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PURIFICATION OF GINGER PROTEASE USING AFFINITY CHROMATOGRAPHY AND CHROMATOFOCUSING

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Summary

The active material in dried ginger obtained from Kenya, East Africa, has been extracted using the method described by Awang (1980). The yield of the crude enzyme was found to be about 10 per cent per dried acetone powder. On affinity chromatography the crude preparation separated into five active fractions while on chromatofocusing adsorbent the crude enzyme separated into seven active fractions. The results are in contrast to what has been reported by other workers.

Introduction

Ginger is the name given to the dried rhizome (underground stem of *Zingiber officinale* Rose), a herbaceous perennial belonging to the family of Zingiberaceae. It is believed to be a native of South East Asia (Parry, 1969), though today, it is cultivated in many tropical and sub-tropical countries especially in Africa, West Indies, India, Japan and China.

It is one of the variety of plants containing appreciable amounts of proteolytic enzyme activity. Ginger protease, a proteolytic enzyme obtained from ginger, is a thiol protease and, unlike papain, it is heterogeneous (Awang, 1980; Ichikawa, Sasa & Michi, 1973; Thompson, Wolf & Allen, 1973).

The enzyme is reported to require low concentrations of activators for maximum activity, and it is believed to possess no amidase activity though it shows esterolytic activity (Awang, 1980). Using ion exchange chromatography, Awang (1980) found that his preparation of crude ginger protease had three active components. On affinity chromatographic (AC) adsorbent, the crude enzyme separated into thiol and non-thiol fractions (Awang, 1980).

The aim of this study was to investigate whether using other chromatographic techniques the crude preparation could be separated into more components than already reported. Two chromatographic techniques have been employed, viz. affinity chromatography and chromatofocusing.

Experimental

Isolation of active materials from ginger

Dried ginger (from Kenya, East Africa) was purchased from Leeds (W. Yorkshire) Market and the proteolytically active material was extracted using the method described by Awang (1980). The yield of the crude enzyme was found to be 10.23 ± 0.8 per cent per dried acetone powder. The crude enzyme preparation was then purified using affinity chromatography (AC) and chromatofocusing.

Purification of crude ginger protease

The affinity chromatography (AC) technique used was as described by Sluyterman & Wijdenes (1970), except that in this case the adsorbing protein, i.e. the thiol fraction of the preparation, was eluted by ionic gradient using $0.5 - 2.0 \times 10^{-3}$ *M* $HgCl_2$. The results obtained are presented in Fig.1. a and b.

Chromatofocusing method used was as described by Sluyterman & Elgersma (1978). The adsorbent, DEAE Biogel A, was equilibrated with 3.5 *m M* ammonia-glycine buffer, *p*H 8.5. The

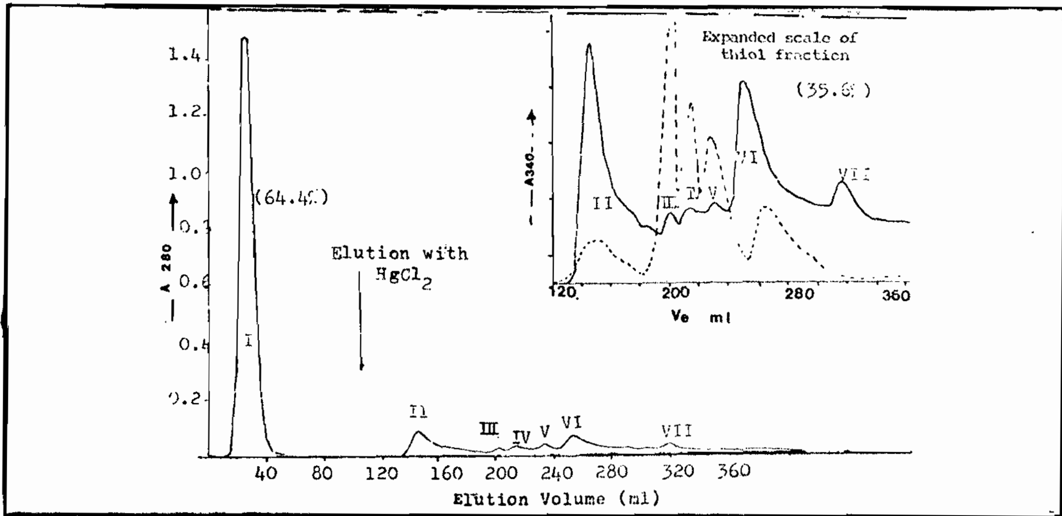

Fig. 1a. Sepharose 4B Column: Elution of bound fraction by $HgCl_2$ gradient (0.5-2.0 *m M* $HgCl_2$)

sample was then applied using a sample applicator and the separation was effected with the elution buffer, 0.15 per cent ampholine, *p*H 5-8, containing 5 per cent dimethylsulphoxide and adjusted to *p*H 5.8 with acetic acid. The results obtained are presented in Fig. 2.

Kinetic studies on the fractions from AC column

The Km and Vmax of the various fractions obtained from the AC column were determined using CGNP in 0.1*M* phosphate buffer *p*H 6.5 at 37 per cent. The results obtained are presented in Fig. 3 and Table 2.

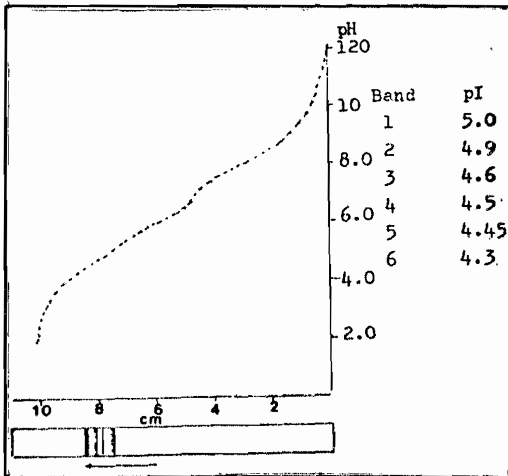

Fig. 1 b. Sepharose 4B Column: 1EF of nonthiol fraction of ginger proteases.

Results

Seven fractions were obtained from the affinity chromatography column (Fig.1). Fractions 1, which was the non-thiol component of the crude enzyme, constituted 64.5 per cent of the total protein applied. The thiol components, fractions II - VII constituted 35.5 per cent of the total protein applied. Fraction 1 had very little ac-

TABLE 1

Comparison of activities of various fractions obtained from AC column

Fraction	*Activity (A340/min)*	*Specific activity (A340/min/mg protein)*	*Purification factor*
I	4.64×10^{-3}	4.16×10^{-3}	0.19 ×
II	3.02×10^{-3}	38.97×10^{-3}	1.73
III	6.73×10^{-3}	299.11×10^{-3}	13.29
IV	4.97×10^{-3}	194.90×10^{-3}	8.66
V	4.81×10^{-3}	173.93×10^{-3}	7.73
VI	5.12×10^{-3}	73.14×10^{-3}	3.25
VII	No activity	-	-

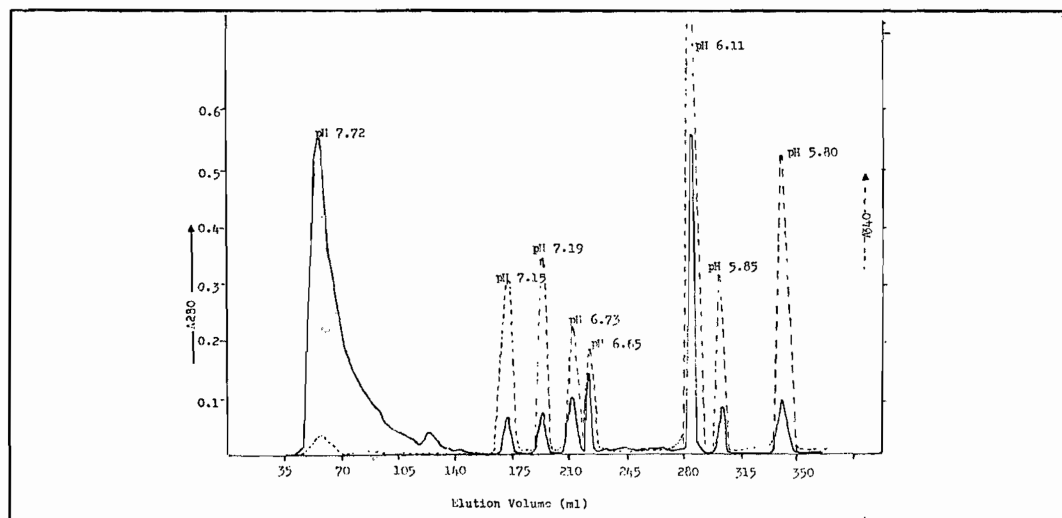

Fig. 2. Chromatofocusing of crude ginger protease (*p*H 8.5 - 5.85)

TABLE 2

Comparison of Km and Vmax of the various fractions obtained from AC column

Component	*Km (M)*	*Vmax (A340/min)*
Crude	9.3×10^{-3}	0.39
F_{II}	8.5×10^{-3}	0.20
F_{III}	4.0×10^{-3}	0.21
F_{IV}	4.3×10^{-3}	0.24
F_{V}	3.3×10^{-3}	0.31

tivity; the bulk of the activity was restricted to the thiol components. Table 1 shows the activities of the fractions obtained.

The $HgCl_2$ gradient elution separated the thiol component into six fractions, the major components being fractions II, VI and VII. Fractions III, IV and V were similar in protein concentration. Fraction II had the highest specific activity using CGNP as the substrate. Fraction VII had no activity towards CGNP.

The results presented in Fig. 2 show the behaviour of crude ginger protease on a chromatofocusing column. The crude enzyme fractionated into nine components with eight of them showing activity towards CGNP and casein.

The components obtained from the column were eluted with pI (app) with the given values: (FI) 7.72, (FII) 7.29 (no enzymic activity), (FIII) 7.15, (FIV) 7.19, (FV) 6.73, (FVI) 6.65, (FVII) 6.11 (highest specific activity), (FVIII) 5.85 and (FIX) 5.80.

The results of the kinetic studies carried out on the various fractions obtained from the AC column are presented in Fig. 3 and Table 2.

Discussion

Crude ginger protease was found to be made up of five to seven active fractions. This is in contrast to what has been reported by other workers. Awang (1980) using ion exchange chromatographic technique found that his preparation had three active fractions. The difference in the number of active components of the crude preparation might be due to the methods used in the fractionation, or the ginger roots from different geographical locations might have different active components as found for ficin by Glazer & Smith (1971), Sgarbieri *et al.* (1964) and Bernhard & Gutfreud (1956).

Gradient elution of the thiol components of crude ginger protease adsorbed on

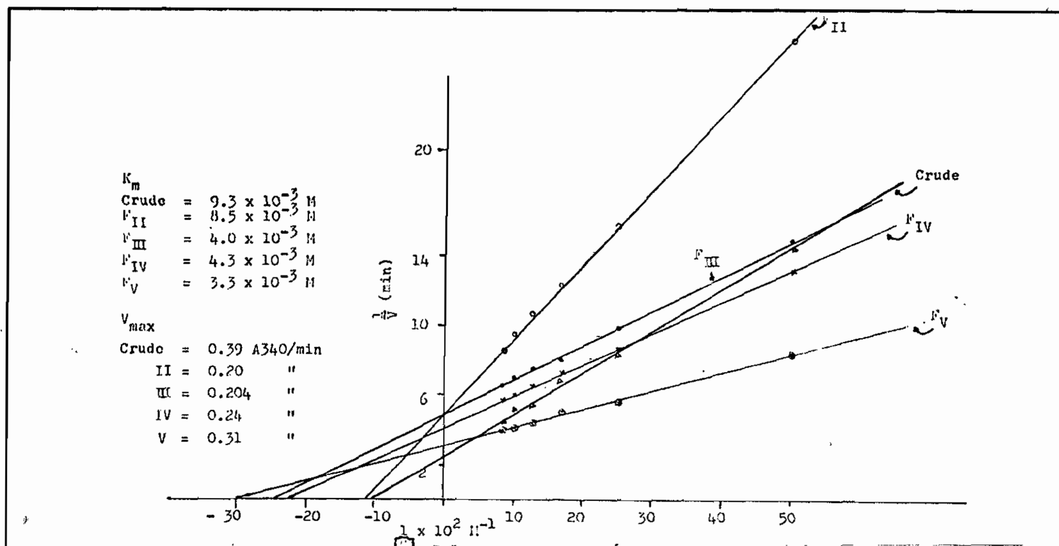

Fig. 3. Lineweaver and Burk plot for the determination of Km and Vmax of ginger protease using CGHP as substrate at *p*H 6.5, 37 °C.

organomercurial agarose adsorbent showed that the various components of the thiol fraction are held on the adsorbent at different ionic strengths. Thus, advantage can be taken of these varying binding strengths to separate and purify the individual components of the thiol fraction using only the AC technique if a proper ionic gradient is used.

Sluyterman & Wijdenes (1970) and Awang (1980) have used the AC technique to separate papain and ginger protease respectively into two fractions, viz. thiol and non-thiol fractions. Studies carried out on the thiol fraction of crude papain eluted from an AC column have revealed that this fraction is not homogeneous but rather a heterogeneous mixture containing at least seven components with pIs ranging between *p*H 4.40 and *p*H 10 (Agyeman, 1983). It follows that if a constant ionic strength of the elution buffer is used, advantage can be taken of the varying binding strengths of the various components of the thiol fraction.

The results suggest that AC can be a very powerful purification technique if differential elution is employed. It may be worth mentioning that F_{II} from the AC column is different from the F_{II} obtained from the chromatofocusing chromatographic column since F_{II} from the latter showed no activity towards the substrate.

Thus, using chromatofocusing technique, ginger protease can be fractionated into its various components with high degree of purity. It can also be deduced that though the enzymic activity of the crude preparation is restricted to the thiol fraction, not all the components of the thiol fraction are active. The different values of Km and Vmax obtained for the various fractions eluted from the AC column suggest that these fractions are not mere contaminations but real active components of crude ginger protease.

From Table 2 it appears that F_{II} showed an affinity similar to that of the crude enzyme for the substrate. Thus F_{II} may be the major component of crude ginger protease. Awang (1980) has reported a Km of 1.2×10^{-4} *M* for his preparation with a Vmax of 0.11 min^{-1} using the same substrate under similar conditions except temperature at 25 °C. Plant proteases show considerable variations in their properties depending on their source. Comparisons among them are,

therefore, very difficult especially if they come from different geographical locations.

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