PHOSPHOPROTEIN-PHOSPHATASE ACTIVITY ASSOCIATED WITH HUMAN PLACENTAL ALKALINE PHOSPHATASE

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SUMMARY. Human placental alkaline phosphatase, a marker protein for some nontrophoblastic neoplasms, was found to have phosphoprotein phosphatase activity. This was demonstrated by the dephosphorylation of ^{32}P-labeled histones, protamine, glycogen synthetase, casein, and phosvitin at various pH values. Unlike the general phosphoprotein phosphatase, the placental alkaline phosphatase does not have phosphorylase a phosphatase activity.

Placental proteins such as placental lactogen, chorionic gonadotrophin, and alkaline phosphatase (EC. 3.1.3.1) are normally present in the serum of pregnant women. However, their presence in the serum of a man or nonpregnant woman indicates neoplasm (1). Many nontrophoblastic tumors and tumor-derived cell lines synthesize these placental proteins. It has been suggested that malignant transformation results in the derepression of the genome for these proteins (2). On the other hand, synthesis of placental alkaline phosphatase is repressed in cells derived from choriocarcinoma, a neoplasm of placenta (3,4). Induction of placental alkaline phosphatase in choriocarcinoma cells can be achieved by addition of 5-bromodeoxyuridine, or a variety of other compounds, to the culture (3,4). Because of the interesting phenomenon of the repression and derepression of placental alkaline phosphatase as related to choriocarcinoma and many nontrophoblastic tumors, we examined this enzyme for its phosphoprotein-phosphatase activity. Phosphoprotein-phosphatase is thought to play a significant role in regulating cellular metabolism and gene expression (5). In the present study, we report that placental alkaline phosphatase is indeed a phosphoprotein phosphatase.

MATERIALS AND METHODS Calf thymus histone, type II-A (histone mixture) and type III-S (lysine-rich fraction); protamine; and phosvitin were purchased from Sigma Chemical Company. [γ-^{32}P]ATP was obtained from New England Nuclear Corporation. Vitamin-free casein (Nutritional Biochemicals) was suspended in water according to the method of Reimann, et al. (6). Human placental alkaline phosphatase was purified and assayed according to the method of Sussman, et al. (7). Cyclic AMP-dependent protein kinase from rabbit muscle was purified according to the method of Yamamura, et al. (8); the muscle protein-kinase B_1 fraction was used. Phosvitin kinase from the same tissue was prepared according to the method of Goldstein and Hasty (9). ^{32}P-Labeled proteins were prepared by incubation of the protein substrates with cyclic AMP-dependent protein kinase (for preparing ^{32}P-histones and ^{32}P-protamine) or phosvitin kinase (for preparing ^{32}P-phosvitin and ^{32}P-casein) as described previously (10). ^{32}P-Labeled glycogen synthetase was prepared by incubation of I-form enzyme with both cyclic AMP-dependent protein kinase and phosvitin kinase (11). ^{32}P-Phosphorylase a was kindly provided by Dr. F. W. Huang of this laboratory. Phosphoprotein phosphatase activity was measured at 37° in a reaction mixture of 25 µl containing 200 mM sodium acetate, Tris-Cl, or 2-amino-2-methyl-1-propanol; 0.04 to 0.8 mg/ml of ^{32}P-labeled protein (2 to 6 x 10^4 CPM); and 0.2 to 2 µg of purified alkaline phosphatase (specific activity of 400 U/mg with p-nitrophenylphosphate). The reaction was stopped by the addition of 5 µl glacial acetic acid, and $^{32}P_i$ was separated from ^{32}P-protein by the ITLC-chromatography method of Huang and Robinson (10).

RESULTS AND DISCUSSION At alkaline pH placental alkaline phosphatase hydrolyzes a variety of compounds having the phosphomonoester linkage (12). However, when phosphoproteins served as substrate, the optimum pH values for these reactions varied broadly, ranging from acid to alkaline (Fig. 1). The optimum pH for the hydrolysis of the acidic proteins, ^{32}P-phosvitin and ^{32}P-casein, was between 5 and 6. The optimum pH for the hydrolysis of the basic proteins, ^{32}P-histones, was very broad, extending between 6 and 10. The optimum pH for the hydrolysis of a very basic protein, ^{32}P-protamine, was 11.5, which was similar to the optimum pH when p-nitrophenylphosphate was used as substrate. The wide variation in the optimum pH for the hydrolysis of various protein substrates by alkaline phosphatase may reflect the accessibility of the phosphate on the substrate and the state of ionization of the substrate at various pH values.

^{32}P-Phosphorylase a which is readily hydrolyzed by rabbit muscle phosphoprotein phosphatase, was, in contrast to the phosphoproteins tested, resistant to alkaline phosphatase throughout the entire pH range

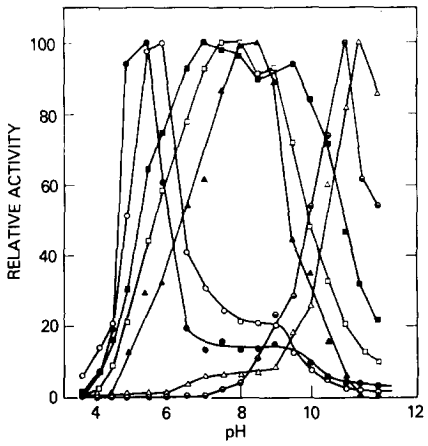

Fig. 1. Dependence of phosphoprotein phosphatase activity on pH. The buffers used were sodium acetate (from pH 3.5 to 6.5), Tris-Cl (from pH 7.0 to 9.0), and 2-amino-2-methyl-1-propanol (from pH 9.5 to 12.0). The substrates were; ^{32}P-casein (-O-); ^{32}P-histone mixture (- ■ -); lysine-rich ^{32}P-histone (- □ -); ^{32}P-phosvitin (- ● -); ^{32}P-glycogen synthetase (- ▲ -); ^{32}P-protamine (- △ -); p-nitrophenyl phosphate (- ◐ -).

(from 3.5 to 12). It seems that the phosphoprotein-phosphatase activity associated with placental alkaline phosphatase is different from the general phosphoprotein-phosphatase (13,14) which hydrolyzes a variety of phosphoproteins including phosphorylase a. Furthermore, the hydrolysis of ^{32}P-glycogen synthetase by general phosphoprotein phosphatase is activated by divalent cations (Mn^{++}, Mg^{++}, and Ca^{++}), whereas the dephosphorylation by alkaline phosphatase is not affected by these metal ions.

In order to demonstrate that the phosphoprotein-phosphatase activity is associated with alkaline phosphatase, the enzyme was subjected to disc gel electrophoresis. Fig. 2 shows that enzyme activity for the hydrolysis of p-nitrophenylphosphate coincides with all the phosphoprotein-phosphatase activities. Furthermore, all the phosphatase activities were associated with the major protein band of the gel. These experiments show that placental alkaline phosphatase indeed has phosphoprotein-phosphatase activity.

The extent of dephosphorylation of ^{32}-labeled proteins by alkaline phosphatase is illustrated in Fig. 3. After prolonged incubation, over

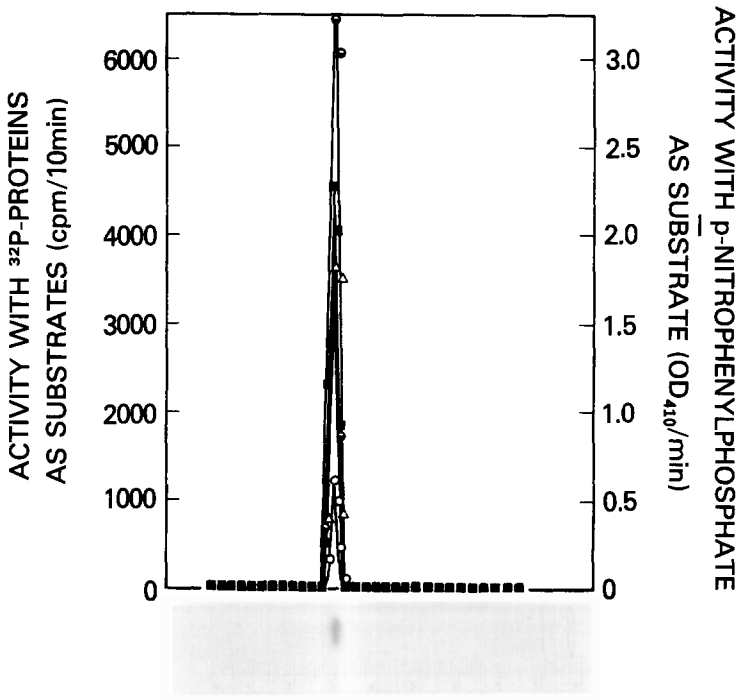

Fig. 2. Disc gel electrophoresis of the purified placental alkaline phosphatase and the measurement of phosphatase activities in the fractionated gel. The electrophoresis was carried out according to the method of Davis (15). After electrophoresis the gel was fractionated into 1-mm fractions in a gel slicer, and the fractions were assayed for phosphatase activities at the optimum pH for each substrate. The duplicate gel was stained for protein with Coomassie blue. The symbols for the phosphatase activities are the same as those shown in Fig. 1.

80 percent of the phosphate from ^{32}P-labeled protamine, histones, glycogen synthetase, and phosvitin was released; however, only approximately 60 percent of $^{32}P_i$ was released from ^{32}P-casein. We analyzed the acid lability (0.1 N HCl at 100° for 15 min) and alkali lability (0.1 N NaOH at 100° for 15 min) of the phosphate of these proteins and concluded that only those phosphates which were alkali-labile but acid-stable, characteristic of serine and threonine phosphates, could be hydrolyzed. Although phosphorylase a also contains serine phosphate, it cannot be dephosphorylated by placental alkaline phosphatase. The phosphate site on phosphorylase a may be in a conformation that it is unaccessible to the phosphatase.

Kinetic constants of the phosphatase for a variety of substrates are shown in Table 1. The K_m values for the protein substrates were in

TABLE 1. K_m and V_{max} Values for Placental Alkaline Phosphatase Using Phosphoproteins as Substrates

	Histone (mixture)	Histone (lysine-rich)	Protamine	Glycogen Synthetase	Casein	Phosvitin
K_m (mg/ml)	0.16	0.4	0.02	0.02	0.1	0.02
K_m (μM) [a]	10	25	5.0	0.23	4.3	0.47
V_{max} (nmole/min/mg)	25	16.5	7.5	6.3	5.5	1.43

[a] Assumes that the average subunit molecular weight of histone is 16,000; protamine, 4,000; glycogen synthetase, 85,000; casein, 23,000; and phosvitin, 42,000.

The reactions were carried out under standard assay conditions at the optimum pH for each substrate: Tris-Cl, pH 8.0, for histones and glycogen synthetase; sodium acetate, pH 5.0, for phosvitin; sodium acetate, pH 5.8, for casein; and 2-amino-2-methyl-1-propanol, pH 11.4, for protamine.

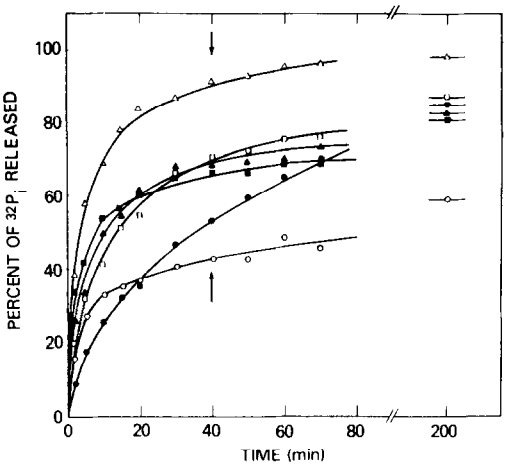

Fig. 3. Extent of dephosphorylation of ^{32}P-labeled proteins by purified alkaline phosphatase. The reactions were carried out under standard assay conditions in a volume of 0.125 ml with 20 µg of enzyme at the optimum pH for each substrate. At timed intervals 10-µl samples were withdrawn for the determination of $^{32}P_i$ released. Arrow indicates that 10 µg phosphatase was again added to the reaction mixtures. The symbols for the ^{32}P-protein substrates are the same as those shown in Fig. 1.

the µM range. The V_{max} values of the phosphatase using ^{32}P-histone and ^{32}P-glycogen synthetase as substrates are comparable to those of partially purified phosphoprotein phosphatase from rabbit muscle (13).

Since histones and glycogen synthetase are substrates of placental alkaline phosphatase at physiological pH, this enzyme might be of significance in the regulation of glycogen synthesis and gene transcription in mammalian tissue.

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