Intracellular Localization of β -Fructofuranosidase in Tomato Fruit Tissue

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A number of studies have been reported on the distribution of enzymes among the different organelles of plant cells and of the activities of individual types of organelle.¹⁻⁴⁾ The presence of enzymes in plant cell walls have been widely discussed,⁵⁻⁹⁾ and it has been demonstrated that invertases in higher plants were largely located in cell wall fraction by differential centrifugation.⁷⁻¹¹⁾ However, reports on the precise intracellular localization of many enzymes have been scarce because of the difficulties to isolate undamaged or uncontaminated organelles and of the possibility that some soluble enzymes are adsorbed on the cell surface or cytoplasmic particles during the fractionation procedure.

Previously, we reported that β -fructofuranosidase (β -FFase, EC 3. 2. 1. 26) in tomato fruit was located in cell wall of pericarp tissue as an insoluble form by the use of differential centrifugation.¹²⁾

In this paper, we report the direct evidence of localization *in vivo* of β -FFase in tomato fruit using immunofluorescent antibody technique.

β -FFase extracted from cell wall fraction of red ripe stage of tomato fruits was purified by the method of Nakagawa *et al.*¹³⁾ In order to avoid the antibody formation against contaminated minor proteins, immunoelectrosynthesis¹⁴⁾ was carried out to prepare the antigen, β -FFase, and rabbit antibody complex. The precipitin bands with β -FFase activity were cut out and washed several times with 0.9% NaCl solution. The homogenate containing antigen-antibody complex was used for the further immunization as the antigen. Rabbits were immunized at intervals of 2 weeks by 5 injections with a mixture of 1.5 ml of antigen (*ca.* 1 mg) and 1.5 ml of Freund's complete adjuvant. A potent antiserum was obtained 2 weeks after the last injection and its specificity towards β -FFase was confirmed by double immunodiffusion analysis and immunoelectrophoretic analysis.

The immunofluorescence procedure used (indirect

method) was similar to that described by Kawamura¹⁵⁾ and Knox *et al.*¹⁶⁻¹⁸⁾ Fresh pericarp tissues from red ripe stage of tomato fruit were fixed in the graded series of ethanols at 4°C. The fixed tissues were sectioned with freehand razor blade as thin as possible. The sections were mounted on slides and air-dried. Anti- β -FFase antibody (full strength) was layered onto the sections and the slides were held in a moist chamber for 2 hr at 37°C. The excess serum was then removed by rinsing with phosphate buffered saline (PBS), pH 7.0 for 5~10 min. Fluorescein isothiocyanate (FITC)-labelled goat-rabbit γ -globulin antibody was spread on the sections. The slides were again incubated for a further period of 2 hr in a moist chamber at 37°C, rinsed thoroughly with PBS for 5~10 min and air-dried. Controls were made by using normal (pre-immunization) serum in place of anti- β -FFase serum. The sections were mounted in non-fluorescent elvanol-buffered glycerin mixture, and observed by fluorescent microscopy (Olympus-FLM) using UV excitation system.

The clarity of the cell wall localization of β -FFase in tomato fruit can be seen in Fig. 1. Some of the wall

FIG. 1. Intracellular Localization of β -FFase in Tomato Fruit Tissue.

The fluorescence which is due to the presence of β -FFase occurs in cell wall (CW), and is shown by arrows in the microphotograph.

fluorescence shown in Fig. 1 (arrows) was truly due to the presence of β -FFase, but much of it was auto-fluorescence which was distinguished from the fluorescence due to FITC, because auto-fluorescence was greenish in colour, whereas the FITC fluorescence was yellowish.

Although we presented the evidence that β -FFase in tomato fruit was located in cell wall by fluorescent antibody technique, more detailed evidence by the use of immunoelectron microscopy is necessary for more

definite localization of cell wall-bound β -FFase in cell wall of tomato fruit.

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