ISOLATION AND PROPERTIES OF BROMELIN PROTEASE

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1. Introduction

Bromelin (E.C. 3.4.4.24) is a mixture of several enzymes obtained from the stems of *Ananas comosus* [1]. Several authors, using different methods, succeeded in separating this mixture into various components [2–5]. Besides proteases bromelin contains acid phosphatase and ribonuclease [6]. This report is concerned with the enzymatic properties of bromelin main peak protease (denoted "bromelin protease" in this paper).

2. Experimental

Isolation of bromelin protease from crude bromelin by gel chromatography on Sephadex® G-75 in 0.06 M phosphate buffer pH 5.7 (fig. 1). Separation of bromelin protease into components E and A by gel chromatography on DEAE-Sephadex® A-50 in 0.05 M phosphate buffer pH 7.5 using a 2 M KCl gradient (fig. 3). Preparation of heat inactivated bromelin protease by heating the protease solution for 10 min at 80°C.

Test for enzymatic activity with substrates L-leucine-*p*-nitranilide (Leupa), L-alanine-*p*-nitranilide (Alapa), L-phenylalanine-*p*-nitranilide (Phenpa), and *N*-benzoyl-D,L-arginine-*p*-nitranilide (Bapa): 0.2 ml enzyme solution (10 mg/ml), 0.2 ml dithiothreitol solution (0.8 mg/ml), preincubation 5 min, addition of 2.1 ml buffer solution, start of reaction with 0.3 ml substrate solution ($1-5 \times 10^{-3}$ M), pH 7.0, 25°C. With Bapa start of reaction with 2.4 ml buffer-substrate solution. The enzyme reaction was measured every two minutes for at least 20 min. Test with hemoglobin substrate according to Anson [7].

3. Results and discussion

Gel chromatography of crude bromelin on Sephadex® G 75 results in 5 peaks. The first peak contains acid phosphatase, the second peak contains the main protease (fig. 1). The activity of this protease against hemoglobin is 3.5–4 m Anson U/mg, the activity against Bapa is about 2.5 mU/mg. Using both substrates the reaction velocity shows linear dependence upon the enzyme concentration. The substrates Alapa,

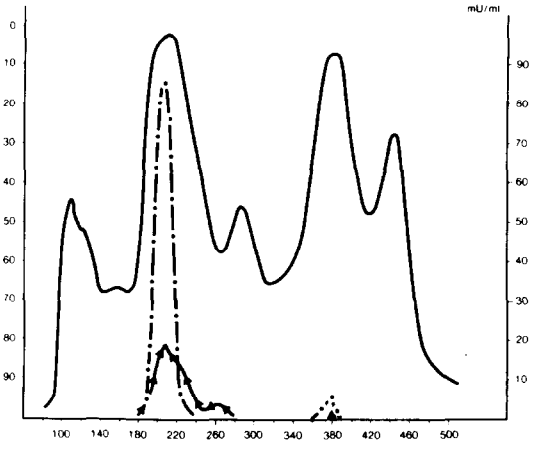

Fig. 1. Gel chromatography of crude bromelin on Sephadex® G-75, column 7 X 120 cm, 0.06 M phosphate pH 5.7, fractions of 10 ml. Bromelin protease = fractions nos. 186–236.
—— transmittance 280 nm
●—● protease activity (mU Leupa)
▲—▲ protease activity (mU Bapa)

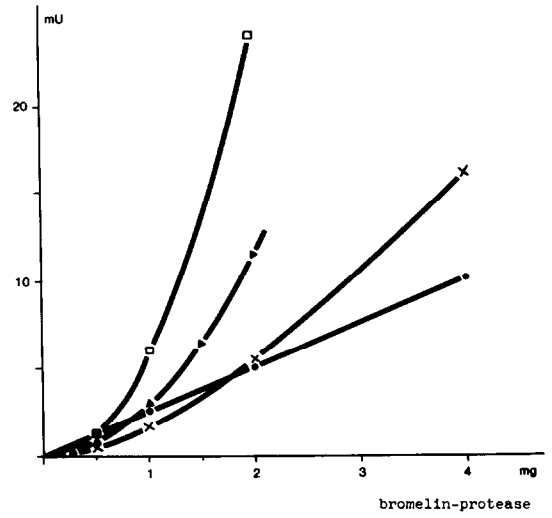

Fig. 2. Dependence of enzymatic activity on concentration of bromelin protease.
▲ Leupa 3×10^{-3} M □ Leupa 10×10^{-3} M
x Alapa 10×10^{-3} M ● Bapa 3.2×10^{-3} M

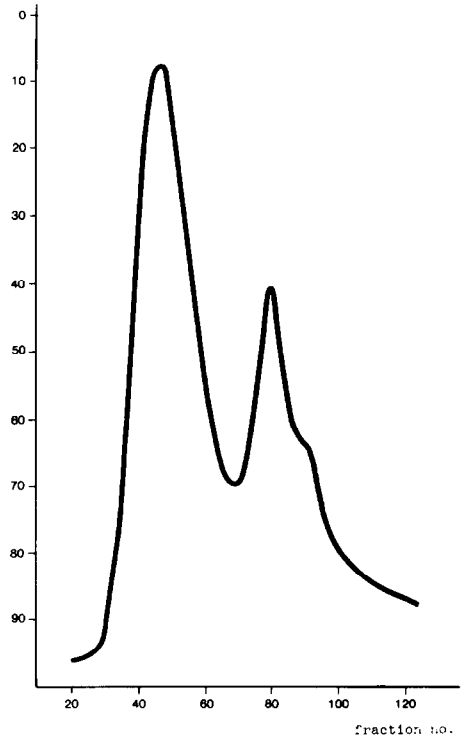

Fig. 3. Ion exchange chromatography of bromelin protease on DEAE-Sephadex® A-50, column 2.5 × 40 cm, 0.05 M phosphate pH 7.5, 2 M KCl gradient, fractions of 3 ml. Component E = fractions nos. 41–54. Component A = fractions nos. 76–86.

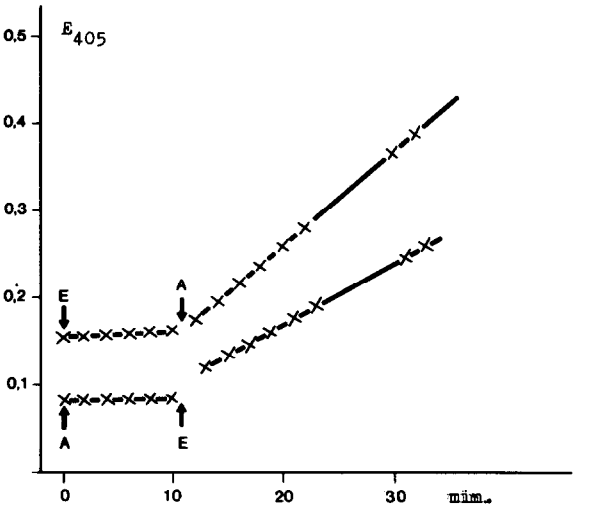

Fig. 4. Hydrolysis of Leupa (3×10^{-3} M) by combinations of components E and A. E: 1 mg component E, A: 0.5 mg component A.

Leupa and Phenpa are hydrolyzed by the protease, but the reaction velocity increases at a higher than linear rate with increasing enzyme concentration (fig. 2). This unusual dependence of the enzyme activity (U/mg) on the enzyme concentration is independent of variations of substrate concentration, buffer and pH. The addition of heat inactivated bromelin protease increases the reaction velocity of bromelin protease against Leupa. The increase of reaction velocity is proportional to the concentration of the heat inactivated protease added.

Bromelin protease is separated on DEAE-Sephadex® A 50 into 2 components (fig. 3). The first component E hydrolyzes hemoglobin (2.5 m Anson U/mg) and Bapa (1 mU/mg), but does not hydrolyze Leupa. The second component A is enzymatically inactive. When component A is added to the not reacting mixture of component E plus Leupa, or vice versa, the enzyme reaction starts (fig. 4). The reaction velocity increases almost linear with the concentration of the added component, if the concentration of the other component is kept constant (table 1). When the concentration of both components is raised in a constant proportion, the reaction velocity increases at a higher than linear rate. This is the same phenomenon as observed with non-fractionated bromelin protease (see table 1 and fig. 2).

The cooperation between components E and A is

Table 1
Hydrolysis of Leupa by combinations of bromelin protease components E and A.

Component E (mg/test)	Component A (mg/test)	Leupa hydrolysis (mU/test)
1.0	0	0.2
1.0	0.25	2.5
1.0	0.5	4.5
1.0	1.0	8.1
2.0	0	0
2.0	0.25	4.6
2.0	0.5	8.2
2.0	1.0	15.6

the reason for the unusual behaviour of the bromelin protease against the substrates Leupa, Alapa and Phenpa. The reaction between component E and hemoglobin or Bapa resp. is not influenced by component A. These findings are consistent with the following interpretation: Component E hydrolyzes substrates of high molecular weight. Bapa is hydrolyzed unaided, because the cleaved bond is not adjacent to the free α amino group. The other tested amino acid derivatives of low molecular weight with a free α amino group are only hydrolyzed by component E if component A is present. Further work is in progress.

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