphatidic acid and TPI under the same conditions excludes phosphatidic acid from being the product of hydrolysis of TPI. TLC experiments also demonstrate phosphatidylinositol to be the product of dephosphorylation of TPI by 50 IU alkaline phosphatase (not shown).

Comparison of alkaline phosphatase activity with *p*-nitrophenyl phosphate and phospholipids under the same reaction conditions (10 mM Tris-HCl, pH 7.4, 140 mM NaCl, 3 mM DOC, 37°C) shows a high correlation (fig.1). In these condi-

Table 1

Hydrolysis of TPI and phosphatidic acid by alkaline phosphatase

Substrate	Experiment	Phosphate soluble in	
		Water phase (%)	Organic phase (%)
TPI	without AP	0.34 ± 0.3	99.66 ± 0.3 (n = 8)
	0.5 IU AP	63.10 ± 4.3	36.90 ± 4.3 (n = 9)
Phosphatidic acid	without AP	2.50 ± 5.0	97.50 ± 5.0 (n = 4)
	0.5 IU AP	95.10 ± 5.0	4.90 ± 5.0 (n = 3)

For reaction conditions and phase separation see section 2. Alkaline phosphatase (AP) was measured under standard reaction conditions. Phosphate values: 100% = sum of total phosphate in the water and organic phases. The water-soluble phosphate after TPI hydrolysis is represented by P_i determined as in [9]

tions the enzyme dephosphorylates *p*-nitrophenyl phosphate and both phospholipids with similar specific activity, 139 and 110 IU/mg, respectively.

Among the detergent concentrations examined, 3 mM DOC produced the highest specific activity with TPI as substrate (table 2). The activity was considerably less when SDS was used and not detectable with Triton X-100 and without detergent. Enzyme denaturation by the detergents investigated could be excluded as the reason for the diminished enzyme activity.

Dephosphorylation of both phospholipids by alkaline phosphatase is inhibited at low Ca^{2+} concentrations (fig.2).

Table 2

Specific activity of alkaline phosphatase with TPI under several conditions

Detergent	Specific activity
1 mM DOC	10.6 IU/mg (n = 2)
3 mM DOC	103.5 ± 26.4 IU/mg (n = 11)
6 mM DOC	69.7 ± 10.5 IU/mg (n = 3)
3 mM SDS	22.6 IU/mg (n = 1)
0.1% Triton X-100	0 (n = 2)
Without detergent	0 (n = 2)

Reaction medium: 10 mM Tris-HCl, pH 7.4, 140 mM NaCl, 87 μM TPI, 37°C. For reaction conditions and phase separation see section 2

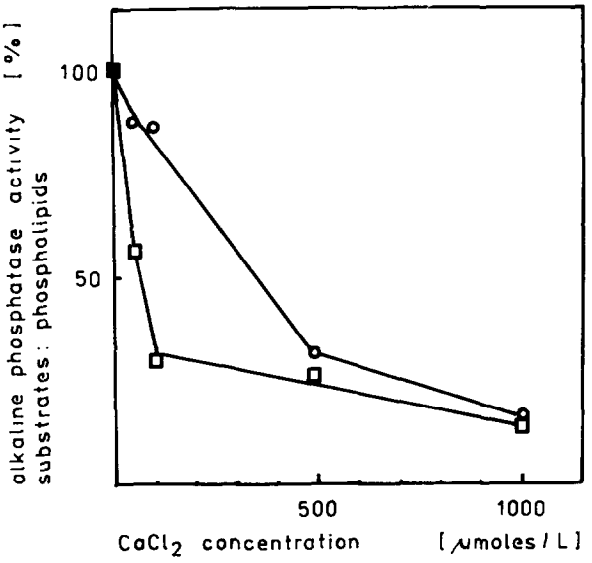

Fig.2. Hydrolysis of phospholipids by alkaline phosphatase at several Ca^{2+} concentrations. For reaction conditions and substrate concentrations see fig.1. 100% activity without $CaCl_2$: TPI, 1.34 mIU; phosphatidic acid, 0.76 mIU. (○) TPI, (□) phosphatidic acid.

4. DISCUSSION

To our knowledge, this is the first time that intestinal alkaline phosphatase has been shown to hydrolyze phospholipids. This activity of the enzyme occurs at neutral pH and 3 mM deoxycholate with a specific activity comparable with that of the hydrolysis of *p*-nitrophenyl phosphate. Our results correspond to the observation that alkaline phosphatase hydrolyzes nearly all phosphomonoesters, ROP, at similar rates regardless of the size or chemical nature of R because dephosphorylation of the E·P intermediate is rate-limiting [10]. The proven ability of alkaline phosphatase to hydrolyze TPI is not in contrast with the insignificant hydrolysis of this phospholipid by the enzyme reported by Dawson et al. [11] because they used non-optimal conditions without detergent.

Concentrations of deoxycholate producing high specific activity are similar to those found in bile [12] and chyme [13].

The mechanism of inhibition by Ca^{2+} is unknown, but may result from complex formation with the substrates. For quantitative interpretation of the results suitable data on complex formation constants are lacking.

The physiological importance of our findings has remained obscure until now. Alkaline phosphatase may be involved in P_i release from all P_i-containing nutrients, in the known rapid turnover of polyphosphoinositide phosphomonoesters or in splitting nutritive phospholipids of exogenous or endogenous origin.

ACKNOWLEDGEMENT

The kind gift of phosphatidic acid from Dr Rüstow (Charité Berlin) is greatefully acknowledged.

REFERENCES

[1] Harkness, D.R. (1968) Arch. Biochem. Biophys. 126, 513–523.
[2] Huang, K.P., Robinson, J.C. and Chou, J.Y. (1976) Biochem. Biophys. Res. Commun. 70, 186–192.
[3] Van Belle, H. (1976) Gen. Pharmacol. 7, 53–58.
[4] Fishman, W.H. (1974) Am. J. Med. 56, 617–650.
[5] Horn, A., Bublitz, R., Fernandez Yero, J.L., Schulze, M. and Ehle, H. (1983) Prog. Clin. Enzymol. 2, 255–259.
[6] Bradford, M.M. (1976) Anal. Biochem. 72, 248–254.
[7] Kiselev, G.V. (1982) Biochim. Biophys. Acta 712, 719–721.
[8] Bartlett, G.R. (1978) J. Chromatogr. 152, 569–576.
[9] Martin, J.R. and Doty, D.M. (1949) Anal. Chem. 21, 965–978.
[10] Coleman, J.E. and Gettins, P. (1983) Adv. Enzymol. 55, 381–452.
[11] Dawson, R.M.C. and Thompson, W. (1964) Biochem. J. 91, 244–250.
[12] Carey, M.C., Small, D.M. and Bliss, C.M. (1983) Annu. Rev. Physiol. 45, 651–677.
[13] Kuksis, A. (1984) in: Intestinal Toxicology (Schiller, C.M. ed.) pp.69–114, Raven, New York.