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# MOLECULAR PROPERTIES AND THERMAL SECRETION OF LUPIN SEED ACID PHOSPHATASE

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**Key Word Index**—*Lupinus albus*; Papilionaceae; seed; acid phosphatase; thermal secretion.

**Abstract**—An acid phosphatase with optimum pH at 6 was found in the total albumin extract of mature dry *Lupinus albus* seeds. Three groups of phosphatase isoenzymes were identified by IEF. The active bands in the IEF gel were run in 2D-SDS–PAGE under reducing conditions and found to contain one major (*ca* 70%) polypeptide chain of $M_r$ 68 000 and one minor (*ca* 30%) of 61 000. When lupin seeds were incubated in water for 3 hr at 60°, residual phosphatase activity was detectable in the medium suggesting that the enzyme was secreted together with other already identified polypeptides. The IEF and 2D-SDS-PAGE patterns of the constitutive and heat-secreted phosphatase(s) coincided.

## INTRODUCTION

Seed acid phosphatases (APase) [EC 3.1.3.2] are hydrolytic enzymes of broad activity spectrum usually involved in the mobilization of phosphate reserves [1]. A number of such enzymes has been purified and characterized from various plant sources [2, 3]. Their pH optima vary between 4.5 and 6.5 [4]. They often consist of several isoenzymes which differ in their p*I*s [2], location in the cells [5–7], $M_r$ [8–9], extent of glycosylation [10] and specificity [6, 9].

Within lupin genus, *Lupinus luteus* and *L. angustifolius* acid phosphatases have already been described and partially characterized [4, 10]. However this enzyme from *L. albus* seeds has received no attention so far. In this work we investigated the presence of acid phosphatase(s) in dry *L. albus* seeds and identified this enzyme activity associated with a polypeptide(s) secreted upon severe heat treatment of the mature seeds.

## RESULTS AND DISCUSSION

Optimum pH of the enzyme(s) showing activity on *p*-nitrophenylphosphate (*p*NPP) in the total albumin extract of mature lupin seeds was 6.0 with a half maximal activity at 5.5 and 7.6. This qualifies the hydrolytic activity as an acid phosphatase, though this value is slightly higher than that found for yellow lupin [4] and pea [9] seed phosphatases.

When the total albumin extract (TAE) was submitted to IEF under non-denaturing conditions and APase activity was assayed on the gel, as described below, three groups of active bands in the p*I* range of 4.6–5.5, 5.9–6.4 and 7.3–7.6 were found, making up to about 10 active bands. Asghar and DeMason [4] found nine APase isoenzymes in *Lupinus luteus* in the pH range from 4.8 to 5.6. All of the active bands found in IEF showed in 2D-SDS–PAGE, under reducing conditions, two polypeptides with $M_r$ 68 000 (*ca* 70%) and 61 000 (*ca* 30%) respectively. Although great diversity has been found to exist in the $M_r$ values of denatured and reduced APases, various APases consisted of one polypeptide with $M_r$ in the range that we determined [6, 11].

The enzyme was relatively heat-stable, its activity decreasing from 60 to 13% between 0.5 and 3 hr exposure of the total albumin extract at 60°. When lupin seeds were immersed in water at 60° for at least 3 hr, secretion of various polypeptides occurred [12]. Most of them (90%) consisted of conglutin $\gamma$ [12], a glycoprotein previously referred to as a storage protein [13]. However other polypeptides were visible in the SDS–PAGE pattern of the water medium [12]. A significant APase activity was found in the incubation medium of heat-treated lupin seeds after correction for the inactivation losses. The IEF pattern, *in situ* activity detection and 2D-SDS–PAGE of the TAE and the heat-secreted fraction coincided. This indicates that part of the protein material secreted upon heat treatment of mature lupin seeds consisted of APase enzyme(s). Secretion of APases by roots [11], cultures [3], developing seeds [14] and cell walls [6] have already been described and is invariably related to the utilization of phosphate compounds under stress conditions.

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## EXPERIMENTAL

Defatted flour of lupin (*Lupinus albus*, L.) cotyledons was extracted in $H_2O$ (1:10 w/v) for 30 min at 4°. The slurry was centrifuged (10000 *g*) for 20 min at 4°. The supernatant (TAE) was immediately used for enzyme assays and electrophoretic separations. Aliquots of TAE were treated at 60° for various times. The insoluble material formed was removed by centrifugation as above and the activity in the supernatant immediately measured. Heat treatment of mature lupin seeds was carried out in $H_2O$ at 60° for various times as described in ref. [12]. Protein concn was determined by the method of ref. [15].

Phosphatase activity was assayed at 37° by measuring the changes in *A* 405 nm upon addition of aliquots of enzyme preparations to solns containing 0.38 M *p*-nitrophenyl phosphate (*p*NPP) and 1.4 mM $ZnCl_2$ in 0.1 M Na citrate buffer, pH 6. Denatured and reduced protein samples were analysed in SDS–PAGE on 12.5% acrylamide gels according to ref. [16]. The protein bands were stained with Coomassie Brilliant Blue R250 and scanned on a Cream Image Processing System (KemEn Tech, Copenhagen).

IEF was carried out on 6% acrylamide gel with 0.3% (v/v) aq. ampholines (pH 3.5–10) and 10% glycerol. The protein bands were stained with Coomassie Blue. Alternatively the gel or part of it was placed in 0.1 M citrate buffer, pH 6, containing 1.4 mM $ZnCl_2$, 20 mM Na $\beta$-naphthyl phosphate, 1 g $l^{-1}$ Fast Red. The gel was kept at 4° overnight for development of the colour. The active bands were excised, incubated in denaturing buffer for 15 min at room temp. and run in SDS–PAGE. The gels were stained with Coomassie Blue and scanned as described above.

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