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INACTIVE β-FRUCTOFURANOSIDASE MOLECULES IN SENESCENT TOMATO FRUIT

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Key Word Index—*Lycopersicon esculentum*; Solanaceae; tomato; β-fructofuranosidase; aging; single radial immunodiffusion.

Abstract—The present paper deals with the formation of altered molecules of β-fructofuranosidase (β-FFase, EC 3.2.1.26) in the cell wall fraction of tomato fruit in relation to aging. The monospecific antibody prepared from rabbits was used to characterize enzymes at ripened and senescent stages of tomato fruits. Although the activity on a fresh weight basis and the specific activity of the crude extract declined as the fruit aged, no difference was observed in the amount of the enzyme protein on a fr. wt basis between the two stages. With purified enzyme, there was little difference in such properties as K_m, heat stability and optimum pH. However, the purified β-FFase from the senescent fruits had a lower specific activity. It is concluded from the results that the decline in the enzyme activity in the senescent fruits is due to the occurrence of immunologically active but catalytically inactive molecules of β-FFase.

INTRODUCTION

One of the biochemical processes involved in aging may be an accumulation of altered enzymes and other proteins [1]. Among many theories on aging, the error catastrophy theory first proposed by Orgel [2–4] has received considerable attention [5–9]. It suggests that as an animal ages the frequency of errors increases at either the transcriptional or translational level of protein synthesis, resulting in the synthesis or accumulation of partially or totally inactive enzymes [2–4] with decreased specific activity. Much information has been obtained with animal enzymes in support of Orgel's error theory [5–9]. However, no data are available for plant enzymes which confirm the theory. In the present work the possible presence of catalytically altered or inactive enzyme molecules in relation to the aging of tomato fruit has been examined. β-FFase was purified from ripened and senescent tomato fruits and immunological techniques were utilized.

RESULTS

β-FFase activity and concentration in extracts from ripened and senescent tomato fruits

Activity was expressed as enzyme units per g fr. wt, while the concentration was expressed as μg enzyme protein determined by a single immunological diffusion method [10]. The results are given in Table 1. β-FFase in the cell wall of tomato fruits showed an age-related decrease in the specific activity (enzyme activity/enzyme concentration), although there was no difference in the concentration of enzyme protein.

Table 1. The level of β-FFase activity and content in the extracts from ripened and senescent tomato fruits

Sample	Activity (units/ g*fr. wt)	Protein (μg/ g*fr. wt)	Specific activity (units/μg protein)
Ripened tomato	1.6 ± 0.06	27 ± 1.4	0.059 ± 0.005
Senescent tomato	0.8 ± 0.05	23 ± 1.5	0.035 ± 0.0045

*g fr. wt of pericarp of tomato fruit. Protein was calculated as immunological activity. Immunological activity was measured by single radial immunodiffusion.

Preliminary experiments also confirmed the parallel decrease of enzyme activity and specific activity as the fruit aged. Mixing experiments suggested that the results obtained were not due to the presence of inhibitors or activators of β-FFase activity (Nakagawa, H., unpublished results).

Properties of purified β-FFase from ripened and senescent tomato fruits

In order to compare the properties of the enzyme extracted from ripened and senescent tomatoes, β-FFase was purified under the same conditions from both 12-day and 30-day ripened fruit. The specific activity of β-FFase in senescent fruits of tomato was lower than that of ripened fruits (Table 2). However, there was little difference between the two ages in other

Table 2. Comparison of β-FFase from ripened and senescent tomato fruits

	Ripened tomato (12 days)	Senescent tomato (30 days)
Specific activity*	54	36
K_m for sucrose	9.5 mM	8.9 mM
Heat stability†	53°	52°
Optimum pH	4.5	4.5

*Units per mg of total protein.

†Temperature required to produce 50% inactivation of β-FFase activity in 10 min.

properties, i.e. heat stability K_m for sucrose, and opimum pH.

Double immunodiffusion analysis

The difference in specific activity of β-FFase in extracts of ripened and senescent tomatoes (Table 1) may be interpreted as an indication for the presence of abnormal enzyme molecules that are inactive or partially active. In an attempt to investigate this possibility, antibody specific for tomato fruit β-FFase was used to search for the presence of cross-reacting material (CRM). This antibody recognized purified β-FFase from ripened tomato, and the β-FFase in extracts of both ripened and senescent tomatoes, all as a single antigen.

Fig. 1 shows the immunotitration of the antibody with β-FFase extracted from the ripened and senescent tomato fruits. If β-FFase from both ages of tomato fruits were exactly the same in its catalytic activity as well as in its antigenic response, the equivalence point should also be the same. However, the equivalance point obtained for senescent tomato fruit was smaller than that for ripened tomato fruit, indicating more antigenic recognition sites for the enzyme from the senescent tomato fruits. The results provide clear evidence for the presence of totally or partially inactive β-FFase molecules in senescent tomato fruits.

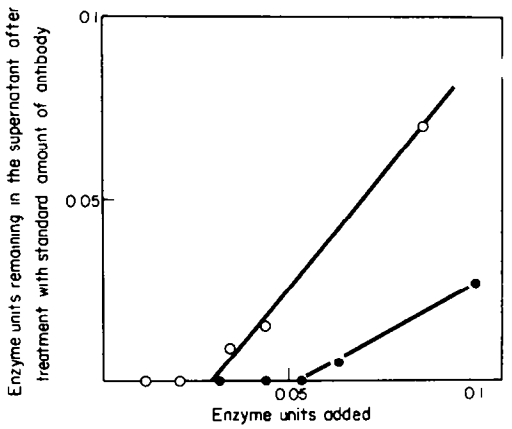

Fig. 1. Immunotitration of antibody with β-FFase extracted from ripened and senescent tomato fruits. ●, Crude extract of ripened tomato fruit; ○, crude extract of senescent tomato fruit.

DISCUSSION

Previous work [11,12] showed that β-FFase activity of tomato fruit increased markedly during maturation, reaching the highest level about 12 days after harvest. In contrast, the present results demonstrated a decline of the enzyme activity over the next 18 days. This decline may be attributable to a decrease in the number of β-FFase molecules per cell, to qualitative modification of the enzyme, or it may be due to a progressive formation of β-FFase inhibitor. The last possibility is unlikely because mixing experiments failed to demonstrate the formation of any inhibitors or activators of the enzyme. A comparison in CRM between the ripened and the senescent tomato fruits revealed little difference in the amount of enzyme protein on a fr. wt basis (Table 1). However, a significant difference was observed in the specific activity implying the presence of a catalytically inactive but immunologically active form of the enzyme. When β-FFase was purified from both stages of tomato fruits and some of the properties were examined, there was no significant difference in K_m for sucrose, optimum pH, and heat stability (Table 2). The only difference observed was again in the specific activity, excluding the second possibility raised above. Therefore, the decline of the enzyme activity in the senescent tomato fruits can be explained only by the first possibility, i.e. the decrease in the number of active β-FFase molecules per cell. The decrease in the specific activity suggests that tomato fruit at the senescent age possesses β-FFase molecules which are catalytically inactive but immunologically active.

The formation of altered molecules of β-FFase in the aging tomato fruits is not surprising because similar phenomena have already been reported with the other organisms. Gershon and Gershon [7] found the existance of inactive molecules of isocitrate lyase in the aging nematode *Turbatrix acti*. The same authors [8] also reported the presence of non-functional molecules of fructose-1,6-diphosphate aldolase in aging mouse liver.

The present paper presents evidence for enzyme alterations with age in tomato fruits. The mechanism by which the β-FFase is altered with age is not known. However, the age-related alteration of enzyme molecules may be a universal and systematic phenomenon for higher plants. Accumulation of more information of this phenomenon using purified enzymes may facilitate the understanding of the mechanisms of aging in plants.

EXPERIMENTAL

Plant materials. Tomato fruits (*lycopersicon esculentum* cv Kyoryokugoko) were harvested at the mature green stage from the Experimental Farm of Faculty of Horticulture, Chiba University. The fruits were allowed to ripen at room temp. for 12 and 30 days after harvest, and were designated as ripened and senescent stages, respectively.

β-FFase preparation. Crude extracts were prepared from the cell wall fraction of ripened and senescent tomato fruits [13]. β-FFase was purified by the method of ref. [14]. *Preparation of antiserum.* An antiserum against tomato β-FFase was prepared as previously describes [12]. *Enzyme assay.* β FFase activity was assayed by the colorimetric proce-

dure described in ref. [13]. *Determination of protein.* Protein was determined by the method of ref. [15]. *Quantitative determination of β-FFase by single radial immunodiffusion.* Single radial immunodiffusion was carried out according to the method of ref. [10]. *Double immunodiffusion analysis.* The double immunodiffusion analysis was performed as given in ref. [16]. Development of precipitin bands was usually completed in 24 hr at 4°. *Immunotitration.* Immunotitration of β-FFase extracted from ripened and senescent tomato fruits was carried out by the method of ref. [12].

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