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FICIN E, A SERINE-CENTRED PROTEASE FROM *FICUS ELASTICA**

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Key Word Index—*Ficus elastica*; fig; Moroceae ficin e; serine protease.

Abstract—A protease of M_r 50000 was purified from the latex of *Ficus elastica*. The enzyme has a pI of 3.7 and pH optimum at 6.0. Its activity is dependent on serine and histidine residues. The amino acid composition is reported.

INTRODUCTION

Ficin (EC 3.4.22.3) is a proteolytic enzyme with an essential cysteine at the active site [1], which is commonly isolated from *Ficus glabrata* [2]. A compositionally similar enzyme was purified from *F. anthelmintica* [2, 3], though that from *F. carica* differs in amino acid composition [4] while still having an essential cysteinyl residue at the active site. The enzyme from *F. glabrata*, which is the most extensively studied, exists in a number of charged forms [4, 5] and is related by sequence studies, in the regions of both the vital cysteine and histidine residues, to papain [6] obtained from the latex of papaya (*Carica papaya* L.).

The protease of *Ficus elastica*, a common house plant, has not formerly been investigated, and during a continuing study of the proteases from a variety of plant latices [7–13] we have lately examined the latex of that species. Unlike the proteolytic enzymes of *F. glabrata* and the other members of this genus listed above, protease activity in *F. elastica* was unaffected by the presence of mild reducing agents or such sulphydryl-specific agents as *p*-chloromercuribenzoate, suggesting that enzymic activity is not determined by a vital cysteinyl-residue.

We have recently isolated and described [7–13] a number of trypsin-like proteases of the serine class from latices of the genera *Euphorbia*, *Elaeophorbia* and *Hevea* of the family Euphorbiaceae. As that family is of the order Urticales, which comprises also the Moraceae to which *Ficus* belongs, it was of interest to examine the protease of *F. elastica* for comparison with the analogous enzymes previously characterized [7–13].

We shall report here the isolation and purification to homogeneity of a protease from the latex of *F. elastica* and describe some properties of that enzyme. To distinguish it from those previously isolated from *Ficus* species [2–4] we have given it the trivial name ficin e.

RESULTS AND DISCUSSION

The product from HPLC had an M_r of 50000, estimated using a standard line composed of M_rs *versus* elution times for immunoglobulin G, bovine serum albumin, ovalbumin, myoglobin and lysozyme. This is double the M_r of the enzyme from *F. glabrata* [2]. On SDS-gel electrophoresis the enzyme fragmented to a unit of M_r 29000 with a minor band of 27000. Isoelectric focusing [14] showed that ficin e consisted of a single charged form with a pI of 3.7. The comparable measurement for ficin from *F. glabrata* is not available, but the pI of the related enzymes papain and bromelain [1] are 8.75 and 9.5 respectively. Clearly ficin e is distinct from previously examined ficins.

Ficin e is reactive with the proteolytic substrates azocollagen (0.05 units/mg enzyme) azocasein (0.04 units/mg) and hemoglobin (0.03 units/mg), demonstrating its activity as a protease. This is further demonstrated in Fig. 1 which contains two dimensional thin layer maps of digests of insulin B chain made with ficin e and with ficin from the latex of *F. glabrata*. It is evident that the two proteolyses are different, and as that catalysed by the latter ficin is related to those reported for other sulphydryl proteases [15] it is again demonstrated that ficin e is a different enzyme.

While such sulphydryl specific reagents as *p*-chloromercuribenzoate inhibit ficin and other cysteinyl proteases, they are without effect on ficin e. However that enzyme was rapidly and completely inhibited by the classic serine-specific reagent di-isopropyl fluorophosphate [16]: 96% inhibition in 40 min with a 100-fold molar excess of reagent. A second, less powerful inhibitor of serine enzymes, phenylmethane sulphonyl fluoride [17] caused 75% inhibition of ficin e when in 10^6-fold excess. Diethyl pyrocarbonate, a reagent with high specificity for histidine residues [18] completely inhibited ficin e when in 10^4-fold molar excess. The protease from *F. elastica* thus apparently has essential serine and histidine residues, and so resembles some of the trypsin-like enzymes isolated from the family Euphorbiaceae [7–13]. Other inhibitors of the serine class of proteases—chymostatin, elastatinal, potato inhibitor I and soybean trypsin inhibitor—did not affect ficin e activity. This was also unaffected by two reagents specific for carboxyl-centred proteases such as pepsin, namely pepstatin A and diazo-DL-norleucine methyl esters.

Ficin e has an optimal pH at 6.0, measured with azocollagen as substrate. In Table 1 the results from amino acid analyses of the protease ficin e are presented. Shortage of material prevented determinations of cysteine,

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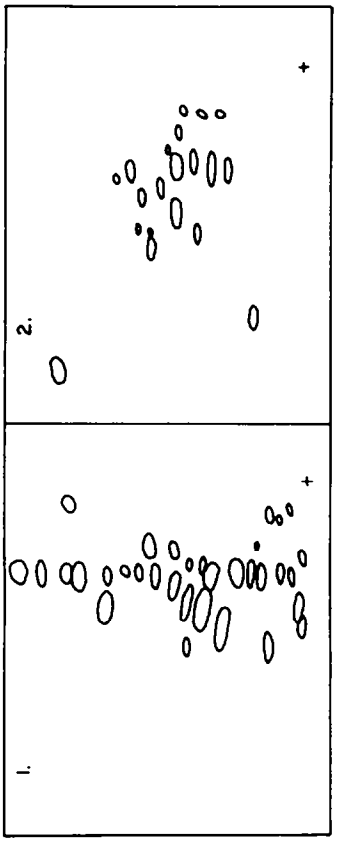

Fig. 1. Two dimensional thin layer peptide maps of the digestion of insulin B chain with (1) ficin from *F. glabrata* and (2) ficin e.

tryptophan and amino sugars, but the data clearly show that this enzyme has a composition distinctly different from that of the ficins from *F. glabrata* [2] and *F. carica* [4] as well as *F. anthelmintica* [2, 3]. Nor is it closely related, in composition, to the proteases from Euphorbiaceae [7–13].

Table 1. Amino acid composition of ficin e

Amino acid	Number	Weight % composition
Cys	n.m.*	n.m.
Asx	74	17.4
Thr	38	7.8
Ser	37	6.7
Glx	44	11.6
Pro	25	5.0
Gly	38	4.4
Ala	32	4.6
Val	25	5.1
Met	2	0.5
Ile	22	5.1
Leu	39	9.0
Tyr	14	4.7
Phe	23	6.9
His	7	2.0
Lys	12	3.1
Arg	19	6.1
Trp	n.m.	n.m.

*Not measured.

EXPERIMENTAL

All reagents employed were of analytical grade except as noted. BioGel P150, BioLyte ampholytes and BioRad protein reagent were obtained from BioRad. Sigma supplied the mercaptoethanol, diazo-DL-norleucine methyl ester, diethylpyrocarbonate, *p*-chloromercuribenzoate, CBZ-glycine-*p*-nitrophenyl ester (CGN), azocollagen, azocasein, hemoglobin, insulin B chain, di-isopropyl fluorophosphate, soybean trypsin inhibitor, leupeptin and pepstatin A. Antipain, elastatinal and chymostatin were purchased from the Protein Research Foundation, Osaka, Japan. Potato inhibitor I was prepared as described in ref. [19].

Assay methods. Procedures described were used with CGN [7], azocollagen [7], azocasein [8] and hemoglobin [13]. Protein content was measured as A_{280} or using the Folin–Lowry procedure. Various inhibitors of the several classes of proteases were tested by measuring the residual activity of ficin e under standard conditions with CGN [7] in the presence and absence of the inhibitors in varying concns.

SDS-polyacrylamide gel electrophoresis. The procedure of ref. [14] was used.

Isoelectric focusing. BioLyte ampholytes were employed in the method of ref. [20].

M_r *determination with HPLC.* A column (300 × 7.5 mm) of TSK Spherogel G3000SW, equipped with a 10 mm precolumn (BioRad) was used with a Beckman Model 110A solvent metering pump and a Varian Model 2050 UV detector. The buffer employed was 200 mM KPi, pH 7 at 21° and a flow rate of 0.5 ml/min.

Amino acid analyses. Shortage of purified enzyme only allowed duplicate standard hydrolysates in 6 M HCl under vacuum at 110° for 22 hr. Amino acid analyses were made on a Durrum D-500 automatic analyser using previously calibrated columns. Norleucine was used as an internal standard. The independently determined M_r was employed in the calculation of the amino acid residues.

Mapping digests of insulin B chain. Digestions were made with 600 μg/ml insulin B chain in 100 mM NH_4OAc, pH 7, at 37° for 18 hr. The enzyme/substrate ratio was 1 : 100 by wt. Peptide maps were prepared on thin layer cellulose sheets (Macherey–Nagel Polygram Cel 400) and developed, as described in ref. [21].

Purification of ficin e. Ficus elastica Roxb. & Homem (var. decora) plants were obtained commercially and latex collected from incisions in the trunk and stems. Larger trees of the same species at the greenhouse, Carleton University, Ottawa were similarly 'milked'. The latex obtained was clarified by centrifugation (20 000 *g*, 1 hr) immediately following collection, and stored at −20°. The protein content of the latex was only 600 μg/ml, which limited the availability of ficin e.

Latex serum (26 ml, sp. activity 3.3 units/mg) was applied to a column (1.5 × 90 cm) of BioGel P 150 in 10 mM NaOAc, pH 6, at 4°. The esterolytically active peak was concd and subjected to HPLC on TSK G3000SW at room temp. in 200 mM KPi buffer, pH 7. A second cycle through the same HPLC column produced homogeneous enzyme. The sp. act. of the ficin e was 45 units/mg (13.6 fold purification); the yield of protein was 2 mg (1.8%).

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