

## Acid phosphatase from stored *Poa pratensis* caryopses and its ability for binding to lectins

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### Abstract

The effect of the storage period of *Poa pratensis* caryopses on acid phosphatase activity and on the ability of this enzyme to interact with lectins has been studied. It has been shown that after ten years of caryopses storage, the activity of acid phosphatase decreased about 50 per cent, while the content of proteins and carbohydrates did not change. The decrease of enzyme activity during the long period of storage was found only in seeds, but not in chaffs. Acid phosphatase was isolated from caryopses stored one, two, three, five and ten years. The enzyme showed the ability to bind to immobilized as well as to free conA during the whole period of storage, but did not react with Wheat Germen Agglutinin (WGA). The activation of acid phosphatase by binding to conA decreased with the length of storage period.

*Key words:* Acid phosphatase, lectins, stored caryopses, *Poa pratensis*.

### INTRODUCTION

It has been shown that acid phosphatases of grass caryopses are glycoproteins (Wieczorek et al. 1977, Lorenc-Kubis and Morawiecka 1980) with the ability to interact with lectins, especially with conA (Lorenc-Kubis and Bøg-Hansen 1981, Lorenc-Kubis et al. 1982) and that the enzymes are activated or inhibited by binding to lectins (Lorenc-Kubis et al. 1981). The acid phosphatases from grass seeds differ in the number of polymorphic forms. The occurrence of multiple forms has been shown by gel electrophoresis (Morawiecka et al. 1976, Lorenc-Kubis et al. 1975, Wieczorek et al. 1980), chromatography (Wieczorek et al. 1978, Lorenc-Kubis 1983), and immunoelectrophoresis (Lorenc-Kubis and Bøg-Hansen 1981, Lorenc-Kubis et al. 1982). Acid phosphatase

tases show a great variability in the germination process of caryopses. Wieczorek et al. (1975) showed a distinct increase of acid phosphatase activity in the initial part of the germination period of barley seeds. The changes in the enzymatic activity were accompanied by an increase of enzyme fractions visible in polyacrylamide gel electrophoresis. On the other hand, little is known about the activity of acid phosphatases and their ability to interact with lectins during the period of caryopses storage.

In the present paper acid phosphatase was isolated from *Poa pratensis* caryopses stored from one to ten years. The activity, multiple forms and interaction of the enzyme with lectins have been investigated.

## MATERIALS AND METHODS

### PLANT MATERIAL

The investigations were performed on *Poa pratensis* caryopses (variety Skrzyszowicka) from the 1972, 1974, 1975, 1977, 1979, 1980 and 1981 harvest. The caryopses were stored from 1 to 10 years at 20°C. Ground caryopses, seeds and chaffs were extracted with water, 0.9% sodium chloride solution or 100 mM acetate buffer, pH 5.1 at 0°C as described earlier (Lorenc-Kubis and Morawiecka 1973). Germination capacity was evaluated on samples of 50 or 100 caryopses (in 3 replicates). The caryopses stored during 1, 2, 3, 5, 8 and 10 years were germinated in Petri dishes on two layers of filter paper saturated with water.

### ESTIMATION OF PROTEINS AND SUGARS

Protein was determined by the method of Lowry et al. (1951) with serum albumin as a standard. Neutral sugars were estimated by phenol-sulphuric acid assay (Dubois et al. 1956) and pentoses by method of Meibaum (1939).

### ACID PHOSPHATASE ACTIVITY

Acid phosphatase activity was measured spectrophotometrically by liberation of p-nitrophenol from p-nitrophenyl phosphate at 37°C at pH 5.1 (Yoshida and Tamiya 1971). One unit of enzyme activity was defined as the amount which liberated 1  $\mu$ mol of p-nitrophenol per 1 min at 37°C in 100 mM acetate buffer, pH 5.1. Specific activity was defined as the number of units per mg of protein.

## ISOLATION OF ACID PHOSPHATASE FROM CARYOPSES

ConA-binding acid phosphatase (AcPase B) from *Poa pratensis* caryopses stored during 1, 2, 3, 5 and 10 years was isolated on conA-Sepharose and Bio-Gel P-100 as described earlier (Lorenc-Kubis et al. 1981).

## AFFINITY CHROMATOGRAPHY

Affinity chromatography was performed on ConA-Sepharose and WGA-Sepharose (Pharmacia Fine Chemicals, Uppsala). The bound proteins were liberated with 10% alpha-methyl-D-mannopyranoside or 10% N-acetyl glucosamine (pH 5.6), respectively.

## GEL ELECTROPHORESIS

Polyacrylamide gel electrophoresis was carried out in 7.5% polyacrylamide gels (pH 8.4) according to the method of Davis (1964). Samples containing 100-200  $\mu$ g proteins were subjected to electrophoresis. Protein fractions were visualized by 1% Amido Black 10 B in 7% acetic acid. Acid phosphatase activity was identified in the gels by a histochemical staining method (Lorenc-Kubis and Bøg-Hansen 1981).

## QUANTITATIVE IMMUNOELECTROPHORESIS

Crossed immunoelectrophoresis was performed according to Weeke (1973), fused rocket immunoelectrophoresis by the method of Svendsen (1973). Lectin affinity immunoelectrophoresis was carried out as described by Bøg-Hansen and Brogren (1975). Quantitative immunoelectrophoresis was performed at pH 8.6 in thin layers of agarose (Litex, Glostrup, Denmark). Antibodies were purified rabbit antiserum (Harboe and Ingild 1973) raised against protein extracts of *Poa pratensis* caryopses and against purified acid phosphatase from *Poa pratensis* (Lorenc-Kubis et al. 1981). Acid phosphatase activity was revealed by the histochemical staining method used for acid phosphatase (incubation at 37°C in 100 mM acetate buffer pH 5.1 with 0.1% sodium alpha-naphthyl phosphate and 0.04% Fast Blue B) as described earlier (Lorenc-Kubis and Bøg-Hansen 1981).

## RESULTS AND DISCUSSION

## THE EFFECT OF STORAGE PERIOD OF POA PRATENSIS CARYOPSES ON THE ACID PHOSPHATASE ACTIVITY

Table 1 presents the acid phosphatase activity of *Poa pratensis* caryopses stored during a period from one to ten years. It was shown that the specific enzyme activity decreased about 50% after ten years of sto-

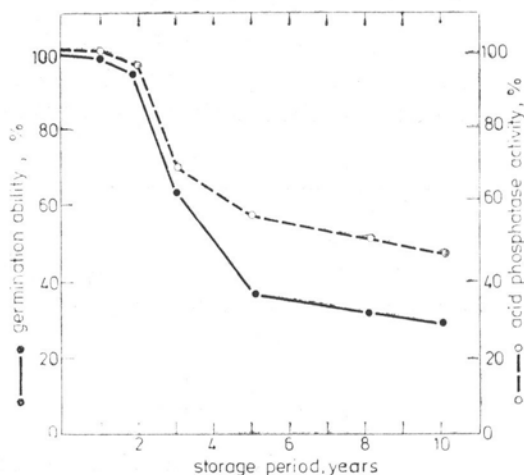


Fig. 1. Effect of storage period of *Poa pratensis* caryopses on the germination capacity ●—● and acid phosphatase activity ○—○ of proteins extracted into 100 mM acetate buffer, pH 5.1

rage. The highest enzyme activity was noted in the extracts into acetate buffer, the lowest into water, independent of the storage period. A similar correlation between enzyme activity and solvent used was also observed for other grass caryopses (Lorenc-Kubis and Wieczorek 1973).

The effect of the storage period of caryopses on acid phosphatase activity as well as on their germination capacity is shown in Fig. 1. It

Table 1

The effect of storage period of *Poa pratensis* caryopses on acid phosphatase activity

The period of storage, years	Acid phosphatase activity, $U \cdot mg^{-1}$ protein, extracted with		
	water	sodium chloride, 0.9%	acetate buffer, 100 mM, pH 5.1
1	0.97	1.18	1.90
2	0.97	1.10	1.82
3	0.67	0.78	1.34
5	0.57	0.69	1.10
8	0.50	0.61	1.00
10	0.45	0.60	0.90

The presented data are mean values calculated from four determinations of enzyme activity.

was shown that an extension of the storage period of caryopses resulted in the decrease of germination capacity and of the specific enzyme activity. Tamura et al. (1982) have shown that acid phosphatase plays an important role in phosphorus metabolism during the early phase of seed germination. A marked decrease of enzyme activity accompanying the decrease of germination capacity of caryopses may be used as an indicator of seed viability.

ACID PHOSPHATASE ACTIVITY OF SEEDS AND CHAFFS  
OF *POA PRATENSIS* CARYOPSES

Seeds and chaffs from caryopses stored one or ten years were extracted with water, 0.9% sodium chloride and 100 mM acetate buffer pH 5.1 (Table 2). Acid phosphatase activity was localized both in seeds and chaffs which constitute 56.4% and 43.6% of the weight of *Poa pratensis* caryopses, respectively. The specific enzyme activity of the seeds extracted into acetate buffer pH 5.1 was about 1.4 units per mg of protein and was twice as high as in chaffs. During the period of storage of caryopses, a decrease of acid phosphatase activity in seeds was observed. After ten years the enzyme activity in seeds decreased about 50% while the activity of acid phosphatase in chaffs remained unchanged.

Table 2

The activity of acid phosphatase of seeds and chaffs from *Poa pratensis* caryopses  
stored 1 and 10 years

Storage material	Duration of storage, years	Acid phosphatase activity, $\text{U} \cdot \text{mg}^{-1}$ protein extracted with		
		water	sodium chloride, 0.9%	acetate buffer, 100 mM
Seeds	1	0.90	1.20	1.42
	10	0.42	0.53	0.53
Chaffs	1	0.50	0.47	0.68
	10	0.48	0.46	0.68

The presented data are mean values calculated from four determinations of enzyme activity.

PROTEIN AND SUGAR CONTENT OF SEEDS AND CHAFFS FROM STORED  
*POA PRATENSIS* CARYOPSES

The content of proteins and carbohydrates did not change essentially during the investigated storage period. The level of proteins ranged from 0.18 to 0.37 g per 100 g and sugar from 2.2 to 2.4 g per 100 g of caryopses depending upon the extracting solvent. The highest content of the

investigated substances was found in the extracts into sodium chloride solution. The content of proteins in seeds ranged from 0.14 to 0.22 g per 100 g and sugar 1.6 to 1.9 g per 100 g of seeds. The level of the investigated substances was about two to three times higher in seeds than that in the chaffs. Similar results were also obtained for seeds and chaffs of *Dactylis glomerata* caryopses (Wieczorek et al. 1980). The level of sugar extracted from seeds with acetate buffer pH 5.1 was five times higher than in chaffs of *Poa pratensis* caryopses.

#### ELECTROPHORETIC ANALYSIS OF THE PROTEINS FROM CARYOPSES, SEEDS AND CHAFFS

Proteins of caryopses stored for one or ten years did not show essential differences in content as well as in the number of fractions in polyacrylamide gel electrophoresis at pH 8.4.

Analysis of proteins from chaffs by polyacrylamide gel electrophoresis at pH 8.4 (Fig. 2) showed eight protein fractions of which four were histochemically stained for acid phosphatase activity. Similarly to caryopses protein, no changes in the electrophoretic pattern of chaff proteins were observed. Proteins of stored seeds were separated by polyacrylamide gel electrophoresis into five fractions. Two of them with the greatest anodic mobility showed acid phosphatase activity. It was shown that ten years of storage did not change the number of protein or acid phosphatase fractions. Differences occurred only in the staining intensity of enzyme activity bands (Fig. 2).

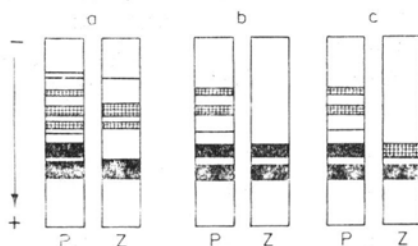


Fig. 2. Polyacrylamide gel electrophoresis of proteins of *Poa pratensis* chaffs and seeds extracted into 100 mM acetate buffer, pH 5.1. The chaffs and seeds were obtained from caryopses stored 1 and 10 years

100-150  $\mu$ g of proteins were used for polyacrylamide gel electrophoresis at 7.5% gel concentration at pH 8.4. Other experimental conditions are described in Methods. a — proteins of chaffs stored 1 or 10 years, b — proteins of seeds stored 1 year, c — proteins of seeds stored 10 years, P — proteinogram stained with amido black 10 B, Z — zymogram of acid phosphatase activity

When crude extracts of *Poa pratensis* caryopses stored for one or ten years were analysed by crossed immunoelectrophoresis with antibodies

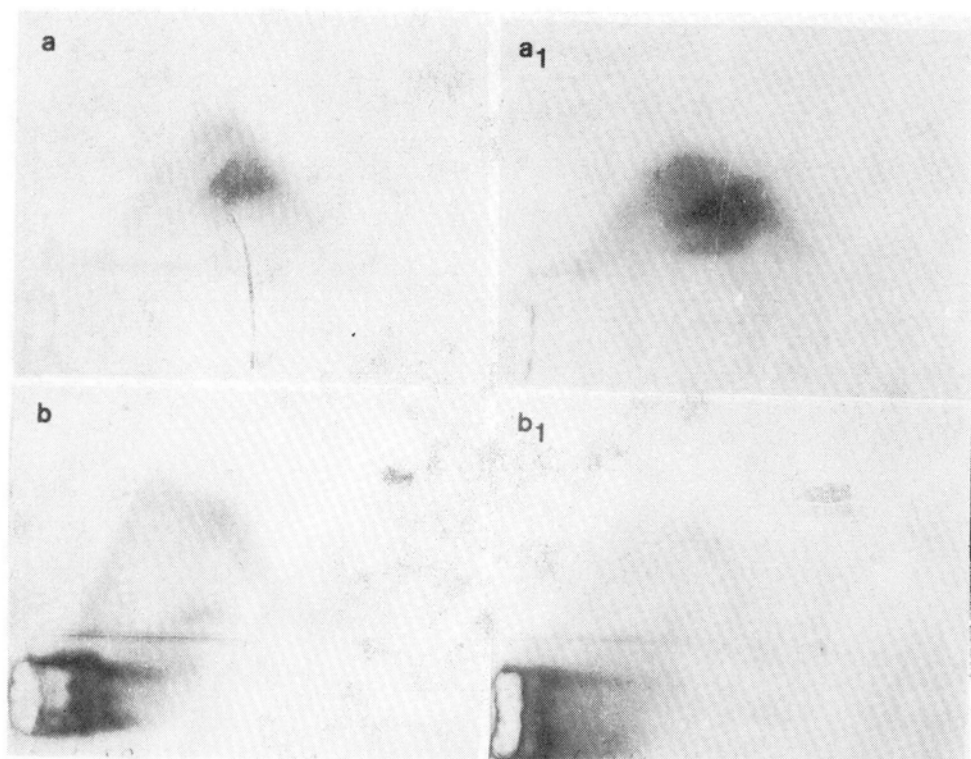


Fig. 3. Crossed immunoelectrophoresis and lectin affinity immunoelectrophoresis of acid phosphatases of *Poa pratensis* caryopses stored over 10 years

A crude extract of proteins (200  $\mu\text{g}$ ) from *Poa pratensis* caryopses was electrophoresed for 80 min in the first dimension at  $10 \text{ V} \cdot \text{cm}^{-1}$  (anode to the right), then into antibody-containing gel for 18 h at  $2 \text{ V} \cdot \text{cm}^{-1}$  (17.5 mm<sup>3</sup> purified immunoglobulin fraction per cm<sup>2</sup>, anode at the top). The plates were stained for acid phosphatase activity as described in Methods. a — crossed immunoelectrophoresis of acid phosphatases of caryopses stored 1 year, a<sub>1</sub> — stored 10 years, b — lectin affinity immunoelectrophoresis of acid phosphatases of caryopses stored 1 year, b<sub>1</sub> — stored 10 years. ConA in the first dimension gel (50  $\mu\text{g} \cdot \text{cm}^{-2}$ )

against proteins of *Poa pratensis*, two acid phosphatases were revealed (Fig. 3). The enzymes differed in their migration velocities, intensity of staining and in their binding to conA independent of the storage period (Fig. 3b).

#### THE AFFINITY OF CARYOPSE ACID PHOSPHATASES TO LECTINS AND THEIR EFFECT ON ENZYME ACTIVITY

Affinity chromatography on conA-Sepharose of proteins from caryopses stored two, three, five and ten years analysed by spectrophotometric assay and by fused rocket immunoelectrophoresis showed, similarly to proteins from caryopses stored one year (Lorenc-Kubis and Bøg-Hansen 1981), the presence of two acid phosphatases: the conA non-binding (AcPase A) and the conA-binding fraction (AcPase B) which was liberated from the column with 10% alpha-methyl-D-mannopyranoside. It was shown that the glycoprotein acid phosphatase B, after many years of caryopses storage, still preserves its affinity to immobilized as well as to free conA (Fig. 3b), but did not bind to immobilized or free WGA.

It has been reported earlier that conA-binding acid phosphatase B of *Poa pratensis* (Lorenc-Kubis and Bøg-Hansen 1981) and other grass caryopses was activated by binding to conA (Lorenc-Kubis et al. 1981, 1982). The study on the effect of conA on the activity of partly purified AcPase B of *Poa pratensis* caryopses stored one, two, three, five and ten years has shown that the activation of this enzyme in the presence of conA decreased with the length of the storage period. AcPase B of caryopses stored for five years showed a very slight increase of its activity in the presence of conA. The effect of caryopses storage on acid phosphatase, namely the decrease in its activity (Table 1) and changes in its response to free conA activation, may be connected with changes in the molecular properties of this enzyme.

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### *Kwaśna fosfataza ziarniaków wiechliny łąkowej i jej zdolność do wiązania lektyn*

#### Streszczenie

Badano wpływ okresu przechowywania ziarniaków wiechliny łąkowej (*Poa pratensis*) na zachowanie się aktywności kwaśnej fosfatazy oraz jej zdolność do interakcji z lektynami. Wykazano, że po 10-letnim okresie przechowywania ziarniaków aktywność enzymu spada około 50%, a zawartość białek i cukrów nie ulega zmianie. Spadek aktywności enzymu stwierdzono jedynie w nasionach. Aktywność kwaśnej fosfatazy plewek nie ulegała zmianie. Kwaśną fosfatazę izolowano z ziarniaków przechowywanych rok oraz 2, 3, 5 i 10 lat. Enzym ten zachowywał zdolność do wiązania zarówno immobilizowanej jak i wolnej conA, lecz nie wykazywał powinowactwa do lektyny z zarodków pszenicy (WGA). Aktywacja enzymu w obecności conA spada w miarę przedłużania okresu przechowywania ziarniaków.