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MULTIPLE FORMS OF α-GALATOSIDASE IN CHICK PEA SEEDLINGS

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Key Word Index—*Cicer arietinum*; Leguminoseae; chick peas; multiple forms of α-galactosidase.

Abstract—Two molecular species of α-galactosidase with apparent MWs of 134000 and 43000 were separated and partially purified from germinating *Cicer arietinum* seeds. The two forms of the enzyme showed different kinetic and thermodynamic properties, and responses towards the specific inhibitors, but their pH optima and pH stabilities were almost identical.

Multimolecular forms of α-galactosidase (α-D-galactoside galactohydrolase, EC 3.2.1.22) occur in dormant seeds of higher plants [1–5]. Recently, Dey and Pridham [4–6] have reported the purification and properties of two forms of α-galactosidase from dormant broad bean seeds. Little information is available about this enzyme from seedlings and in the present communication we report the separation, partial purification and some properties of two species of α-galactosidase from germinating seeds of chick peas.

The separation and partial purification of the multiple forms was achieved in the following manner. The Me_2CO powder of 6-day-old chick pea seedlings was extracted with 0.1 M citrate buffer (pH 5.2). The crude extract was then adjusted to pH 3 with 1 M citric acid and the resulting ppt (inactive protein) was discarded. The supernatant was brought to pH 5.2 and subjected to $(NH_4)_2SO_4$ fractionation. The fraction which precipitated between 0.2 and 0.7 saturation was collected by centrifugation at 20000 *g* for 1 hr at 4°, dissolved in 50 mM citrate buffer (pH 5.2) and dialysed against the same buffer for 18 hr. This fraction was rich in α-galactosidase and was further fractionated by gel filtration using Sephadex G-100 column (2.25 × 80 cm) and 50 mM citrate buffer (pH 5.2) containing 0.1M KCl was the eluting solvent. Protein fraction (1 ml ≡ 100 mg protein by method of ref. [7] containing about 800 units of the enzyme was applied to the column and 2 ml fractions were collected at a flow rate of 0.1 ml/min. Aliquots (0.2 ml) from each tube were assayed for α-galactosidase by the method of ref. [8]. One unit of enzyme was defined as mg protein which would liberate 1 μmol of *p*-nitrophenol from the substrate in 1 hr under the conditions described.

Three α-galactosidase peaks were obtained; A at 115 ml, B as a shoulder of A at 125–130 ml, C at 138 ml. Of these the main peaks A and C were free from cross contamination by other glycosidases, namely, β-galactosidase, α- and β-glucosidase, and α- and β-mannosidase. The fraction-A containing the high MW species of the enzyme and fraction-C containing the low MW form of the enzyme were referred to as α-galactosidase I and α-galactosidase II, respectively. At this point the purification of α-galactosidase I and II was 110 and 53 fold, respectively, with their respective yields of 17 and 2.1% relative to the total α-galactosidase activity in the crude homogenate. Fraction-B appeared to be relatively minor with about 6% of the total α-galactosidase activity and

Table 1. Properties of multimolecular forms of α-galactosidase from germinating seeds of *Cicer arietinum*

Properties	Molecular forms of enzyme	
	α-galactosidase I	α-galactosidase II
MW*	134000	43000
Optimum pH	5.2–6.0	5.0–5.2
pH-Stability†	5–8	5–6
Residual activity after 15 min at 50°(%)	0.0 ±0.5	23.6 ± 3.0
Apparent K_m(mM)	0.25	0.34
V_{max} (μmol/min/mg)	4.3	2.2
Energy of activation (Kcal/mol)	14.6 ± 0.5	22.8 ± 0.5
ΔF (Kcal/mol)	−5.0 ± 0.2	−4.7 ± 0.2
ΔH (Kcal/mol)	−7.9 ± 0.2	12.1 ± 0.3
ΔS (Cal/deg/mol)	−10.3 ± 0.5	−24.7 ± 1.0
Inhibition by		
(i) Ag^+	Competitive ($K_i = 6.7 \times 10^{-3}$ mM)	Competitive ($K_i = 0.78 \times 10^{-3}$ mM)
(ii) Hg^{2+}	Non-competitive ($K_i = 1.0 \times 10^{-3}$ mM)	Competitive ($K_i = 0.6 \times 10^{-3}$ mM)
(iii) Myoinositol (40 mM)	55.1%	44.0%
(iv) D-Xylose (12 mM)	57.0%	47.2%

In all experiments the enzyme was assayed at 25° and pH 5.2 as described in ref. [8]. * MW was determined by the method of ref. [10] using Sephadex G-100 column. † Represents the pH range of 100% stability after 1 hr exposure of the enzyme to various pH levels (2–8); the residual activity was measured at optimum pH and the untreated enzyme was used as control. Energy of activation was determined by Arrhenius plots [11] in the temp. range 15–35°. Other thermodynamic parameters were determined at 25° from K_m values at various temps as described in ref. [6]. K_i values were obtained from Dixon Plots [12].

much lower sp. act. (0.02 units/mg) compared to 70–75% of the total activity and 40.6 sp. act. of enzyme I and 18–21% of the total activity and 5.2 sp. act. of enzyme II. At the moment we are not certain if this fraction is real or an artifact, although this fraction was also present in the freshly prepared crude enzyme extracts obtained from 3- and 6-day-old seedlings and the ripening seeds near maturing [unpublished data]. This fraction was not analysed any further.

Judging from the size of the peaks α-galactosidase I had *ca* 4 × the activity of enzyme II in germinating chick peas seedling. In contrast to this, however, Barham *et al.* [3] reported that the high MW forms of α-galactosidase, which are generally predominant in dormant seeds, were readily replaced by the low MW forms on germination. Thus, multiple forms of α-galactosidase in chick peas appear to function differently from those in *Vicia faba* seeds. In this respect it may be pointed out that Sephadex gel filtration of the freshly prepared crude extracts from 3- and 6-day-old germinating chick peas also showed a predominance of the enzyme I, and the activity ratio of enzyme I with respect to the enzyme II increased in favour of enzyme I [unpublished data]. Thus, the predominance of high MW form of α-galactosidase I in the germinating chick peas was thought to be real and perhaps *in vivo* conversion of the low MW species to high MW enzyme takes place during germination as the latter is *ca* × 3 the MW of the former (Table 1). However, the *in vitro* conversion of enzyme II to enzyme I as observed by Dey and Pridham [9] is possible and further work is needed to clarify this point.

A comparison of the properties of the two forms of the enzyme is shown in Table 1. The two enzymes from chick pea appear to follow the pattern reported by Dey and Pridham [4] except that in the present case the thermal stability of the low MW species of the enzyme was somewhat greater than that of high MW. Furthermore, the two forms can be distinguished from one another with respect to their MWs, thermal stability, energy of activation, ΔH and ΔS values, and responses to specific inhibitors.

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THE ESSENTIAL OIL OF *ARTEMISIA CAPILLARIS**

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Key Word Index—*Artemisia capillaris*; Compositae; essential oil; terpenoids; phenylacetylenes; phenols; fatty acids.

Abstract—Twenty-five terpenoids, 6-phenylacetylenes, 7 phenols, and 15 fatty acids were characterized in this oil. It differs considerably in composition from the oils of *A. kurromensis*, *A. maritima* and *A. fukudo*, which have α- and β-thujone as the major constituents.

INTRODUCTION

350 species of *Artemisia* occur throughout the world, about 30 of which are known in Japan. In our previous papers, [1–4], the new acetylenic compounds, 1-(2′-methoxy phenyl)-2,4-hexadiyne (*o*-methoxycapillen), capillanol, norcapillen and neocapillen, were reported in the essential oil of the stalks and leaves or extract of roots of *A. capillaris* Thunb. (Kawarayomogi). The volatile constituents from the stalks and leaves of *A. capillaris* have also been reported by Harada *et al.* [5–8], but the terpenoid, phenol and fatty acid constituents have not so far been studied. Mono- and sesqui-terpenes have been identified in other *Artemisia* species [9–13]. This

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