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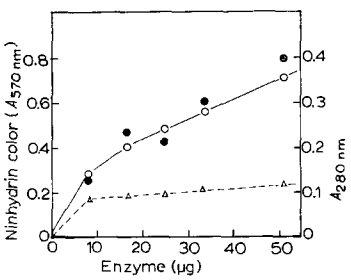
A unique carboxypeptidase activity of yeast proteinase C

Baker's yeast contains three intracellular proteinases, A, B and C. One of them, proteinase C, was isolated in a pure form and its enzymatic and physicochemical natures have been described previously[1]. This enzyme has been regarded as an endopeptidase since it can hydrolyze casein at pH 6 and *N*-acetyltyrosine* ethyl ester, the specific substrate of chymotrypsin, at pH 8.0. However, the enzyme also rapidly hydrolyzes the substrates of carboxypeptidase, *e.g.* Z–Gly–Phe, Z–Gly–Leu and Z–Glu–Tyr. So, a unique specificity has been expected for yeast proteinase C.

Recently, COHEN[2] suggested that a new type of proteinase was required for the intracellular protein turnover. Such an enzyme would be characterized as an exopeptidase and would be able to hydrolyze all types of peptide bonds, liberating only free amino acids as the products. Because yeast proteinase C is an intracellular enzyme, we investigated the possibility that the enzyme might correspond to his suggested enzyme. In this communication, we demonstrate that yeast proteinase C should be characterized as a carboxypeptidase having a broader specificity than bovine carboxypeptidases.

Proteinase C was isolated from baker's yeast (Oriental Yeast Co.) by the method described previously[1] with a slight modification. This enzyme hydrolyzes a small amount of Hammarsten casein at pH 6.2 under the experimental conditions standard-



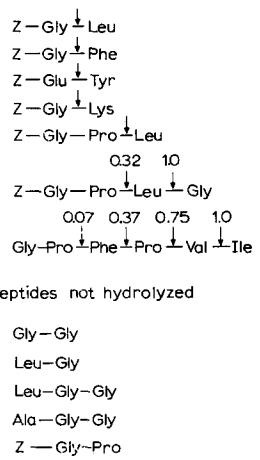


Fig. 1. Hydrolysis of casein by yeast proteinase C. 0.5% casein in 0.02 M sodium phosphate buffer (pH 6.2) was incubated with the indicated amounts of the enzyme for 30 min at 25° as described in the text. 0.2 M trichloroacetic acid-soluble fractions were obtained by filtration and each fraction was divided into two portions. One of these was employed in the measurement of absorbance at 280 nm (△ - - - △). The other portion was washed 3 times with ether and used for ninhydrin color reactions[5] before (○—○) and after (●—●) alkaline hydrolysis.

Fig. 2. Actions of yeast proteinase C on various peptides. The enzymatic hydrolysis of the peptides was made in 0.05 M pyridine–acetate buffer (pH 4.6) for 60–120 min at 25°. The substrate to enzyme ratio was about 90 on the basis of weight. The figures indicate the relative quantity of liberated amino acids.

Abbreviation: Z–, carbobenzoxy.

* All amino acids in the present paper have the L-configuration except for glycine.

ized by HAGIHARA *et al.*[3]. As shown in Fig. 1, trichloroacetic acid-soluble compounds having absorbance at 280 nm increased only slightly with increasing amounts of the enzyme. This phenomenon may be explained as due to the narrow substrate specificity of the enzyme, if it is an endopeptidase. However, the ninhydrin-reactive substances in the trichloroacetic acid-soluble fractions normally increased with the enzyme quantity. Further, ninhydrin color did not change after alkaline hydrolysis of the trichloroacetic acid-soluble fractions. These results indicate that the enzyme can liberate only free amino acids from casein. At least fourteen amino acids were detected with certainty in the trichloroacetic acid-soluble fraction by an amino acid analyzer. Therefore, the enzyme would be regarded as an exopeptidase rather than an endopeptidase.

In Fig. 2, the actions of yeast proteinase C on various peptides are summarized. The peptides, Gly–Pro–Phe–Pro–Val–Ile and Phe–Phe–Val–Ala–Pro–Phe–Pro–Glu–Val–Phe–Gly–Lys, were isolated from the tryptic hydrolysate of casein as the compounds having a bitter taste[4]. These peptides were designated bitter peptides I and II, respectively. The other peptides were purchased from the Institute for Protein Research, Osaka University, Osaka. Splitting bonds of the peptides were estimated by analyzing the assay mixture by thin-layer chromatography or using an automatic amino acid analyzer (Yanagimoto Type LC-5S). Yeast proteinase C hydrolyzed the synthetic substrates of carboxypeptidases A and B, while the enzyme hardly attacked the substrates of aminopeptidase, *e.g.* Leu-Gly–Gly and Ala–Gly–Gly*. It is noticeable that the present enzyme released proline, which was never liberated by carboxypeptidase A[4], from bitter peptide I. Although yeast proteinase C little hydrolyzed Z–Gly–Pro under the experimental conditions, the enzyme seemed to liberate proline which was located within the peptide chain.

Fig. 3 shows the time-course of the hydrolysis of bitter peptide II. Even in a

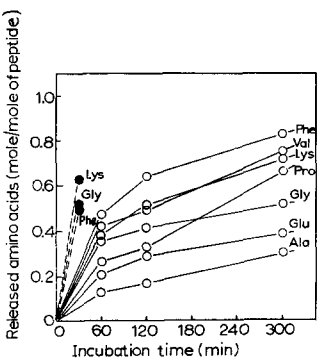


Fig. 3. Action of yeast proteinase C on bitter peptide II: Phe–Phe–Val–Ala–Pro–Phe–Pro–Glu–Val–Phe–Gly–Lys. 1.5 mg of the peptide were incubated with 16.8 μg of the enzyme under the same conditions as in Fig. 2. An aliquot of the assay mixture was removed at specified time intervals and analyzed using the amino acid analyzer. ●, hydrolysis of the peptide by carboxypeptidase B from bovine pancreas[4].

* Yeast proteinase C hydrolyzed Cbz–Gly–Phe and Cbz–Gly–Leu at a slightly more acidic region having a broad pH optimum. However, hydrolysis of Cbz–Glu–Tyr occurred at pH 5 or below where the negative charge of glutamic acid was decreased. So in this case the effects of the enzyme on the peptides were tested at pH 4.6.

short incubation, all kinds of amino acids composing the peptide were released by yeast proteinase C, while carboxypeptidase B liberated only three amino acids around the C-terminal of the peptide. However, yeast proteinase C would not necessarily completely degrade this peptide into free amino acids, as might be expected from its behavior toward the peptides having a free N-terminal (see Fig. 2). Since alanine, which is only one of the amino acids in bitter peptide II, was slowly released, the enzyme would degrade the peptide in amino acid units from its C-terminal to the Val–Ala bond at least.

From these results, yeast proteinase C is concluded to be a carboxypeptidase having a broader specificity than bovine carboxypeptidases. In a preliminary experiment, the present enzyme was found to hydrolyze horse apo-myoglobin and to release all amino acids composing the protein, though a complete degradation leading to free amino acids has not yet been achieved. This result further supports the conclusion that the enzyme has a very broad specificity. However, bovine carboxypeptidase B successively released C-terminal amino acids of bitter peptide II, while yeast proteinase C did not release amino acids in the order expected from the C-terminal sequence of the peptide (Fig. 3). In order to be able to use the present enzyme for structural studies, more detailed experiments are required.

Yeast proteinase C possesses an inactive form the molecular weight of which is higher by about 20 000 than that of the active enzyme[6]. Considering COHEN'S[2] suggestion, this fact and the present result will provide interesting problems concerning the regulation and mechanism of intracellular protein degradation in yeast.

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