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SELECTIVE DEGRADATION OF ALTERED TOMATO β-FRUCTOFURANOSIDASE MOLECULES BY NEUTRAL PROTEASE

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Key Word Index—*Lycopersicon esculentum*; Solanaceae; tomato; β-fructofuranosidase; aging; neutral protease; protein degradation; radioimmunoassay.

Abstract—Evidence is presented for the selective breakdown of altered tomato β-fructofuranosidase molecules by a neutral protease from *Bacillus subtilis*.

INTRODUCTION

It is well known that in bacteria and animal cells abnormal proteins, such as those produced by denaturation of cell proteins, by errors in transcription or translation, or by genetic mutation, are degraded selectively [1–6]. In senescent tomato fruit, the accumulation of an altered β-fructofuranosidase (β-FFase, EC 3.2.1.26) which retained immunological activity but lost its catalytic activity was reported [7]. The mechanism by which this transformation occurs is not known. In the present communication, a possible difference in susceptibility between the altered and normal tomato β-FFases to proteolytic attack was studied.

RESULTS AND DISCUSSION

Digestion of β-FFase with some proteases

Susceptibility of purified tomato β-FFase to attack by a variety of proteases including trypsin, papain, bromelin and neutral protease from *Bacillus subtilis* was examined. It was found that only the neutral protease was able to inhibit tomato β-FFase activity.

Loss of β-FFase activity and immunological activity during proteolytic digestion

In previous papers, we have suggested that the molecular species of β-FFase were composed of the normal type of β-FFase in ripened tomato fruit [8], while those in senescent tomato fruit were composed of normal and altered β-FFases [7]. Therefore, if one assumes that the altered β-FFase molecules are degraded more rapidly than normal β-FFase molecules by proteolysis, the catalytic activity of the β-FFase from senescent tomato fruit would decrease more slowly than that of the β-FFase from ripened tomato fruit when subjected to proteolysis. Fig. 1 demonstrates that catalytic activity of ripened tomato β-FFase decreased faster than its immunological activity on digestion with neutral protease. In contrast, a more rapid decrease was observed in the immunological activity than in the catalytic activity with senescent tomato β-FFase (Fig. 2). Comparing these two results, it is obvious that the catalytic activity of the β-FFase in senescent tomato decreased more slowly than in ripened tomato, as was expected. Therefore, the data suggest that the altered β-FFase, which is one of the molecular components of β-FFase in senescent tomato fruit cells, is attacked more actively than the normal counterpart by the neutral protease. It is not known what structural features of these altered polypeptides lead to their selective degradation. Studies of intracellular proteins with highly abnormal conformations demonstrated that protein structures determine half lives of the proteins involved [2, 4, 6]. In both bacterial and animal cells it has

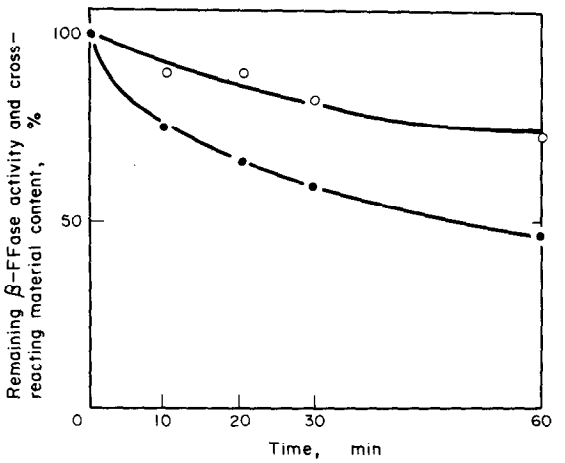

Fig. 1. Effect of neutral protease on catalytic activity (●) and immunological activity (○) of β-FFase of ripened tomato fruit. The reaction mixture contained 28 μg (1.5 units) of β-FFase, 420 units of immobilized neutral protease and 10 mM potassium phosphate buffer, pH 6.5, in a final volume of 3 ml. After incubation for 10, 20, 30 and 60 min, immobilized neutral protease was removed from the reaction mixture by centrifugation (10 000 g for 30 min). The remaining activity of β-FFase and the amount of cross-reacting material in the supernatant were determined. The values in the figure are the means of two experiments.

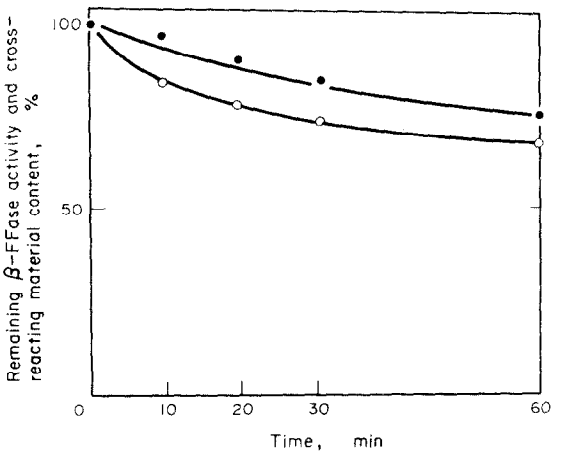

Fig. 2. Effect of neutral protease on catalytic activity (●) and immunological activity (○) of β-FFase of senescent tomato fruit. The reaction mixture contained 42 μg (1.5 units) of β-FFase. The experimental conditions were otherwise as given in Fig. 1.

already been shown that altered polypeptides are degraded rapidly. This process appears to represent a sort of 'cellular sanitation mechanism'. This prevents the accumulation of highly aberrant molecules which could be potentially harmful to the cell. If the 'cellular sanitation mechanism' takes place in tomato fruit cells, the decrease in the activity which selectively degrades altered enzyme molecules in senescent tomato fruits could be one of the causes of the accumulation of altered β-FFase. In contrast, the reduced content of altered β-FFase in ripened tomato, as was actually shown previously [8], could be due to its preferential hydrolysis by proteases naturally occurring in the fruit at this stage of ripeness.

EXPERIMENTAL

Plant material. Tomato plants (*Lycopersicon esculentum* cv zuiko) were grown in the farm of Faculty of Horticulture of Chiba University.

Enzyme preparations. The β-FFase from ripened and senescent tomato fruits were prepared by the method of ref. [7]. Trypsin, papain, bromelin and neutral protease from *B. subtilis* were obtained from Nagase Co. Ltd, Japan.

Enzyme assay. β-FFase activity was measured by the method of ref. [9]. Proteolytic activity was measured by the method of ref. [10]. One unit of protease was defined as the amount of enzyme catalysing the formation of 1 μg of tyrosine per min. Tyrosine was determined by the method of ref. [11].

Immobilization of neutral protease. Immobilized neutral protease was prepared by covalent coupling to Sepharose 4B according to the method by ref. [12].

Preparation of antiserum. Antiserum against tomato β-FFase was prepared as described in ref. [8].

Radioimmunoassay (double antibody method). The radioimmunochemical method for measurement of the amount of β-FFase has been described previously [8].

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