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A LYSOZYME FROM THE FRUIT OF *ACTINIDIA CHINENSIS**

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Key Word Index—*Actinidia chinensis*; Actinidiaceae; kiwi fruit.

Abstract—A lysozyme of M_r 28 000 was purified to homogeneity from *Actinidia chinensis*. It is readily inhibited by histamine, but is less sensitive to *N*-acetyl-D-glucosamine. The amino acid composition is reported.

INTRODUCTION

Lysozymes of plant origin have not been extensively studied. From *Papaya carica* [1], *Hevea brasiliensis* [2], a member of the *Ficus* family [3] and from the latex of *Asclepias syriaca* [4] such enzymes have been isolated, purified to homogeneity and characterized. Lysozymes from other plant sources have been reported ([5] and refs therein), but compared with the avian and mammalian enzymes few plant lysozymes have been described.

During studies with the cysteinyl protease actinidin from *Actinidia chinensis* (kiwi fruit) a lysozyme was found in the juice. That enzyme has now been purified to homogeneity by the method described here. Some properties, and the amino acid composition of the lysozyme, are reported and compared with those of other lysozymes.

RESULTS AND DISCUSSION

The lysozyme from kiwi fruit eluted as a single, symmetrical peak when rechromatographed on a BioSil TSK SW-250 column in HPLC, with a retention time of 36 min. As was found in the chromatography of other plant lysozymes in that system [4] the retention time cannot be used to determine the M_r, as the enzymes apparently bind to the column packing anomalously. The homogeneity of the lysozyme was also demonstrated by disc gel electrophoresis when a single sharp protein band with R_f of 0.44 was observed.

Passage through a calibrated BioGel P-100 column gave a single symmetrical peak with M_r 28 000, which is comparable with the M_rs of the lysozymes isolated from other plant sources [1–5]. That figure was essentially confirmed by SDS-gel electrophoresis when a M_r of 30 000 was estimated. The enzyme had a broad range for maximal pH, from 4 to 5 in a conventional 'bell' shaped curve.

The amino acid composition of the lysozyme isolated here is reported in Table 1. The enzyme is not obviously related to those from other plant sources [2, 3, 5] except that similarities to two of the four lysozymes purified from the latex of *Asclepias syriaca* [4] were observed [6].

No tryptophan residue was detected in the lysozyme from the *A. chinensis*, using three assay procedures [7–9].

Table 1. Amino acid composition of lysozyme from *Actinidia chinensis*

Residue	No. of residues/mol	(% weight composition)
Cys	11	4.2
Asx	35	14.2
Thr	18	6.4
Ser	13	4.0
Glx	22	10.1
Pro	15	5.1
Gly	41	8.4
Ala	22	5.5
Val	10	3.5
Met	2	1.0
Ile	11	4.6
Leu	14	5.6
Tyr	9	5.0
Phe	10	5.4
His	6	2.9
Lys	10	4.7
Arg	12	6.9
Trp	0	0

In the four lysozymes isolated from the latex of *Asclepias syriaca*, also, no tryptophan could be detected [4], although that residue is found in the analogous enzymes in latices of papaya and fig [3]. While a tryptophan apparently has an important role in the mode of action of the lysozymes from avian sources, this is not so in those from plants as evidence reported above and elsewhere [3] shows. Neither glucosamine nor galactosamine were evident in the lysozyme from *A. chinensis* following mild acid hydrolysis: the enzyme is, then, probably not a glycoprotein.

Histamine is an effective inhibitor of both avian and plant lysozymes [3]. It is reactive with the enzyme from *A. chinensis* which is completely inhibited by 9 mM histamine and 50% inhibited by 1.6 mM reagent. This enzyme is, then, as sensitive to histamine as the lysozymes from fig [3] and milkweed [4].

Avian lysozymes are inhibited by low concentrations of *N*-acetyl-D-glucosamine while those from plants are relatively unaffected [3]. The enzyme from the kiwi fruit

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resembles the other plant lysozymes in this respect, being only 50% inhibited by 400 mM *N*-acetyl-D-glucosamine. That is, it is comparable with the enzymes from papaya and fig [3] and more sensitive than those from *Asclepias syriaca* [4].

The lysozymes from papaya and fig, like those from eggs, are also chitinases, the two plant enzymes being more active towards chitin than the lysozymes from eggs [3]. Lysozyme from *A. chinensis* is a relatively inactive chitinase, the homogeneous enzyme having a specific activity of 0.4 μg glucose equiv./min/mg, compared with 1 unit for egg white lysozyme when measured under the same conditions.

EXPERIMENTAL

Reagents. Dried cells of *Micrococcus lysodeikticus*, histamine and *N*-acetyl-D-glucosamine were obtained from Sigma, and Bio-Rad supplied the reagents for preparing, running and staining the cationic electrophoresis gels, as well as the BioGel P-100 and P-30. Pharmacia supplied reagents for the 'PhastGel' system. The CM-52 cellulose was from Whatman. The Pierce Chemical Co. provided the methane sulphonic acid, and Amicon the Diaflo membranes.

Methods. Assays: A suspension of 10 mg dried *M. lysodeikticus* cells in 100 ml of 100 mM NaOAc at pH 4.6 was used to measure the initial rate of digestion, at 37°, turbidimetrically at 440 nm [10]. The results are expressed as units of A_{440}/min/ml enzyme soln. Adjustment of digestion times and enzyme concns. ensured that measurements were of initial rates. Chitinase activity was assayed with colloidal chitin in digestions at 37° [3] using 100 mM NaOAc at pH 5.6 as buffer. The reducing groups so produced were measured, and activity is expressed in units of μg glucose equivalents/min/ml enzyme soln [11]. Protein content was measured as A_{280}, or the Folin procedure was used with bovine serum albumin as standard. Cationic electrophoresis gels were prepared following ref. [12]; the Pharmacia 'Phastgel' system was used for the SDS gels. M_r determinations were made on a column (1.5 × 100 cm) of BioGel P100 equilibrated with 100 mM NaOAc buffer, pH 4.6, at 4°. This was calibrated with bovine plasma albumin, ovalbumin, trypsinogen and egg white lysozyme [13]. Amino acid analyses were run on a Durrum D-500 automatic analyser, which was also used to determine amino sugars after hydrolysis in 4 M HCl for 6 hr at 110° under vacuum. Conventional hydrolysates were prepared in 6 M HCl under vacuum for 22 hr. Tryptophan was determined after hydrolysis at 110° in 4 M methane sulphonic acid, under vacuum for 22 hr [8]. That residue was also assayed using *N*-bromosuccinimide [9] and a spectrophotometric method at alkaline pH [7]. Cysteine was determined using the procedure of ref. [14].

Purification of lysozyme from *Actinidia chinensis*. The juice and flesh were removed from the skin of the commercially available fruit, macerated and centrifuged at 15000 *g* for 30 min. The supernatant (35 ml) contained 4 A_{440}/min total lysozyme activity. Half of this was loaded on a Bio-Rad P-30 column (60 × 3 cm) equilibrated with 100 mM NaOAc, pH 4.6 at 4°, and the active peak collected (8 A_{440}/min total) when there was apparent separation from an inhibitor. The pooled eluate was concd on a PM10 Diaflo membrane, with complete recovery of activity, and applied to a column of CM-52 cellulose (2.5 × 40 cm) equilibrated with 100 mM NaOAc pH 4.6 at 4°. After washing with 100 ml of that buffer, the lysozyme was eluted with a linear gradient composed of 100 ml each 100 mM NaOAc, pH 4.6 to 300 mM NaOAc, pH 5.6 at 4°. The recovery of activity was 15%, and this product was combined with several other similar collections before the purification proceeded.

After concn of those combined pools on a Diaflo membrane, when there was 70% recovery of activity, the concentrate was dialysed against 150 mM NaOAc, pH 5.2 and recycled through a CM-52 cellulose column (25 × 1.5 cm) at 4°. A linear gradient from 50 ml 150 mM NaOAc, pH 5.2 to 50 ml 300 mM acetate pH 5.6 was used, and followed by washing with 50 ml of the latter buffer. The yield was 68% of the applied lysozyme.

After concn on the Diaflo membrane, the lysozyme was subjected to HPLC on a Bio-Sil TSK-250 column with 200 mM K-Pi, pH 7, as solvent, at 22°, using a Beckman Model 110A pump and Varian Model 2050 UV detector. The sole enzymically active peak, from the several separated, was collected and recycled on the HPLC apparatus. The final yield was 0.22 A_{440}/min–11% of that measured in the juice used.

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