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RYE GERM ACID PHOSPHATASE: PROPERTIES OF THE ENZYME AND ITS ACTIVATION BY LECTINS

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Key Word Index—*Secale cereale*; Gramineae; rye germ; acid phosphatase; lectin; ConA*.

Abstract—Acid phosphatase (EC 3.1.3.2) from rye germs is a glycoprotein of M_r 90 000 with subunit structure. The pH optimum for *p*NPP hydrolysis is 5.4. The best substrates for the enzyme are *p*NPP, PP_i and ATP. In the presence of plant lectins an increase in AcPase activity was found. ConA causes a 20% decrease of K_m^{app} and a 50% increase of V_{max}^{app} with *p*NPP as substrate.

INTRODUCTION

Acid phosphatases are considered to play a role in the mobilization of phosphorus reserves during germination [1, 2]. Although these enzymes have been purified from several plants [3–7], little is known about those localized in cereal germs. In rye germ, acid phosphatase is more abundant than in any other part of the seed.

Lectins are particularly abundant in germs of Gramineae seeds [8] and as plant acid phosphatases are glycoproteins, their binding to lectins is not surprising. However, the change in hydrolytic activity, which was observed for AcPases from grass seeds and potato tubers in the presence of lectins [9–11], is of great interest and suggests the possibility of a regulatory role for lectins.

In the present paper we report the characteristics of rye germ acid phosphatase and modification of its activity by several plant lectins.

RESULTS AND DISCUSSION

Purification and molecular properties

The purification procedure described in this paper enables isolation of the two main forms of AcPase. The partially purified AcPase has two active bands in PAGE at pH 4.5 and a single band at pH 9.5. In isoelectric focussing two zones with enzymatic activity were found with isoelectric points between 5.4–5.6 and 6.0–6.4, respectively. The less acidic zone shows additional microheterogeneity. The M_rs of the two forms occurring in PAGE at pH 4.5 were estimated as 35 000 and 60 000. The two main protein bands in SDS–PAGE also show M_rs of 30 000 and 60 000, although trace contaminants are present in the gels. In gel filtration, AcPase occurs as a single peak with the elution volume corresponding to M_r 90 000 ± 5000. We assume that in acidic PAGE conditions AcPase dissociates into two active forms. The dimeric form of the enzyme is the glycoprotein containing about 9% neutral sugars, similar to the AcPases isolated from sweet potato bulbs [3] and soybean [12]. Pentoses compose 25% of the carbohydrate part of the enzyme. Such a high content of pentoses seems to be typical for AcPases from higher plants [6, 7, 13].

Catalytic properties

Rye germ AcPase hydrolyses *p*NPP with an optimal pH at 5.4; The relative activity towards different phosphorylated compounds (expressed as a percentage of that for *p*NPP assumed as 100%) is: phenylphosphate 120%, α-naphthylphosphate 25%, α-glycerophosphate 20%, β-glycerophosphate 30%, glucose-6-phosphate 10%, FDP 25%, phytic acid 7%, ATP −80%, ADP −40% and bis-*p*NPP 2%. The broad substrate specifity of the enzyme is similar to that of other plant AcPase [1, 2, 5, 12, 14]. The enzyme is efficient with *p*NPP, ATP and PP_i, as the K_{cat}/K_m ratio is relatively high in these cases (Table 1). Phytate is a rather weak substrate for rye germ AcPase. Comparing our results with those of Yamagata *et al.* [1] and Tamura *et al.* [2] we suggest that AcPases show tissue and organ specificity. The enzymes isolated from coty-

Table 1. Approximate kinetic values for some AcPase substrates

Phosphoric compound	K_m (mM)	V_{max} (nM P_i)	K_{cat} $\left(\frac{\text{nM } P_i}{\text{nME·min}}\right)$	$\frac{K_{cat}}{K_m}$
*p*NPP	0.98	48.7	919	938
β-Glycero-phosphate	2.90	18.1	342	118
PP_i	1.00	39.9	753	753
FDP	0.54	10.1	190	352
ATP	0.84	35.5	670	798
Phytate	5.54	5.2	98	18

*Abbreviations used: AcPase, acid phosphatase; *p*NPP, *p*-nitrophenyl phosphate; *p*NP, *p*-nitrophenyl; PP_i, inorganic pyrophosphate; P_i, inorganic phosphate; FDP, fructose 1,6-biphosphate; FDP, fructose 1,6-biphosphate; PAGE, polyacrylamide gel electrophoresis; *St*A, *Solanum tuberosum* agglutinin; ConA, concanavalin A; LL, lentil lectin; SBA, soybean agglutinin; WGA, wheat germ agglutinin; RGA, rye germ agglutinin.

ledons and aleurone particles are more active towards phytate, which is stored in these tissues.

AcPase inhibitors.

The heavy metal ions Cu^{2+}, Hg^{2+} and Zn^{2+} are uncompetitive inhibitors of rye germ AcPase, and inhibition constants (K_i) are calculated to be: 26, 32 and 155 μm, respectively. Phosphate as well as tartrate and molybdate are competitive inhibitors of the phosphatase. The inhibition constants (K_i) are 90, 1600 and 1.6 μm, respectively. The loss of enzyme activity in the presence of iodoacetamide and *p*CMB suggests a role for free –SH groups in supporting the active conformation or in the catalytic process.

Lectins as AcPase activators

The AcPase in crude rye germ extract is accompanied by agglutinating activity. The separation of AcPase from the agglutinating protein causes a significant loss of enzyme activity. The addition of crude endogenous lectin preparation to the AcPase causes a gradual increase of enzyme activity (Fig. 1).

The purified AcPase from rye germs is activated by several plant lectins: WGA, SBA, LL, ConA and *St*A (Fig. 2). The increase of activity ranges from 15 to 55% and requires excess lectin. The addition of monosaccharide specifically interacting with the lectin to the reaction mixture reverses the enzyme activation (Fig. 3). This indicates that the carbohydrate part of the enzyme is engaged in the process of activation. A similar activation was observed previously in our laboratory for AcPases from grass seeds which are stimulated by ConA and LL [9, 10] and for potato AcPase, which is activated by *St*A [10].

In the presence of ConA the rye germ AcPase is less sensitive to thermal inactivation. Similar effects are found when the influence of pH on the interaction is examined (Fig. 4). There is no shift in pH optimum. At pH 3.5 AcPase is 3-fold higher if ConA is added to the medium. At pH 7.0 activation reaches 60% and it is weakest at the pH optimum. These features suggest a protective action of lectin, perhaps by stabilization of the most active enzyme conformation. The presence of ConA changes the main

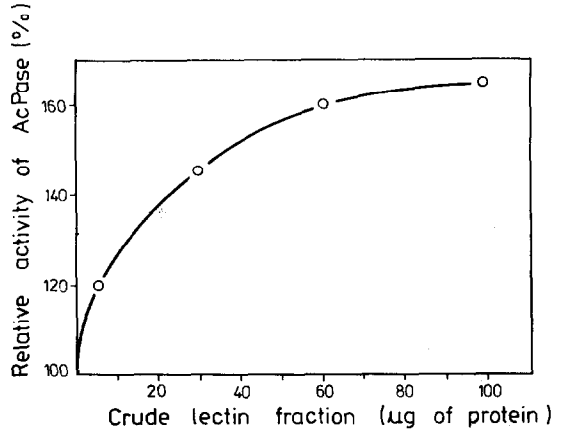

Fig. 1. Activation of rye germ AcPase in the presence of crude RGA. For details see the Experimental section.

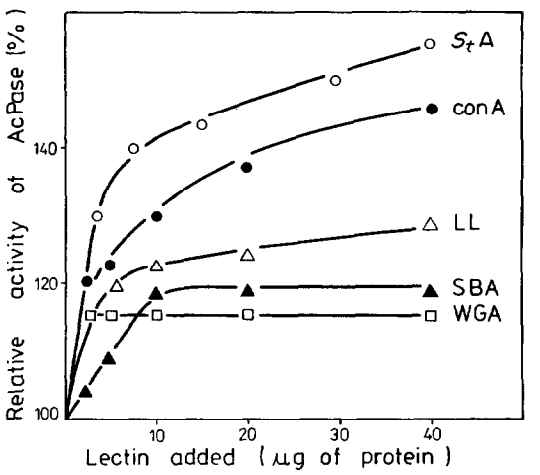

Fig. 2. Activation of rye germ AcPase caused by lectins. ○, AcPase – *St*A; ●, AcPase – ConA; △, AcPase – LL; ▲, AcPase – SBA; □, AcPase – WGA.

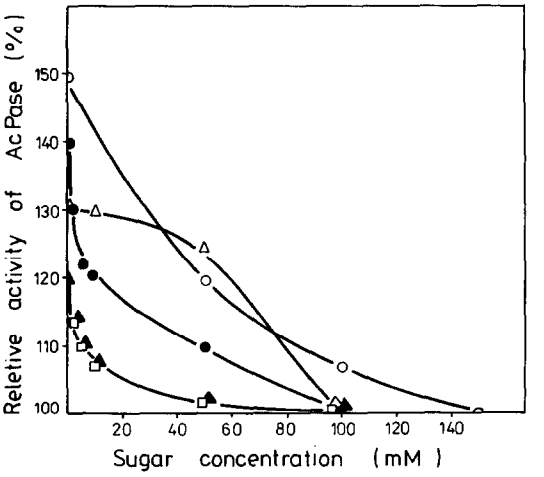

Fig. 3. Changes in AcPase activation caused by specific monosaccharides. ○, AcPase – *St*A + GlcNAc; ●, AcPase – ConA + Glc; △, AcPase – LL + Glc; ▲, AcPase – SBA + GalNAc; □, AcPase – WGA + GlcNAc. In the controls containing the enzyme and carbohydrates, no effect on the AcPase activity was observed.

kinetic values of *p*NPP hydrolysis. The K_m^{app} value towards *p*NPP decreases to 0.77 mM (*ca* 20%) and the increase of V_{max}^{app} reaches 40%. The decrease of K_m^{app} caused by lectin was also reported for AcPases from *Poa pratensis* [9] and potato [15].

EXPERIMENTAL

Analytical. Protein was determined according to ref. [16], neutral sugars according to ref. [17] and pentoses according to ref. [18]. AcPase activity was estimated in 0.1 M NaOAc buffer at pH 5.1 at 37° using 5 mM *p*NPP as substrate in a total vol. of 2 ml. After 10 min incubation the reaction was stopped by addition of 10 ml 0.1 M NaOH. The amount of *p*NP was measured spectrophotometrically at 410 nm. The enzyme activity with other substrates was estimated in a 2 ml reaction mixture

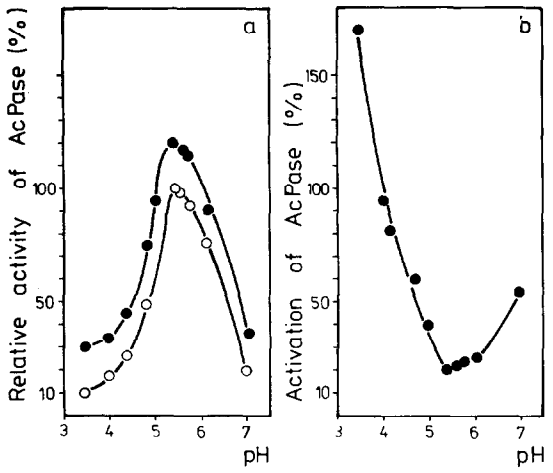

Fig. 4. The effect of pH on AcPase activation caused by ConA. (a) The pH optimum curve without ConA (○) and in the presence of the lectin (●). (b) Activation of AcPase with ConA in different pH conditions.

containing 5 mM substrate, incubated for 30 min at 37°. The reaction was stopped by addition of 2 ml of 5% TCA and the released P_i was determined by the method of ref. [19].

Electrophoresis. PAGE was performed according to ref. [20] at pH 4.5 in 15% gel, according to ref. [21], at pH 9.5 in 10% gel, and according to ref. [22] with SDS in 9% gel. Proteins were stained with Coomassie Brilliant Blue R 250. The method of ref. [23] was used for glycoprotein staining. The enzyme activity was localized in gels using the diazo-coupling method with α-naphthylphosphate as substrate and Fast Blue B salt. Isoelectric focussing was performed in a thin layer of 5% polyacrylamide gel containing 6% of ampholine, with a pH-gradient from 4.1 to 7.6.

Purification procedure: *Step* 1. Proteins were extracted from germ flour by shaking for 2 hr at room temp. with 10 vol. of 0.1 M NaOAc buffer pH 5.1 (buffer A). The extract was centrifuged at 4000 *g* for 30 min at +5°. The supernatant was precipitated with solid $(NH_4)_2SO_4$ to 60% satn. The pellet after centrifugation was dissolved in buffer A. *Step* 2. The supernatant from step 1 was precipitated with cold EtOH to 60% concn and stored overnight. The pellet after centrifugation was dissolved in buffer A, and dialysed overnight in the same buffer. *Step* 3. The material after dialysis was applied to a SP-Sephadex G-50 column (7 × 2.5 cm) equilibrated with buffer A. The active fractions were eluted with 0.5 M NaOAc buffer pH 5.1, dialysed against H_2O and then to 0.1 M NaOAc buffer (pH 5.8) containing Mg^{2+}, Mn^{2+} and Ca^{2+} at 1 mM (buffer B). *Step* 4. Roughly purified AcPase was subjected to affinity chromatography on a ConA–Sepharose 4B column (7 × 0.5 cm) equilibrated with buffer B. The active fractions were eluted with 5% α-methyl D-glucoside. After dialysis, the enzyme prepn was freeze-dried. *Step* 5. The protein was dissolved in buffer A and passed through a Bio-gel P-150 column (100 × 0.8 cm) equilibrated with the same buffer. The first of the two peaks contained AcPase activity. The active fractions were pooled, dialysed and freeze-dried.

Estimation of M_r. Gel filtration was performed on a Bio-gel P-150 column. The marker proteins used for column calibration were haptoglobin (90000), bovine serum albumin (68000), ovalbumin (45000), chymotrypsinogen from bovine pancreas (25000) and cytochrome *c* from equine heart (12500). The M_rs of the two AcPase forms observed in PAGE at pH 4.5 were estimated according to ref. [24]. The marker proteins were equine myoglobin (17800), trypsinogen from bovine pancreas (24000), ovalbumin and bovine serum albumin (monomer 68000, dimer 136000). Standard proteins for SDS–PAGE were phosphorylase *b* from rabbit muscle (94000), bovine serum albumin, ovalbumin, carbonic anhydrase from bovine erythrocytes (30000), soybean trypsin inhibitor (20100) and bovine α-lactalbumin (14400).

Other experiments. Substrate concn in all experiments ranged from 1 to 5 mM. Apparent K_m and V_{max} values were calculated from the equation in ref. [25]. The inhibition constants were estimated to the presence of 1–5 mM *p*NPP and variable inhibitor concn.

In all lectin–AcPase interaction experiments 0.5 μg AcPase and 5–50 μg of ConA or other lectins were preincubated for 15 min at 37° before substrate addition. In K_m^{app} estimations substrate concn from 1 to 5 mM were used.

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