Pergamon

0031-9422(95)00099-2

Phytochemistry, Vol. 39, No. 6, pp. 1301–1303, 1995

0031-9422/95 $9.50 + 0.00

MYROSINASE FROM ROOTS OF *RAPHANUS SATIVUS*

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(*Received in revised form* 3 *January* 1995)

Key Word Index—*Raphanus sativus*; Cruciferae; radish roots; myrosinase characterization; sinigrin.

Abstract—Two myrosinase isoenzymes (EC 3. 2. 3. 1) were extracted and purified by $(NH_4)_2SO_4$ precipitation and DEAE-cellulose and Sephadex G-200 chromatography from radish root tissues with M_rs 28 800 and 58 900. The most active one (120 U mg^{-1}) was identified and characterized. The yield of the purified myrosinases was 21 mg (2398 U) of pure enzymes from 100 g of dry root tissues. The purity was ascertained by obtaining a single sharp band by disc electrophoresis. Optimal myrosinase activity on sinigrin was recorded in phosphate buffer, pH 6–6.5 at 37°. The K_m for myrosinase with sinigrin as substrate was 0.47 mM at pH 6. The enzyme was stable for 45 min at 30 and 40° (18% denaturation).

INTRODUCTION

Glucosinolates (glucose-S-R=N-O-SO_3), are widely distributed in the Cruciferae. They are degraded by thioglucoside glucohydrolase (myrosinase) which at neutral pH values produces free glucose, sulphate, and an isothiocyanate from the glucosinolate substrate, sinigrin [1, 2]. The study of myrosinase has important applications for both the biological and technological aspects of the food and feed stuff industries and for the assessment of the safety of cruciferous materials in general [3]. Myrosinase occurs in all plants that contain glucosinolates [2, 4], fungi [5], insects [6] and mammals [7]. Purification and characterization of myrosinase from seeds has been extensively reported [8, 9], but rarely from leaf tissues [10]. Although in roots it has been detected and partially purified previously [1, 2], the present work describes the properties and purification to homogeneity of the myrosinase from Egyptian radish (*Raphanus sativus*) roots.

RESULTS AND DISCUSSION

A crude extract of high myrosinase activity was obtained by water extraction of fresh radish roots. The activity in the extracts was 35–42 U/g dry weight tissues. Extraction with 1% NaCl or with water from frozen roots led to the loss of 60–90% of its activity. A typical purification scheme is shown in Table 1. The $(NH_4)_2SO_4$ precipitate hydrolysed not only thioglucoside linkage (sinigrin) but also other glucosides (amylose, glucogen and sucrose). Adsorption of the $(NH_4)_2SO_4$ precipitate on DEAE-cellulose column gave myrosinase at 0.2 M NaCl fraction. It also hydrolysed sucrose. Applying the 0.2 M NaCl fraction on a Sephadex G-200 column resolved the myrosinase into two isoenzymes. These results are in accordance with those of others who found that myrosinase in the Cruciferae often exists in multiple forms [1, 2, 10–12]. The most active isoenzyme (120 U mg^{-1}) was selected for studying its physiochemical properties. On lyophilization, the purified enzyme gave a white powder highly soluble in water. A single band obtained by disc electrophoresis confirmed the homogeneity of the most active isoenzyme isolated.

Several features of the purified radish root myrosinase distinguished it from that isolated from seed and leaves. The M_rs of the two isoenzymes were estimated to be 28.8 $\times 10^3$ and 58.9 $\times 10^3$. These values are lower than those of isoenzymes isolated from *Sinapis alba* seeds [9], but similar to those of isoenzymes of turnip leaves [13]. Myrosinase from seeds has been reported to have a different K_m for sinigrin ranging from 0.03 to 0.18 mM depending on the nature and the ionic strength of the buffer used [14–16] while the K_m value of turnip leaf myrosinase was 0.054 mM [13]. Radish root myrosinase K_m was 0.47 mM using sinigrin as substrate, which indicates a high affinity towards sinigrin.

The purified radish myrosinase also shows many properties in common with other myrosinases. Its activity was determined using sinigrin as a substrate with different buffers (pH 4–8). It exhibited maximum activity at pH 6.0–6.5 with 0.1 M phosphate buffer and half maximal activity at pH 5.2, but showed a sharp decrease at pH 7. This optimum range of pH was in accordance with those reported by other investigators [13, 14]. The optimum temp. was 37° and complete deactivation of the enzyme was achieved over 45°. The activation energy (E_a) was 10.6 kcal mol^{-1}. This value (37°) was also found with turnip leaf myrosinase [13], while the optimum temperature of *Sinapis alba* and *Brassica napus* seed myrosinases was 60° [15]. When heating the myrosinase at 30 and 40°

Table 1. Typical myrosinase purification

Steps	Total protein (mg)	Total activity (U)	Total activity (U mg^{-1})	Purification (Fold)	Yield (%)
Crude extract	262	236	1	1	100
$(NH_4)_2SO_4$ fraction	88	209	2	2	89
DEAE-cellulose	6	206	34	34	87
Sephadex G-200					
1st peak	0.8	72	90	90	31
2nd peak	0.6	72	120	120	31

Each value is the average of three to six experiments for different batches.

Table 2. Effect of divalent ions and some reagents on the myrosinase activity

Compounds (1 mM)	% relative activity*
$CuSO_4$	70
$ZnSO_4$	63
$FeSO_4$	39
$CoNO_3$	34
$CaSO_4$	45
Pb acetate	40
K_2SO_4	0
$MgSO_4$	7
$AgNO_3$	21
$Hg(NO_3)_2$	101
Ascorbic acid	0
EDTA	88
Mercaptoethanol	111
Cysteine	43
p-chloromercuribenzoate†	35
Iodoacetic acid	102

*Myrosinase activity is expressed as a percentage of the control enzyme activity (100%) under standard assay conditions.

†Concentration of *p*-chloromercuribenzoate is 2×10^7 mM.

for 45 min, 18% loss of activity was observed. At 30°, the enzyme remains stable for 90 min. Under the standard assay conditions, the reaction was linear up to 33 μg protein/reaction mixture and 120 min. The substrate specificity of radish root myrosinase is like that of other myrosinase [13, 17] but contradicts the results of ref. [12]. It hydrolysed different glucosinolates and did not digest *O*-glycosides (amylose, glycogen, sucrose, lactose and maltose). Sinigrin was degraded at a faster rate than glucosinalbin and progoitrin. The UV absorbance of the purified enzyme exhibited a maximum at 265–270 nm. No significant *A* could be traced in the visible region indicating the abscence of a chromophore [18]. The ratio of *A* at 280–260 nm was 0.986. This indicated that the prepared enzyme is nearly free of nucleotides and nucleic acids [19]. The results in Table 2 indicated that all metal ions and reagents tested caused either a decrease or no effect on the enzyme activity. The inhibitory effect may be due to blocking of the binding site of the substrate or instability of the enzyme–substrate complex.

EXPERIMENTAL

Raphanus sativus roots purchased from local market during winter.

Extraction of myrosinase. As described previously [10]. Fresh roots (100 g, 6 g dry wt) were cut into small pieces and ground with H_2O and sea sand in a mortar at 5°. The homogenate was filtered through cheesecloth, centrifuged at 13000 *g* (4°) and dialysed against H_2O (4°) for 2 days.

Purification of myrosinase. The dialysate was treated with $(NH_4)_2SO_4$ (100% satn) at 4° and the ppt. collected at 13000 *g* for 15 min. This was redissolved in H_2O and fractionated on a DEAE-cellulose column. The adsorbed protein was eluted with a linear gradient of 0.1–0.5 M NaCl in Na–Pi buffer (pH 7, 10 mM). The active enzyme eluate was applied to a Sephadex G-200 column and eluted with Na citrate–Pi buffer (pH 6, 10 mM). Myrosinase purity was ascertained by disc electrophoresis [20].

Myrosinase activity was determined by incubating 0.2 ml enzyme soln with 0.1 ml sinigrin (1%, in 0.1 M Na–Pi buffer, pH 6) for 1 hr at 37°. The glucose liberated was determined following refs [21, 22]. One unit of enzyme activity was defined as 1 μmol of reducing sugar (glucose) liberated per hour under the assay conditions. Sinigrin (thioglucosinolate) was prepared according to the method of ref. [23]. Protein content was determined by the method of ref. [24] using bovine serum albumin as a standard.

Properties. The M_r of the myrosinase was determined by a Sephadex G-200 column [25]. Bovine serum albumin, pepsin, trypsin, disaccharidase, turnip and beetroot β-amylase, and turnip myrosinase were used as standards. The K_m was determined by plotting reciprocals of substrate concs (0.5–5 mM) vs velocity (*U*) at 37° [26]. The optimum temp. was determined by performing the standard assay within the range 25–70°. Thermal stability was determined by holding the enzyme soln at various temps (30–60°) for up to 90 min. Remaining activity was measured under standard assay conditions. To study the effect of metal ions and various reagents, the purified enzyme was preincubated with each one at 15° for 30 min, and the remaining activity was measured relative to control.

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