The Action of Acid Proteinase from *Aspergillus oryzae* on Soybean Proteins[†]

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Until recently it has long been considered that large amounts of free amino acids are liberated from proteins by the hydrolytic action of acid protease of *Aspergillus oryzae*.¹⁻⁵⁾ Yoshii has reported that free amino acids are liberated from soybean proteins by acid protease in miso (soybean paste) brewing.⁶⁾ Recently however, crude acid proteinases are reported to be contaminated with acid carboxypeptidase in cases of *A. oryzae*,⁷⁾ *A. saitoi*,⁸⁾ and *A. niger*.^{9,10)} This suggests that liberation of free amino acid in miso brewing is due to the action of carboxypeptidase of the mold.

In this connection, the present paper deals with separation of acid proteinase from acid carboxypeptidase and hydrolytic action of each enzyme on ALPHA-protein.

Acid proteinase from *A. oryzae* 460 was not separated from acid carboxypeptidase IV by column chromatography on DEAE-cellulose, DEAE-Sephadex A-50, hydroxylapatite, P-cellulose and gel filtration through Sephadex G-100. The partially purified enzyme preparation separated by the above-mentioned methods was dissolved in 150 μ l of 20% sucrose and spacer gels were omitted. Each of 10 μ l of enzyme solution was applied to polyacrylamide gel disc electrophoresis at pH 4.0 (12 columns).¹¹⁾ Electrophoresis was run in a cold room (4°C) for 7 hr at 4 mA per gel column. Frozen gels were sliced laterally into discs 2 mm thick, extracted overnight at 4°C with 1 ml of 0.05 M acetate buffer at pH 4.5 and enzyme activity was assayed with an aliquot of each extract. Figure 1 shows typical electrophoretic patterns of acid proteinase and acid carboxypeptidase IV¹²⁾ from *A. oryzae*. Fractions 7 to 9 were collected, desalted by dialysis and used as acid proteinase.

Acid proteinase has an optimum pH of 3.7 for milk casein. The molecular weight of the enzyme was estimated to be about 39,000 by the gel filtration method.¹³⁾

Table I shows some results on hydrolysis of soybean ALPHA-protein by 18.1 PU of purified acid proteinase (254 μ g) containing trace (526×10^{-5} unit, protein;

FIG. 1. Polyacrylamide Gel Electrophoresis of Acid Proteinase.

Electrophoresis was run in a cold room (4°C) for 7 hr at 4 mA per gel column at pH 4.0.

○—○, proteinase activity on casein (pH 3.0); ●—●, carboxypeptidase activity on Cbz-Glu-Tyr (pH 3.0).

TABLE I. HYDROLYSIS OF α -PROTEIN BY ACID PROTEINASE FROM *A. oryzae*

T. N.	0.0252%
F. N.	0.0028%
Glu.	0 mg/ml
N. P. ^{a)}	0.227 mg/ml
HCl-N. P. ^{b)}	1.525 mg/ml
F. N./T. N.	11.0%
T. N./F. N. ^{c)}	9.00
HCl-N. P./N. P.	6.71

^{a)} Ninhydrin positive substances determined as glutamic acid.

^{b)} Ninhydrin positive substances determined as glutamic acid after re-hydrolysis with HCl of enzyme digested products.

^{c)} Indicates an average number of amino acids in peptides molecules.

0.39 μ g) of acid carboxypeptidase IV¹²⁾ at pH 5.0 for 12 days at 30°C.¹⁴⁾ Commercially available ALPHA-protein (trade-mark) derived from soybeans was obtained from Nutritional Biochemicals Corporation (T. N.; 14.24%). No glutamic acid (Glu.) was found in the hydrolyzate by acid proteinase and only very low value of formol nitrogen (F. N.) was obtained. The hydrolyzed products seemed to be peptides consisted of 7 to 9 amino acids, but not to be free amino acids based on the ratio of T. N./F. N. and on the increase of ninhydrin positive substances after re-hydrolysis with HCl.

Next, ALPHA-protein was hydrolyzed by a mixture of 157 PU of acid proteinase and 5.38 units of acid

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TABLE II. HYDROLYSIS OF α -PROTEIN BY THE MIXTURE OF ACID PROTEINASE AND ACID CARBOXYPEPTIDASE IV FROM *A. oryzae*

T. N.	0.0642%
F. N.	0.0175%
Glu.	0.233 mg/ml
F. N./T. N.	27.2%
T. N./F. N. ^{a)}	3.67

^{a)} Indicates an average number of amino acids in peptide molecules.

carboxypeptidase IV partially purified without disc electrophoresis at pH 5.0 and 30°C for 3 days. As shown in Table II, some glutamic acid was detected in the hydrolyzate and a comparable value of formol nitrogen to total nitrogen was found in this case.

Acid proteinase showed neither carboxypeptidase nor aminopeptidase activity. Acid proteinase was found to be a typical endopeptidase liberating only little free amino acids from protein. The mixed preparation of acid proteinase and acid carboxypeptidase IV formed large amounts of free amino acids.

These results clearly indicate that free amino acids are liberated from soybean protein by the hydrolytic action of both acid proteinase and acid carboxypeptidase of *A. oryzae* in the miso brewing, but not liberated by the hydrolytic action of only acid proteinase.

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