Phytochemistry, Vol 27, No 5, pp 1301–1302, 1988
Printed in Great Britain

0031-9422/88 $3.00 + 0.00
Pergamon Press plc

SUBUNIT STRUCTURE AND AMINO ACID ANALYSES OF β-GALACTOSIDASE PURIFIED FROM CARROT CELL CULTURES

HARUYOSHI KONNO,* KENJI KATOH† and ICHIRO KUBOTA‡

Institute for Agricultural and Biological Sciences, Okayama University, Kurashiki-shi, Okayama 710, Japan, †Suntory Institute for Bioorganic Research, ‡Suntory Institute for Biomedical Research, Shimamoto-cho, Mishima-gun, Osaka 618, Japan

(*Received* 17 *September* 1987)

Key Word Index—*Daucus carota*; Umbelliferae, amino acid analyses, cell culture; β-galactosidase, subunit structure

Abstract—β-Galactosidase has been purified to electrophoretic homogeneity from cell suspension cultures of carrot. It has an apparent M_r of 104 000 and consists of two identical subunits with an apparent M_r of 50 000. The amino acid composition of the enzyme is characterized by a relatively large content of Asx and Leu, and a small content of His and Met. The amino-terminal amino acid of the enzyme is Glu.

INTRODUCTION

It appears that various hydrolytic enzymes in plants play a crucial role in cell wall metabolism in order to remodel and/or loosen the wall structure. The cell walls are constructed from cellulose microfibrils, several noncellulosic carbohydrate polymers and glycoproteins. β-D-Galactosyl linkages are the main constituent of the carbohydrate polymers such as hemicellulose and pectic polysaccharides. A β-galactosidase (β-D-galactoside galactohydrolase, EC 3.2.1.23), responsible for hydrolysing the β-D-galactosyl linkages, is widely distributed in nature, and has been purified from microorganisms and characterized [1]. In contrast to the β-galactosidase from microorganisms, many studies on this enzyme in plant tissues have been limited to the enzymology during fruit ripening process [2–5]. So far the β-galactosidase that has been purified to homogeneity from plant cells has been obtained from jack bean meal [6] and liverwort cell cultures [7], but its molecular characteristics and function *in situ* are still unknown.

In a preceding paper [8], we demonstrated that β-galactosidase, highly purified from carrot cell cultures, was implicated in the degradation of galactose-rich polymer of corresponding cell walls Here, we describe the subunit structure and amino acid analyses of β-galactosidase purified from carrot cell cultures.

RESULTS AND DISCUSSION

The extractability of several glycosidases in cell homogenates was examined by successive extraction in 0.1 M potassium phosphate buffer (pH 7.0) and in the buffer plus 2 M sodium or lithium chloride [8]. Nearly all of the total β-galactosidase activity was readily extracted by buffer alone, and so the enzyme was isolated from a protein fraction soluble in phosphate buffer in a highly purified state, as described previously [8]. The enzyme at this step revealed one intense and two faint protein bands on polyacrylamide gel electrophoresis and, therefore, was further purified by the method of preparative polyacrylamide gel electrophoresis. The enzyme purified in this manner was homogeneous, a single protein band which coincides with the β-galactosidase activity observable on the polyacrylamide gel electrophoresis. The enzyme also has weak hydrolysing activity against other substrates, i.e. lactose and citrus galactan (89 weight % galactosyl residues) when compared to *p*-nitrophenyl β-D-galactopyranoside used as substrate for β-galactosidase (data not shown). Even after an exhaustive reaction conditions, other glycosidic linkages in the substrate, as described previously [8], were not hydrolysed by this enzyme. This result also supports the conclusion that the enzyme preparation is homogeneous.

The purified β-galactosidase was subjected to gel filtration on Sephadex G-200 calibrated with proteins of the known M_r value, and showed an apparent M_r value of 104 000. The reduced and denatured enzyme migrated as a single distinct band when electrophoresed in the 10%SDS-polyacrylamide gel had a M_r value of *ca* 50 000, as calculated by the use of marker proteins. The results show that the native β-galactosidase is a dimer with M_r 104 000 and is composed of two identical subunits, not previously noted for β-galactosidase. During our purification of the carrot enzymes, i.e. β-galactosidase, 36% of the total activity was recovered when passed through the CM Sephadex C-50 column. This fraction was further separated into two active peaks with Sephadex G-200 gel filtration chromatography. As previously reported [8], the carrot cell cultures would produce multiple forms of this enzyme with different M_r values. It is conceivable that these multiple forms arise from the combination and dissociation of the M_r 50 000 subunits, suggesting the post-translational processing of a single gene product.

The amino acid composition was obtained by acid hydrolysis of purified β-galactosidase as shown in Table 1 The total number of amino acid residues was 455 The predominant amino acids were Asx (aspartic acid plus asparagine; 65 residues or 14.3%) and Leu (65 residues or 14.3%), followed by Ser (37 residues or 8.1%),

*Author to whom correspondence should be addressed.

Table 1 Amino acid composition of β-galactosidase

Amino acid	No of residues/molecule	
	Carrot*	*Escherichia coli*†
Asx	65	105
Thr‡	26	59
Ser‡	37	60
Glx	36	124
Pro	32	62
Gly	30	72
Ala	27	81
Cys-1/2	n d §	15
Val	22	64
Met	2	23
Ile	26	38
Leu	65	96
Tyr	14	29
Phe	26	38
Lys	20	23
His	9	31
Arg	18	64
Trp	n d	27
Total residues	455	1011

*Based upon an estimated subunit M_r of 50000
†From Fowler and Zabin [9]
‡Extrapolated to zero time
§Not determined

Glx (glutamic acid plus glutamine, 36 residues or 7.9%) and Pro (32 residues or 7.0%). The fact that the content of Met (2 residues or 0.4%) was markedly low, was particularly noteworthy Tyr (14 residues or 3.1%) and His (9 residues or 2 0%) were also found in small quantities The amino-terminal amino acid was Glu. It is clear that the amino acid analyses of carrot β-galactosidase differ considerably from the analyses of *Escherichia coli* β-galactosidase reported by Fowler and Zabin [9]

EXPERIMENTAL

Cell suspension cultures of carrot (*Daucus carota* L. cv Kintoki) were cultured in the medium of ref [10] containing 3% (w/v) sucrose and 4 5 μM 2,4-dichlorophenoxy acetic acid [11] After 15 days the cells were harvested by filtration on paper, washed with H_2O, lyophilized and stored at −20°

Lyophilized cells were suspended in 0 1 M K-Pi buffer (pH 7) containing 9 mM 2-mercaptoethanol and disrupted by sonication for 10 min at 0° The β-galactosidase was fractionated from cell homogenates by a procedure involving $(NH_4)_2SO_4$ fractionation, CM-Sephadex C-50 ion-exchange chromatography, DEAE-Sephadex A-50 batch wise and Sephadex G-200 gel filtration chromatography, as described in ref. [8] The enzyme was purified by prep PAGE using the system of ref [12]

Native β-galactosidase examined by PAGE (7 5 %gel) at pH 9 5 SDS-PAGE (10% gel) of denatured enzyme under denaturing conditions in the presence of SDS was according to ref [13] The M_r of protein subunits was estimated from Sigma calibration kit (M_r range 14400–94000) The apparent relative mobility (M) was expressed as the ratio of the distance (mm) migrated by protein to that of total gel length (60 mm)

For amino acid analysis, purified β-galactosidase was dialysed against H_2O thoroughly, lyophilized, and hydrolysed with 6 M HCl containing 0 1% (v/v) phenol in sealed tubes for 24 and 72 hr at 110° After hydrolysis of the enzyme (0 1 nmol), amino acid composition was determined by an amino acid analyser (model 835–50, Hitachi, Tokyo) The amino-terminal amino acid was determined by stepwise Edman degradation using a gas-phase protein sequencer (model 470A, Applied Biosystems, Foster City, Calif)

The β-galactosidase activity was determined by measuring the amount of *p*-nitrophenol released from *p*-nitrophenyl β-D-galactopyranoside The reaction conditions were as described previously [8] Protein concn was measured by the method of ref [14] using bovine serum albumin as a standard

Acknowledgement—We appreciate helpful advice about the paper from Dr Y Yamasaki (Okayama University)

REFERENCES

1 Wallenfels, K and Weil, R (1972) in *The Enzymes* Vol 7, 3rd Edn (Boyer, P D, ed), p 617 Academic Press, New York
2. Bartley, I M (1974) *Phytochemistry* **13**, 2107
3 Wallner, S J and Walker, J E (1975) *Plant Physiol* **55**, 94
4 Ahmed, A E. and Labavitch, J H (1980) *Plant Physiol* **65**, 1014
5 Pressey, R (1983) *Plant Physiol* **71**, 132
6 Li, S-C, Mazzotta, M Y, Chien, S-F and Li, Y-T (1975) *J Biol Chem* **250**, 6786
7 Konno, H, Yamasaki, Y and Katoh, K (1986) *Plant. Sci* **44**, 97
8 Konno, H, Yamasaki, Y and Katoh, K (1986) *Physiol Plant* **68**, 46
9 Fowler, A. V and Zabin, I (1977) *Proc Natl Acad Sci U S A* **74**, 1507
10 Murashige, T. and Skoog, F (1962) *Physiol Plant* **15**, 473
11 Konno, H, Katoh, K and Yamasaki, Y (1981) *Plant Cell Physiol* **22**, 899
12 Konno, H, Yamasaki, Y and Katoh, K (1984) *Physiol Plant* **61**, 20
13. Weber, K and Osborn, M (1969) *J Biol Chem* **244**, 4406
14 Lowry, O H, Rosebrough, N J., Farr, A L and Randall, R J (1951) *J Biol Chem* **193**, 265