Phytochemistry, 1978, Vol. 17, pp. 655–657. Pergamon Presl. Printed in England.

GALACTOSIDASES IN CULTIVATED AND WILD PEAS

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(*Received* 8 *November* 1977)

Key Word Index—*Pisum sativum*; *P. elatius*; Leguminosae; α-galactosidase; β-galactosidase; germination.

Abstract—The level of α- and β-galactosidase was followed in the cotyledons and embryos of germinating seeds of *Pisum sativum* and *P. elatius*. α-Galactosidase is preformed in the cotyledons but its activity increases during germination in the embryos. β-Galactosidase activity in embryos increases during germination but shows little change in cotyledons. The possible function of α- and β-galactosidase is discussed.

INTRODUCTION

In previous research we compared some biochemical events in the germination of wild and cultivated peas [1–3]. Disappearance of reserve proteins and starch was faster in the cultivated pea especially in the first 24 hr [1]. Oligosaccharides some of which contain galactose are also known as reserve materials, which serve as a source of energy during the early phase of seed germination. During seed germination the level of the oligosaccharides decreases in many seeds [4–7]. Galactosidase activity is frequently involved in the breakdown of these oligosaccharides [6, 8, 9]. In addition galactans and galactomannans are broken down during germination and their breakdown also involves galactosidase activity [10, 11]. The level of α- and β-galactosidase in cotyledons of some seeds increases during seed germination [6, 12–14] and decreases in others [9].

The change in activity of the galactosidases during germination of seeds therefore is not entirely clear. Moreover, the distinction between α- and β-galactosidase is not always adequately studied. Since in peas galactose containing oligosaccharides are present, we wished to clarify the pattern of galactosidase activity in peas. As the rate of breakdown of reserve materials differs in *P. elatius* and *P. sativum*, we decided to compare the galactosidase activity in cotyledons and embryos of these two species. Galactosidase activity might be controlling the level of respiratory substrate in the early stages of germination.

RESULTS

α-Galactosidase

The specific activity of α-galactosidase in cotyledons of dry seeds of the wild and cultivated peas is high. In the cultivated pea it does not change in the first 24 hr of seed germination but decreases thereafter. In the wild type the decrease in enzyme activity starts between 0 and 24 hr of germination, with a further subsequent decrease (Table 1).

The pH profile of enzyme activity in both species is similar. The results for *P. elatius* are shown in Fig. 1. *P. sativum* enzyme activity was similar. Both show high activity at pH 3.5–5.5 without clear peaks. The pH profile does not change during germination (Fig. 1). The difference in activity around pH 3 between dry seeds and seeds of the cultivated pea germinated for 24 or 48 hr is difficult to account for, but was reproducible.

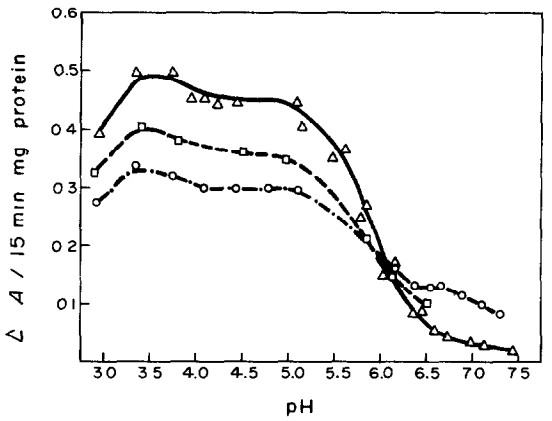

Fig. 1. pH profile of α-galactosidase in cotyledons of *P. elatius* during seed germination. (Activity as Δ *A*/15 min/mg protein. △———△ dry seeds. □-----□ 24 hr of seed germination. ○-·-·-○ 48 hr of seed germination.

Table 1. Changes in α- and β-galactosidase in cotyledons of *P. sativum* and *P. elatius* during germination. Activity expressed as Δ *A*/15 min cotyledon pair

Germination period	α-Galactosidase				β-Galactosidase			
	P. elatius		*P. sativum*		*P. elatius*		*P. sativum*	
	pH 4.1	pH 7.1	pH 4.1	pH 7.1	pH 4.1	pH 7.1	pH 4.1	pH 7.1
0	2.60	0.20	5.38	0.91	2.96	0.79	8.22	2.14
24	2.46	—	5.24	—	4.30	1.45	10.4	2.28
48	1.88	0.58	3.60	1.50	3.91	1.25	8.57	1.82

Table 2. Changes in total α- and β-galactosidase in embryos of *P. elatius* and *P. sativum* during germination. Activity as Δ *A*/15 min/embryo

	Germination period (hr)	α-Galactosidase Range of optimum pH	α-Galactosidase Activity pH 4.1	β-Galactosidase Range of optimum pH	β-Galactosidase Activity pH 4.1
P. sativum	24	3.35–4.85	0.22	3.85–4.5	0.27
	48	2.95–7.3	0.35	4.1–4.8	0.69
P. elatius	24	3.35–5.0	0.08	3.75–4.45	0.16
	48	2.95–7.45	0.17	3.75–4.1	0.21

The changes in total activity towards *p*-nitrophenyl galactoside of the cotyledons are similar to the changes in specific activity (Table1). Total activity per cotyledon pair is higher in the cultivated pea, as may be expected from the greater weight of the cotyledons [3]. We also determined α-galactosidase activity with raffinose as substrate, since the latter is a natural substrate of the enzyme. The activity of α-galactosidase with raffinose as a substrate in cotyledons of the wild and cultivated peas is also high in dry seeds, and hardly changes during seed germination. It may increase a little, by 10–15% during 48 hr of germination.

In embryos (Table 2) of both species the range of pH optimum became wider during seed germination, and the total activity increases in both.

β-*Galactosidase*

The level of β-galactosidase in cotyledons of *P. elatius* and *P. sativum* increases during germination (Table 1, Figs 2 and 3). There is a sharp rise in the specific activity in cotyledons of *P. sativum* in the first 24 hr of seed germination, while the increase of enzyme activity in cotyledons of *P. elatius* is more moderate. Between 24 and 48 hr activity changes very little.

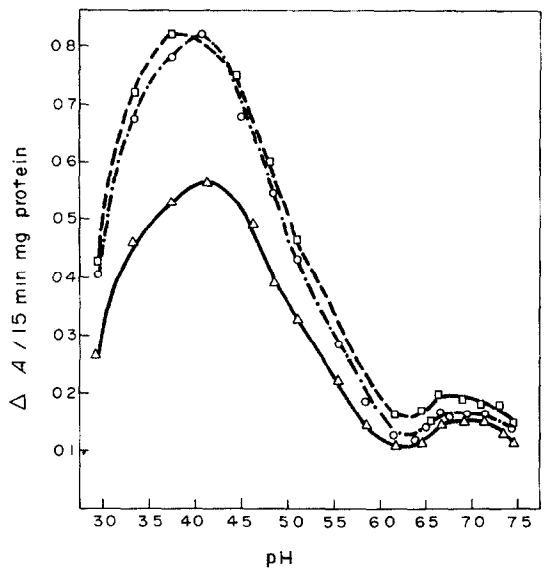

Fig. 2. pH profile of β-galactosidase in cotyledons of *P. sativum* during seed germination. (Activity as Δ *A*/15 min/mg protein). △———△ dry seeds. □-----□ 24 hr of seed germination. ○-·-·-·-○ 48 hr of seed germination.

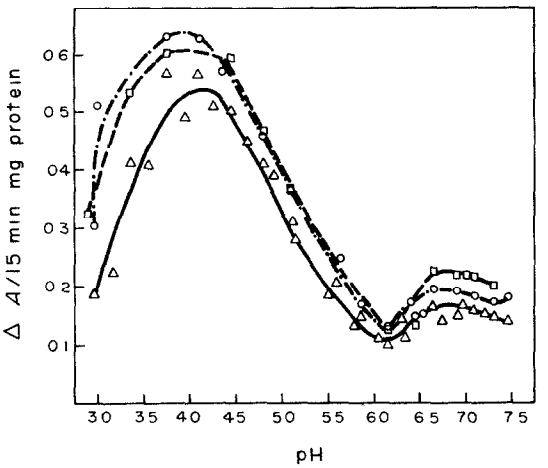

Fig. 3. pH profile of β-galactosidase in cotyledons of *P. elatius* during seed germination. (Activity as Δ *A*/15 min(mg protein). △———△ dry seeds. □-----□ 24 hr of seed germination. ○-·-·-·-○ 48 hr of seed germination.

The pH profile of enzymes from both species is similar. There is a clear peak in enzyme activity at pH 4 whose position changes slightly during seed germination. There is also a small peak at pH 7.1. The position of this peak does not change during seed germination (Figs 2 and 3). The changes in total activity (Table 1) are similar to the changes in specific activity. The activity increases in the first 24 hr of seed germination and after that there is a decrease in enzyme activity, which is more pronounced in *P. sativum* at pH 4.1.

The level of β-galactosidase in 24-hr-old embryos of both species is similar (Table 2). The total activity increases in both species. The increase in the cultivated pea is much greater than the increase in the wild pea. We were unable to determine galactosidase activity in dry embryos since it is practically impossible to prepare clean preparations of dry embryos in amounts sufficient for an assay of enzyme activity.

DISCUSSION

We did not separate α-galactosidase I and II in our experiments [8, 15] and the pH curve of α-galactosidase indicates that both enzymes are present in the preparations. This is in accord with the results of Barham *et al.* [15]. α-Galactosidase activity both towards *p*-nitrophenyl α-D-galactoside and raffinose did not change in the cotyledons of the two species of peas until 24 hr of germination after which activity dropped. On the other hand, in the embryos activity rose between 24 and 48 hr, and by extrapolation may have risen between 0 and 24 hr. This might indicate that α-galactosidase is functional first in the cotyledons, where it is pre-existent. In the embryos it must arise either by activation or synthesis. β-Galactosidase increases slightly during germination, in the cotyledons and drops thereafter, and remains at a steady level in the embryos, between 24 and 48 hr of germination.

The results do not indicate any essential difference in galactosidase activity in the two species, despite the fact that *P. elatius* germinates and develops more slowly than

P. sativum [1]. Thus, whatever the function of α- and β-galactosidases in germinating peas, its activity has no clear regulatory function.

The possible substrates for α-galactosidase are fairly evident, raffinose, stachyose and galactomannan which contain α-D-linkages, which are known to be present in leguminous seeds [5, 7, 9, 17]. Initial activity seems to be sufficient for the initiation of germination. Galactose liberated by galactosidase can apparently be rapidly metabolised [18]. It is more difficult to assign a function to β-galactosidase. This enzyme has been reported to be able to degrade glycopeptides containing a β-galactoside link [13]. Although pea seeds contain glycoproteins as storage materials, the dominant sugar seems to be mannose [19] and no galactose has been reported in them. Thus it seems at least unlikely that β-galactosidase acts on a β-galactoside. However, β-glycosidases are usually considered to be able to synthesize the glycosidic bond. During germination there is considerable synthesis of membrane and such snythesis might also involve formation of galactosyl diglyceride. It seems not impossible that β-galactosidase is crucial in anabolism and catabolism of lipids as also suggested by Gelman [12].

EXPERIMENTAL

Pea seeds, *P. sativum* L. cv Alaska, were purchased from Ferry Morse Seed Company. Seeds of the wild pea *P. elatius* were grown in the garden of the Botany Department in Jerusalem. The seeds were germinated in petri dishes on moistened cotton wool.

Preparation of enzyme extracts. Cotyledons were ground in 50 mM Pi–citrate buffer, pH 5 (20 cotyledons pair of *P. sativum* or 40 cotyledons pairs of P. *elatius* in 40 ml buffer). The homogenate was centrifuged at 1000 *g* for 20 min. The ppt. was discarded and the supernatant retained for determination of enzyme activity. In the case of embryos, 100 embryos were ground in 10 ml buffer.

Enzyme assay. α- and β-galactosidase were determined by following the initial rate of hydrolysis of *p*-nitrophenyl α-D or β-D galactoside [16]. 1 ml reaction mixture contained 2 mM substrate and 40 mM Pi–citrate buffer or Pi buffer at the appropriate pH. The mixture was incubated at 30° for 15 min. 5 ml Na_2CO_3 0.1 M were then added and the *A* measured at 405 nm.

Determination of α-galactosidase with raffinose as substrate. Cotyledons were ground in 50 mM Pi–citrate buffer pH 5. The homogenate was centrifuged at 20 000 *g* for 20 min. The supernatant was fractionated with $(NH_4)_2SO_4$ and the fraction precipitating between 30–60% satn was used as a source of enzyme. The ppt. was washed with Pi–citrate buffer 50 mM, pH 5 and resuspended in 5 ml of the same buffer. Enzyme activity was determined by following the release of reducing sugars during the reaction. 3 ml of reaction mixture contained 8 mM raffinose, 30 mM Pi–citrate buffer or Pi buffer at the appropriate pH and 1 ml enzyme. 0.1 ml Toluene was added and the mixture incubated at 37° for 20 hr. Reducing sugars were determined by the Somogy Nelson reagent [20]. Protein was determined according to Lowry *et al.* [21].

Acknowledgement—This work has been supported in part by a grant from the Agricultural Research Organization, Ministry of Agriculture, Israel.

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