The Development of Activity of Cell Wall Bound β -Fructofuranosidase with Ripening and Senescence of Tomato Fruit

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Many enzyme activities in higher plants are increased by a variety of environmental perturbations.¹⁾

In the past there have been a large number of investigations concerning changes of the enzyme activity associated with fruit ripening.²⁾

It has been reported that the change of activities of some enzymes bound to the cell walls of tomato fruit (cellulase,²⁾ polygalacturonase,²⁾ and pectinesterase³⁾) arises during maturation. β -Fructofuranosidase (β -FFase EC 3.2.1.26) from tomato fruit was also one of the cell wall-bound enzymes and level of β -FFase activity increased steadily during maturation.⁴⁾

This paper shows that the increase in β -FFase activity is due to the synthesis of β -FFase rather than the disappearance of endogenous inhibitors.

Mature green fruits of tomato (*Lycopersicon esculentum* var. Hikari) were ripened at 25°C for 7 days under the dark conditions after the peduncles were immersed in each aqueous solution of chemicals for 2 days. Tomato pericarp tissue was chopped into small pieces and blended for 2 min at the top speed in a Waring blender with an equal amount (w/v) of distilled water. The homogenate was centrifuged at 10,000 $\times g$ for 10 min and the precipitation was extracted in 200 mM potassium phosphate buffer, pH 8.0. The mixture was then centrifuged at 10,000 $\times g$ for 10 min and the supernatant was dialyzed against distilled water.⁵⁾

β -FFase activity was assayed by a colorimetric procedure according to the method of Yabuki.⁶⁾ The reaction mixture for the standard assay method consisted of 0.075 M sucrose, 120 mM acetate buffer (pH 4.5) and enzyme solution in a total volume of 1 ml. The reaction mixture was incubated at 30°C for 15 min. After the incubation, 1.0 ml of the reaction mixture was added to 3,6-dinitrophenolic acid reagent to determine reducing sugar formed.⁷⁾ Activities were

expressed as β -FFase units, micromoles of glucose liberated per min.⁵⁾

The increase in β -FFase activity commenced at the mature green stage of tomato fruit, reached a maximum after about 10 days, and thereafter declined slightly (Fig. 1). After harvest at the mature green stage, the

FIG. 1. Increase in β -FFase Activity in Extracts from Various Stages of Detached Tomato Fruit.

TABLE I. EFFECTS OF VARIOUS FRACTIONS ON β -FFASE ACTIVITY IN EXTRACT OF CELL WALLS OF RED RIPE TOMATO FRUIT

Enzyme assays were carried out under the standard assay conditions. G: Extract of cell walls of green tomato fruit with 200 mM potassium phosphate buffer (pH 8.0). G_s: Supernatant of homogenate from green tomato fruit after the centrifugation at 10,000 $\times g$ for 10 min. R: Extract of cell walls of red ripe tomato fruit with 200 mM potassium phosphate buffer (pH 8.0). R_s: Supernatant of homogenate from red ripe tomato fruit after the centrifugation at 10,000 $\times g$ for 10 min. The heat treatment of G_s was undertaken at 100°C for 10 min. The dialysis of G_s was undertaken for 1 day.

Fraction (ml)	β -FFase activity (units/g fresh weight)	Relative β -FFase activity (%)
G (0.25)	0.96	17.1
G _s (0.25)	0.33	5.9
R (0.25)	5.60	100
R _s (0.25)	0.52	9.2
R (0.25)+G _s (0.25)	3.81	68.0
R (0.25)+G (0.25)	6.54	116.8
R (0.25)+R _s (0.25)	4.60	82.2
R (0.25)+Dialyzed G _s (0.25)	6.17	110.2
R (0.25)+Heated G _s (0.25)	5.64	100.8

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TABLE II. EFFECTS OF CYCLOHEXIMIDE AND THIOURACIL ON β -FFase ACTIVITY OF TOMATO FRUIT

Measurements of enzyme activities were carried out under the standard assay conditions. Tomato fruit were ripened at 25°C for 7 days under the dark conditions after the peduncles were immersed in aqueous solutions of various chemicals for 2 days. Tomato fruit placed in distilled water served as the control.

Treatment	Concentration (M)	Relative β -FFase activity (%)	Inhibition (%)
Control	—	100	0
Cycloheximide	10^{-3}	34.5	65.5
Thiouracil	10^{-3}	31.0	69.0

increase of β -FFase activity in detached fruit ripened at 25°C was the same as that in fruit ripened on the plant.

Extracts of red ripe tomato fruit (R) were prepared and mixed (1:1) with supernatant of mature green tomato fruit (G_s) and subsequently incubated for 10 min at 30°C. The mixture was assayed under the standard assay conditions. Table I shows that the level of β -FFase activity was inhibited about 32% by G_s . G_s seemed to contain heat labile and dialyzable substance from the results of heat treatment and dialysis.

Effects of inhibitors of protein- and nucleic acid-syntheses on the increase in β -FFase activity were shown in Table II. The increase in β -FFase activity was remarkably inhibited by both chemicals *in vivo*. However, in the experiments of the addition of both chemicals (10^{-3} M) to the purified β -FFase solution,⁵⁾ there were little effects on the enzyme activity.

It was suggested that β -FFase activity was inhibited about 32% by G_s (Table I).

In this case, it was impossible to explain the increase in β -FFase activity during the fruit maturation and ripening by means of the decrease in the inhibitor. When the activity of an enzyme has been shown to

increase rapidly in a plant tissue in response to some particular treatment, the introduction of a specific inhibitor of protein synthesis has been used to recognize whether the increase of β -FFase activity is due to synthesis of active enzyme itself.⁸⁾

The level of β -FFase activity was remarkably decreased in whole tomato fruit by cycloheximide or thiouracil (10^{-3} M) (Table II).

The treatment of tomato fruit by cycloheximide and thiouracil, detered color development and softening and did not develop after-ripening normally.

It was concluded that the increase of β -FFase activity during the fruit ripening is due to synthesis of β -FFase rather than disappearance of endogenous inhibitors.

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