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β-GALACTOSIDASE ACTIVITY IN THE GERMINATING SEEDS OF *VIGNA SINENSIS*

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Key Word Index—*Vigna sinensis*; Leguminosae; β-galactosidase; seeds; germination.

Abstract—The β-galactosidase activity in cotyledons of *Vigna sinensis* increases during seed germination and is inhibited by cycloheximide. The increasing activity may be due to the *de novo* synthesis of enzyme protein. The enzyme has been partially purified by gel filtration and characterized with respect to some biochemical parameters. The optimum pH and optimum temperature are 4.5 and 55°, respectively and the enzyme follows typical Michaelis kinetics with K_m and V_{max} of 4.5×10^{-4} M and 2.0×10^{-5} mol/hr respectively. K_i for galactose and lactose are 4.5 and 220 mM, respectively. The energy of activation of the enzyme for *p*-nitrophenyl β-D-galactoside is 9.83 kcal/mol. The apparent relative MW of the enzyme as determined by gel filtration was 56 000.

INTRODUCTION†

The cotyledons of leguminous plants contain reserve materials which serve as a source of energy during the early phase of seed germination. Disappearance of reserve proteins and starch has already been documented in germinating seeds [1]. Oligosaccharides containing α-D-galactoside groups, some of which serve as an energy source, are widely distributed in the plant kingdom. During seed germination, the level of the oligosaccharides decreases in many seeds [2–5]. α-Galactosidase activity is frequently involved in the breakdown of these oligosaccharides [4, 6, 7]. In addition, this activity is also involved in the breakdown of galactans and galactomannans [8, 9]. The level of β-galactosidase activity in the cotyledons of some seeds also increases during seed germination [4, 10–12] and decreases in others [7]. Apart from its ability to split β-galactosidic linkages, its physiological functions remain widely unknown. This enzyme has also been shown to change the antigenic properties of red cells in a particular blood group by removing part of the surface carbohydrate moieties [13, 14]. In spite of these observations the plant β-galactosidase has received little attention. The presence of β-galactosidase activity is reported here, together with some of its characteristics in the germinating seeds of *Vigna sinensis*.

RESULTS

The soluble enzyme was prepared by homogenizing the cotyledons from 96 hr germinating seeds in Tris buffer (pH 7.4). The crude preparation was purified (six-fold) by ammonium sulphate precipitation and gel filtration (Sephadex G-150) to eliminate the endogenous micromolecules. The activity of the β-galactosidase was found to increase nearly two-fold with time of germination up to 100 hr. This increase in activity was inhibited when the seeds were allowed to germinate in the presence of cycloheximide indicating that it may be due to the *de novo* synthesis of the enzyme.

The purified enzyme is active between pH 3.5 and 5.5 and its optimum pH (4.5) is similar to that of some other plant β-galactosidases [11, 15–17] and differs from the optimum pH of bacterial β-galactosidases which are *ca* pH 7.0 [18, 19]. Certain other systems also have an acid β-galactosidase similar to higher plants [20, 21]. The apparent MW of the enzyme was determined as 56 000 by Sephadex G-150 CC using albumin, ovalbumin, trypsin and RNase as standards.

Activity of the enzyme at the temperature optimum (55°) to various glycosides is shown in Table 1. It was active only on β-D-galactopyranosides. The activity for PNPG was four times higher than that for ONPG, the reverse to that found for fungal galactosidase [17]. On heating the β-galactosidase in acetate buffer (pH 5.0) for 10 min pre-incubation it was found that the enzyme is stable up to 50° and then its activity falls sharply and is completely inactivated at 70°.

The effects of various inorganic ions and inhibitors on the enzyme activity are shown in Table 2. All metals except Mg^{2+} inhibited activity. In a few cases EDTA partially overcome the inhibition but Hg^{2+}, cobalt and tungstate, PCMB and iodoacetate inhibited the activity irreversibly and almost completely. Sodium azide and glucose had no effect, but galactose, lactose, ascorbic acid and NEM also inhibited the activity. The effect of PCMB on this enzyme is completely reversed in comparison to fungal β-galactosidase [17] suggesting the involvement of sulphydryl group(s) in the enzyme activity.

The values of apparent K_m and V_{max} of the enzyme for PNPG were 4.5×10^{-4} M and 2.0×10^{-5} mol/hr, respectively. The K_i for galactose and lactose determined by Michaelis–Menten kinetics are 4.5 and 220 mM, respect-

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† Abbreviations: PNPG, *p*-nitrophenyl β-D-galactoside; ONPG, *o*-nitrophenyl β-D-galactoside; EDTA, ethylenediamine tetraacetic acid; PCMB, *p*-chloromercurobenzoate; NEM, *N*-ethylmaleimide.

Table 1. The effects of metal ions, some chemical agents and EDTA on the activity of β-galactosidase

Inhibitors	Inhibitor concentration (mM)	Relative activity	
		Metal*	Metal + EDTA†
Mg^{2+}	1	89	94
Cu^{2+}	1	37	78
Mo^{6+}	1	23	53
Zn^{2+}	1	77	90
Pb^{2+}	1	44	80
Co^{2+}	1	14	11
Arsenate	1	34	64
Tungstate	1	0	0
Sodium fluoride	50	102	—
Sodium azide	50	99	—
Galactose	50	36	—
Lactose	50	78	—
Ascorbic acid	50	78	—
PCMB	50	4	—
Iodoacetate	10	4	—
NEM	10	59	—

Inhibitor or metal ion and enzyme were mixed in 10 mM acetate buffer (pH 5.0) and allowed to stand for 10 min at room temperature. PNPG was added to the reaction mixture and the enzyme activity was determined. Activity was expressed as a percentage of the activity level in the absence of inhibitor or metal ion.

*Enzyme was pre-incubated with 1 mM metal ion.

†Enzyme was pre-incubated with 1 mM metal ion and then with 10 mM EDTA.

Table 2. Substrate specificity of β-galactosidase

Glycoside	Relative activity (%)
p-Nitrophenyl β-D-galactopyranoside	100
o-Nitrophenyl β-D-galactopyranoside	27
p-Nitrophenyl α-D-galactopyranoside	0
p-Nitrophenyl α-D-glucoside	0
p-Nitrophenyl β-D-glucoside	0

The enzyme was incubated with each substrate at 0.1 mM concentration for 10 min at 55°. Activity is expressed as a percentage of the activity level when PNPG was used as a substrate.

ively. Galactose is more inhibitory than lactose (Table 2). At optimum pH, the reaction velocity was determined with PNPG as substrate as a function of temperature over the range 30–50° and gave a value of 9.83 kcal/mol for the energy of activation.

DISCUSSION

The activity of the β-galactosidase increases with increasing time of germination, most likely by the activation or *de novo* synthesis of enzyme protein since cycloheximide inhibits this change. This could indicate that cycloheximide inhibits the *de novo* synthesis of enzyme protein *per se* or of an activator protein which affects a pre-existing inactive form of the enzyme. The enzyme has an optimum pH of 4.5 and temperature optimum of 55°. It preferentially uses PNPG as a substrate rather than ONPG, is heat stable up to 50° and most metals tested are inhibitory. PCMB and iodoacetate, also inhibited enzyme activity. This indicates that sulphydryl groups are necessary for its function. Lactose and galactose inhibit the enzyme activity competitively. Its apparent M_r is 56 000.

EXPERIMENTAL

Vigna sinensis seeds (100 g) were washed (tap water) and immersed in 0.02% $HgCl_2$ for 10 min followed by several washings with H_2O. Seeds were de-coated and allowed to germinate in the dark at 28°. The cotyledons of seeds germinated for 96 hr were separated from the embryo and a 10% homogenate made with ice-cold buffer A (10 mM Tris–HCl, pH 7.4) containing 0.1% Triton X-100. The homogenate (strained through cheese-cloth) was centrifuged at 1000 *g* for 10 min at 4° and the supernatant centrifuged at 10 000 *g* for 20 min. The pellet was discarded and the supernatant fractionated with $(NH_4)SO_4$. The fraction ppting at 40–60% satn was dissolved in a minimum vol. of buffer A. A fraction (1365 units activity and 225 mg protein) was separated on Sephadex G-150 (85 × 1 cm) equilibrated with buffer A. Elution was performed with the same buffer at a flow rate of 6 ml/hr.

Enzyme assay. β-Galactosidase activity was determined with PNPG. The reaction mixture, (1 ml) contained 250 μl 0.1 M NaOAc buffer (pH 5.0) 10 μl 10 mM PNPG and was incubated with enzyme for 10 min at 55° and the reaction stopped by the addition of 4 ml 0.1 M NaOH. The *A* was measured at 410 nm. One unit of activity was defined as the amount of enzyme which hydrolysed 1 μmol/hr of *p*-nitrophenyl β-galactoside at 55° under the standard assay conditions.

Protein determination. Protein was estimated by the method of Lowry *et al.* [22] with bovine serum albumin as a standard.

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