Increase of β-Fructofuranosidase Content in Tomato Fruit during the Ripening Process

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A number of papers have shown that activities of many enzymes in higher plant increase during the fruit ripening.1-2) The activities of some enzymes bound to cell walls of tomato fruit (cellulase,3) polygalacturonase,4) and pectinesterase5) have been reported to increase during the process of fruit ripening. The previous work8) has shown that cycloheximide and thiouracil prevent the increase in β-fructofuranosidase (β-FFase E. C. 3.2.1.26.) activity in tomato fruit, suggesting that protein and nucleic acid syntheses are involved in the ripening process. This, however, has not proven that the increase in enzyme activity is due to the increase of β-FFase content per se, since the observed apparent increase in activity could be also ascribed to the removal or destruction of an endogeneous inhibitor, or to the activation of a preformed protein precursor. However, the evidence for the increase of β-FFase content in higher plants has scarcely been reported by the present time. By the measurements of β-FFase content, we may recognize whether the increase of β-FFase activity is, in fact, due to the increase of β-FFase content.

Rabbits were immunized with a mixture of 1 ml of purified β-FFase (1 mg) and 1 ml of Freund's complete adjuvant. On the 3rd week an enzyme sample (1 ml, containing 0.5 mg of β-FFase) was mixed with 1 ml of Freund's complete adjuvant and injected subcutaneously into a rabbit at intervals of 2 weeks for 6 successive weeks. And the rabbit was bled 2 weeks after the final immunization.

The purified β-FFase was radioiodinated with 125I by the chloramine-T method.9) The specific activity of the labelled β-FFase obtained by this method was 55.8 μCi/μg. From the specific activity it was calculated that about one radioactive iodine atom was tagged per one protein molecule.

The amount of β-FFase was assayed by the double antibody method.10) All reagents were diluted with 0.1 M borate buffer, pH 8.6 containing 0.5% bovine serum albumin. Each 100 μl of standard or sample in a tube was mixed with 100 μl of 1% normal rabbit serum, 100 μl of radioiodinated β-FFase and 100 μl of diluted rabbit anti-β-FFase serum showing an excess of antigen in the reaction mixture. The mixture was incubated for 24 hr at 4°C. After the first incubation, 100 μl of diluted goat anti-rabbit γ-globulin was added to the tube and allowed to react for 24 hr at 4°C. After centrifugation the radio activity of the precipitate was counted by an auto well type scintillation counter (AL-201).

The specificity of antiserum against purified β-FFase was proved by a single precipitin band in an Ouchterlony's double diffusion analysis.

In immunoelectrophoretic analysis, purified β-FFase from red ripe tomato contained immunoreactive material. β-FFase preparation had a single precipitin band in reaction with antiserum against purified β-FFase.

Inhibition curves were obtained from serial dilution of each of three kinds of extracts obtained from tomato fruits of mature green stage, turning stage and red stage, as shown in Fig. 1. The slopes of these curves paralleled to the standard curve of purified β-FFase. Thus, the levels of immunoreactive β-FFase in extracts obtained from fruits of mature green, turning and red ripe stages, can be expressed in terms of known quanti-

![Graph](attachment:image.png)

**Fig. 1. Radioimmunoassay Standard Curve for β-FFase from Tomato Fruit.**

Sample dilution curves as follows: ○—○, purified β-FFase; ▲—▲, extract of mature green stage; ●—●, extract of turning stage; △—△, extract of red ripe stage. % = Precipitin count of various standard point/precipitin count of zero standard × 100.
TABLE I. THE LEVEL OF $\beta$-FFase ACTIVITY AND $\beta$-FFase AMOUNTS IN EXTRACT AT VARIOUS STAGES

Protein was calculated as immunological activity. Immunological activity was measured by radioimmunoassay.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Activity (units per g fresh weight)</th>
<th>Protein (µg per g fresh weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mature green</td>
<td>0.3</td>
<td>2.3</td>
</tr>
<tr>
<td>Turning</td>
<td>1.0</td>
<td>12.4</td>
</tr>
<tr>
<td>Red ripe</td>
<td>4.7</td>
<td>100</td>
</tr>
</tbody>
</table>

activities of the purified $\beta$-FFase from tomato fruit of red ripe stage.

Both $\beta$-FFase activity and $\beta$-FFase content were increased during the process of fruit ripening. The specific activity of the purified $\beta$-FFase from red ripened tomato fruits was coincident with the value of protein basis by the method of radioimmunoassay (Table I).

In the previous work, it was assumed that the increase of $\beta$-FFase activity during the process of fruit ripening was either due to (i) the disappearance of endogenous inhibitor, or to (ii) the synthesis of active $\beta$-FFase. It was impossible to explain the increase in $\beta$-FFase activity during the ripening by means of the decrease in the inhibitor. Cycloheximide and thiouracil remarkably prevent the increase in $\beta$-FFase from tomato fruit, suggesting that protein and nucleic acid syntheses are involved in the increase of the enzyme activity.

In the present experiment, the content of active $\beta$-FFase obtained from various stages of tomato fruits were determined by the use of immunological techniques. The higher $\beta$-FFase activity from tomato fruit indicated the more reacting material with the antiserum against purified $\beta$-FFase was formed.

REFERENCES