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SHORT REPORTS

IMMUNOLOGICAL PROPERTIES OF β-FRUCTOFURANOSIDASE FROM RIPENING TOMATO FRUIT

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Key Word Index—*Lycopersicon esculentum*; Solanaceae; tomato; fruit ripening; β-fructofuranosidase; aging; radioimmunoassay.

Abstract—The amount of tomato fruit β-fructofuranosidase extractable from the cell walls during ripening parallelled the changes in activity of the enzyme. Using the techniques of radioimmunoassay, double immunodiffusion analysis and immunotitration, no differences in immunological properties of β-fructofuranosidase between the various stages of fruit ripening were detected.

INTRODUCTION

Recently, Manning and Maw [1] reported on the increase in the activity of β-fructofuranosidase (β-FFase, EC 3.2.1.26) as tomato fruits mature. However, they did not determine if the increase in enzyme activity is attributed to the *de novo* protein synthesis, activation of the pre-formed enzyme or disappearance of endogeneous inhibitor.

From the above point of view, we have studied the activity level of β-FFase, one of the cell wall-bound enzymes in tomato fruit, in relation to aging and enzyme production. Immunological techniques and inhibitor treatment have been used [2, 3].

In this paper, the amount of β-FFase at various stages of tomato fruits was determined by the methods of radioimmunoassay, double immunodiffusion analysis and immunotitration.

RESULTS AND DISCUSSION

Double immunodiffusion analysis

The antiserum, reacted with crude extracts from various stages of tomato fruits, gave only a single fused precipitin band, suggesting the presence of the same enzyme protein.

Table 1. Level of β-FFase activity and β-FFase content in the extracts obtained at various ripening stages of tomato fruits

Stage	Activity (units/g* fr. wt)	Protein (μg/g* fr. wt)	Specific activity (units/μg protein)
Mature green	0.27 ± 0.03	6.78 ± 0.91	0.040 ± 0.01
Turning	0.50 ± 0.04	8.40 ± 1.46	0.060 ± 0.016
Red ripe	1.29 ± 0.06	25.90 ± 2.1	0.050 ± 0.007

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

Level of β-FFase activity and β-FFase concentration in extracts at various stages

Table 1 shows the increases in the levels of β-FFase activity and β-FFase concentration as fruits mature. It is apparent from these results that the increase in β-FFase activity was accompanied by an increase in the number of β-FFase molecules. Further, the sp. act. seemed similar throughout the ripening process as shown in Table 1.

Immunotitration

Fig. 1 shows the immunotitration of the antibody with β-FFase extracted from the tomato fruits at various stages of maturation. If β-FFase were partially inactivated, or its conformation were changed in some way, yet the enzyme retained some antigenic recognition sites, the equivalence point would be changed. However the

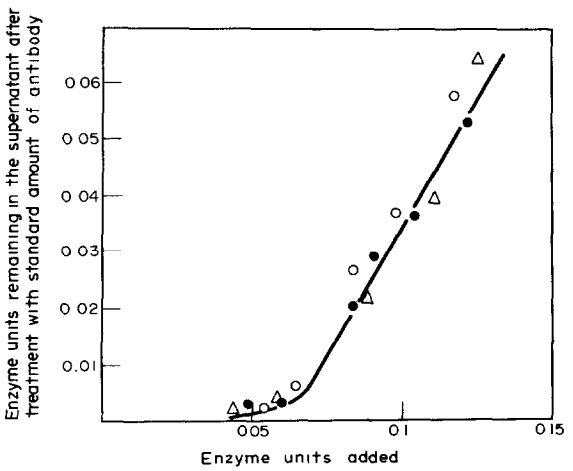

Fig. 1. Immunotitration of antibody with β-FFase extracted from tomato fruits of various maturing stages. ●——● crude extract of mature green stage; △——△ crude extract of turning stage; ○——○ crude extract of red ripe stage.

equivalence point measured several times during the process of fruit ripening was constant, suggesting that the increase in β-FFase as fruits mature is due to the synthesis of an antigenically-identical molecule to that of the mature green stage.

Nakagawa *et al.* [4] found the increase in β-FFase activity as tomato fruits mature. Manning and Maw [1] demonstrated the increase in sp. act. on a protein-N basis with advancing ripeness. In this paper, we reported the immunological identity of β-FFase molecule throughout the ripening process. It is probable that the increase in sp. act. reported by Manning and Maw [1] would be caused by the synthesis of immunologically-identical β-FFase molecule during the ripening of tomato fruit.

When the metabolic function of an enzyme is assessed *in vivo*, it is necessary to take into consideration not only the changes in enzyme activity amidst alterations in total protein concentration, but also possible differences in immunological properties during development.

EXPERIMENTAL

Plant materials. Tomato plants (*Lycopersicon esculentum* cv Kyoryokugoko) were grown in a greenhouse at the Experimental Farm of Chiba Ken Agricultural Experiment Station.

Enzyme assay. β-FFase activity was measured by the method of ref. [5].

Purification of β-FFase. β-FFase extracted from cell wall fraction of red ripe stage of tomato fruits was highly purified by the method of ref. [6]. In order to avoid the antibody formation against contaminated minor proteins, immunoelectrosyneresis [7] was carried out to prepare the antigen which is β-FFase and rabbit antibody complex. The homogenate of the agarose containing antigen–antibody complex was used for the further immunization as the antigen.

Preparation of antiserum. Rabbits were immunized with 5 injections given at 2-weekly interval. A mixture of 1.5 ml of antigen (*ca* 1 mg) and 1.5 ml of Freund's complete adjuvant was used. A potent antiserum was obtained 2 weeks after the last injection and its specificity toward β-FFase was confirmed by both double immunodiffusion analysis [8] and immunoelectrophoretic analysis [9].

Radioiodination of β-FFase. The purified β-FFase was radioiodinated with ^{125}I by the chloramine-T method [10]. The labelled β-FFase was separated from free radioiodine by gel filtration on Sephadex G-25 column (1 × 25 cm). The sp. act. of labelled β-FFase was 46.5 μCi/μg. This value indicates that on average one radioreactive iodine was tagged on each protein molecule.

Radioimmunoassay (double antibody method). The amount of β-FFase, instead of a using unit of activity, was measured by a double antibody method [11]. All reagents were diluted with 0.1 M borate buffer, pH 8.6, containing 0.5% BSA. To each 100μl of standard or sample in a tube, 100μl of radioiodinated β-FFase, 100 μl of 0.1 M borate buffer, pH 8.6 and 100 μl of 1000-fold diluted rabbit anti-β-FFase serum were added, mixed and incubated for 30 hr at 4°. After the first incubation, 100 μl of 1% normal rabbit serum and 100 μl of 5% goat anti-rabbit γ-globulin serum were added to the tube and allowed to stand for 24 hr at 4°. After centrifugation, the radioactivity of the ppt. was counted by an auto well scintillation counter (AL-201).

Immunotitration. Immunotitration of β-FFase extracted from various stages of tomato fruits was carried out by incubation of enzyme soln with the antibody (γ-globulin fraction) in a final vol. of 1.5 ml in 10 mM K–Pi buffer, pH 7.5 at 4° for 18 hr according to the method of ref. [12].

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