PHOSPHOPROTEIN PHOSPHATASE ACTIVITY OF THE PROGESTERONE-INDUCED PURPLE GLYCOPROTEIN OF THE PORCINE UTERUS

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SUMMARY. The iron-containing, progesterone-induced, purple glycoprotein from the pig uterus catalyzes the hydrolysis of phosphate groups from phosphvitin. This enzyme which can be purified in large amounts, has a pH optimum at 4.6 and an apparent K_m of 0.1 mM for bound phosphate. Its V_{max} with phosphvitin as substrate is about 200, μmoles Pi released/min/μmole enzyme and is at least twenty-fold lower than its activity towards p-nitrophenylphosphate. The enzyme is activated by 2-mercaptoethanol and inhibited by molybdate, fluoride and arsenate. The resemblance of this protein to a number of other acid phosphatases of similar substrate specificity in other organisms, suggests that a broad class of such enzymes might exist in nature.

INTRODUCTION. Earlier we have described the purification of a purple-colored, basic glycoprotein from the uterine flushings of ovariectomized sows administered progesterone (1). This protein contains one atom of iron per 32,000 molecular weight polypeptide and has phosphatase activity towards p-nitrophenylphosphate, pyrophosphate and ATP but only negligible activity towards 2-glycerophosphate and glucose 6-phosphate (2). The enzyme is activated by reducing agents such as 2-mercaptoethanol and L-ascorbate. We have also demonstrated that the protein accumulates in large amounts in the pregnant uterus (3) and we have provided evidence that it is transported across the placenta at specialized regions called areolae (4). Nevertheless, its biological function remains unknown. Recently there have been two reports of steroid-induced increases in phosphoprotein phosphatase activity in specific target organs (5,6), including the uterus (5), and there has been speculation that steroid hormones might exert some of their biological effects via the action of such enzymes. Since the purple uterine protein also resembles a phosphoprotein phosphatase isolated from beef spleen, in its spectral properties, iron content and enzymatic activity (7-12), we have

examined the ability of the uterine enzyme to hydrolyze phosphate groups from phosphvitin.

MATERIALS AND METHODS. The purple protein was purified to homogeneity from uterine flushings of ovariectomized sows administered daily doses of progesterone (2). Assays were normally carried out in 1 ml of 0.1 M sodium acetate buffer, pH 4.9 at 37° containing either phosphvitin (1mg/ml) or other phosphoproteins (5mg/ml). Enzyme (1 to 10 µg) was added and the reaction allowed to proceed for 10 min. The reaction was stopped by adding either 1 ml of silicotungstic acid (1 mg/ml) or 1 ml trichloracetic acid (15% w/v). Inorganic or total organic phosphate was measured by the methods of Bartlett (13). The hydrolysis of p-nitrophenylphosphate was assayed as described previously (2) by incubating 0.1 to 0.5 µg of enzyme with 12 mM substrate at 37° and pH 4.9 for either 5 or 10 min.

The activated (pink) form (2) of the induced phosphatase was prepared by treating the purified native (purple) form with 0.1M 2-mercaptoethanol for 10 min. It was then separated from the reducing agent on a short column of Bio-gel P-2 or by dialysis.

RESULTS. Using 10 µg of enzyme and 1 mg/ml of phosphvitin the release of orthophosphate was linear for at least 30 min. In the particular experiment shown in Fig. 1A, the rate of reaction was about 3.0 µmoles orthophosphate released/min/mg of protein. A linear relationship was also obtained between the rate of the reaction over 10 min and the amount of enzyme added (up to 10 µg) (Fig. 1B). After treatment of the enzyme with 0.1 M mercaptoethanol for 10 min, this rate of reaction approximately doubled, whereas pretreatment with the mild oxidizing agent potassium ferricyanide (10^{-2}M) reduced the activity three to four fold. Similar results have been described earlier using p-nitrophenylphosphate as substrate (2). However, with p-nitrophenylphosphate the maximum velocity of the reaction was about 20 times greater than for the rate of release of phosphate from phosphvitin. It has been assumed that the enzyme as isolated from uterine flushings is probably a

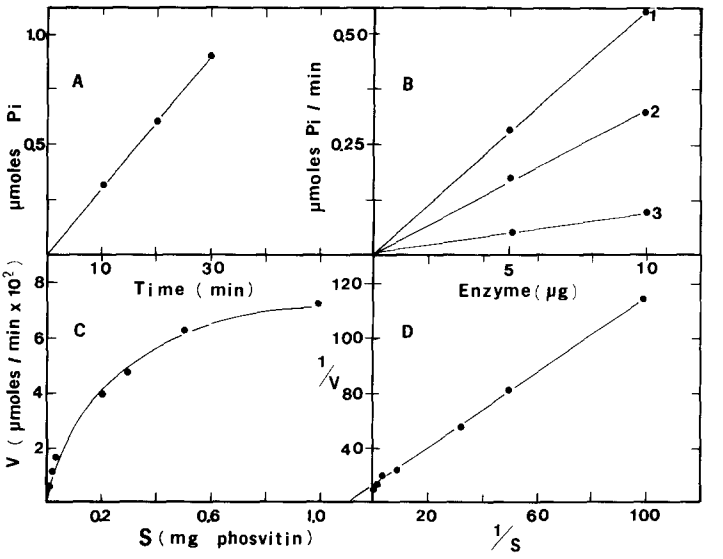

Fig. 1 Phosphoprotein phosphatase activity of the purple protein from the porcine uterus

A. Linearity of reaction with time under standard assay conditions.

B. Effect of increasing amounts of enzyme on release of inorganic phosphate from phosphvitin (1 mg/ml); native enzyme as purified (2); enzyme pretreated with 10^{-1}M 2-mercaptoethanol for 10 min (1); enzyme pretreated with 10^{-3}M potassium ferricyanide for 10 min (3).

C. Relationship between phosphvitin concentration and initial rate of inorganic phosphate release. The enzyme concentration was 10 µg/ml.

D. Lineweaver-Burke plot of results presented in 1C.

mixture of reduced and oxidized forms, with the former being most active (2). The activation by mercaptoethanol is also accompanied by a pronounced spectral shift in the absorption maximum in the visible range from 545 nm to 508 nm (2). Because of this, the protein assumed a pink coloration in presence of mercaptoethanol.

The pH optimum with phosphvitin as substrate was about 4,6 with no measurable activity above pH 6.0. This optimum is slightly lower than that observed towards p-nitrophenylphosphate which is at pH 4.9 (2). Using mercaptoethanol-activated enzyme a normal hyperbolic relationship was observed

between substrate concentration and initial reaction rate (Fig. 1C). The K_m was calculated to be 0.1 mg/ml for phosphvitin which is approximately 0.1mM for protein-bound phosphate (Fig. 1D). This assumes of course, that all of the phosphate groups are equally susceptible to hydrolysis. This value is significantly lower than the K_m for p-nitrophenylphosphate (0.1mM versus 2.2 mM (2)). It is difficult to compare this activity with that of other steroid-induced, phosphoprotein phosphatases that have been described recently (5,6), since for the most part they have not been purified and activity has usually been measured by the release of ^{32}P from labelled substrates that have been prepared by means of protein kinase. The V_{max} for the purple phosphatase is very low, approximating to a turnover number of only about 200 for the mercaptoethanol-treated enzyme. This is comparable, however, to the activity of the phosphoprotein phosphatase from beef spleen towards α-casein (8). The uterine enzyme has less than one-tenth this activity towards α-casein and ovalbumin.

Arsenate, molybdate and fluoride all inhibited activity towards phosphvitin as well as the hydrolysis of p-nitrophenylphosphate (Table 1). Zinc ions which typically inactivate phosphoprotein phosphatase activity (5,14,15) also inhibit. Mercuric chloride interestingly had no effect on phosphvitin hydrolysis when it was added to the substrate prior to addition of enzyme, whereas it was a very potent inhibitor of p-nitrophenylphosphatase activity. We attribute this to protection of the enzyme by the protein substrate since it can also probably bind Hg^{2+}. The metal chelators disodium EDTA and bipyridine did not inhibit activity at concentrations up to $10^{-3}M$.

In conclusion, therefore, the progesterone-induced phosphatase isolated from the pig uterus has well defined phosphoprotein phosphatase activity. Nevertheless some caution seems necessary before interpreting that this activity is related to its function in the early pregnant uterus. Such large quantities (3) of the enzyme are secreted that it is difficult to imagine it having a purely enzymatic role. Further, a number of other acid phosphatases

TABLE 1

The Effects of Various Additives on Phosphoprotein Phosphatase and P-Nitrophenylphosphatase Activities.

Additive	Concentration	% Inhibition Phosphoprotein Phosphatase[1]	% Inhibition p-Nitrophenyl Phosphatase[1]
None	—	0	0
Na arsenate	10^{-3}M	50	73
Na molybdate	10^{-5}M	77	86
Na molybdate	10^{-6}M	30	32
Na fluoride	10^{-3}M	14	75
$MgCl_2$	10^{-4}M	0	0
$MgCl_2$	10^{-4}M	0	0
$HgCl_2$	10^{-6}M	0	70
$ZnCl_2$	10^{-3}M	65	58
$ZnCl_2$	10^{-4}M	13	26
diNa EDTA	10^{-3}M	0	0
bipyridine	10^{-3}M	0	0

[1]Assayed at pH 4.9

including those from the human prostate and intestine (16), bovine milk (17) and spleen (8) and rat liver mitochondria (18) will hydrolyze phosphoproteins. Other phosphatases may well do so but have not been tested. Several of these phosphoprotein phosphatases which have been partially or wholly purified resemble the purple uterine protein in one or more characteristics such as relatively small size, poor activity towards glycerophosphate, purple or violet color, iron content, relatively high basicity and activation by thiols, suggesting a possible common mode of catalysis. In most cases only very small amounts of the enzymes have been purified. Since we can purify up to 0.5g of the uterine protein from a single animal, it may provide not only a useful

model for studying the induction of a well-characterized protein by progesterone in a mammal, but also a source of homogeneous protein for studies on this particular class of phosphatases which may be more abundant in nature than previously suspected.

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