NADP⁺-Malic Enzyme and Organic Acid Levels in Developing Tomato Fruits

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Abstract. Tomato (*Lycopersicon esculentum* Mill., cv. Ohio 7814) fruits were harvested weekly following flowering to relate changes in NADP⁺-malic enzyme (NADP-ME) activity and major organic acids (malate and citrate) to fruit development. Specific activity of NADP-ME and fresh weight concentrations of the acids reached maximal levels at the mature-green stage. During ripening, a decline in malate concentration was followed by decreases in NADP-ME activity and citrate concentration. Activity of NADP-ME and organic acid concentrations were highest in the locular gel, but activity also occurred in tomato leaves, stems, and roots. The data do not exclude a role for NADP-ME in the metabolism of organic acids during fruit ripening. However, it is also possible that the enzyme is involved in cytoplasmic pH regulation.

Like many fruits, tomatoes accumulate organic acids during growth and use the acids as respiratory substrates during ripening. The loss of acidity is one aspect of tomato quality, determining consumer judgment of ripeness. The pH of tomato products is influenced by organic acid and potassium contents and needs to be below a threshold value to prevent microbial spoilage after processing (Powers, 1976). Citric acid is the predominant organic acid in tomatoes; malic acid is also present as a major constituent, and other acids occur in trace quantities (Ulrich, 1970). During ripening, malate declines substantially, whereas citrate has been reported to decline (Thorne and Efiuvwevwe, 1988), remain constant (Davies, 1966), or even increase slightly (Goodenough and Thomas, 1980). Goodenough et al. (1985) suggested that operation of the TCA cycle was restricted in ripening tomato fruit and that the metabolism of malate was switched from the TCA cycle to a cytosolic NADP⁺-malic enzyme (NADP-ME; EC 1.1.1.40), catalyzing the following reaction:

Carbon dioxide generated by the activity of this enzyme was thought to account for much of the climacteric rise in respiration. In pome fruits, an increase in NADP-ME activity is associated with the climacteric and can be stimulated by ethylene (Hulme and Rhodes, 1971). However, in tomatoes, the climacteric can be stimulated by ethylene, whereas NADP-ME activity and acid metabolism appear to be "ethylene independent" (Jeffery et al., 1984). Nonclimacteric fruits, such as cherry (*Prunus avium* L.) and grape (*Vitis vinifera* L.), also develop high activities of NADP-ME (Hartmann, 1975; Ruffner et al., 1976), and elsewhere malic enzymes are responsible for CO₂ generation for photosynthesis in crassulacean acid metabolism plants and in some C₄ plants (Campbell and Black, 1981; Ting, 1985), and possibly for pH regulation (Davies, 1984).

As part of a project to control changes in acid levels in tomato fruits, further information was sought relative to the role of NADP-ME. Enzyme activity and concentrations of malate and

citrate were examined earlier in fruit development than in previous studies, to relate NADP-ME activity to changes in acid levels. NADP-ME activities in other parts of the plant were also measured to determine whether the enzyme is specific to fruit.

Materials and Methods

'Ohio 7814' (Berry and Gould, 1983) tomatoes were grown at The Ohio State Univ. Horticulture Farm, Columbus. Flower trusses were labeled at anthesis, so that fruits of known age could be gathered. Fruits were mature-green from 40 to 45 days after anthesis. At each harvest, the outer pericarp was removed from two samples of five to 10 fruits, and seeds were removed from the remaining inner tissues. Outer pericarp and inner tissues were homogenized at 0°C in 2 vols 0.1 M bicine, 0.1 M MOPS (pH 8.2), 3 mM EDTA, 5 mM mercaptoethanol with 1% (w/v) polyvinylpyrrolidone (40T), using a Polytron (Brinkmann Instruments, Westbury, N.Y.). The homogenate was filtered through four layers of muslin, and the filtrate was centrifuged at 20,000 × g for 20 min. A portion of the supernatant solution was applied to an 18 × 1.5 cm column of Sephadex G-25, equilibrated with 10 mM bicine, 10 mM MOPS (pH 7.0), 1 mM mercaptoethanol, 0.5 mM MnCl₂. Elution was continued with this buffer, and absorbance at 280 nm was monitored; the excluded fraction was collected and assayed for NADP-ME activity in 0.1 M MOPS (pH 7.0) with 0.5 mM NADP, 10 mM malate and 5 mM MnCl₂ (Goodenough et al., 1985). Activity was measured as the increase in concentration of NADPH through the change in absorbance at 340 nm over 3 min. Protein was assayed, by dye-binding (Bradford, 1976), in the original supernatant solution for an estimate of fruit soluble protein content, and in the enzyme preparation after gel filtration so that specific activities could be reported. Organic acids in the supernatant solution were estimated by enzymic procedures, employing malate dehydrogenase (E.C. 1.1.1.37) and glutamic-oxaloacetic transaminase (E.C. 2.6.1.1) with NAD reduction for malate and citrate lyase (EC 4.1.3.6), malate dehydrogenase, and lactate dehydrogenase (E.C. 1.1.1.27) with NADH oxidation for citrate (Bergmeyer, 1983; Moellering and Wolfgang, 1966).

Results and Discussion

Enzyme assay. NADP-ME activity was detected in the supernatant solution after centrifugation of a crude enzyme preparation. Gel permeation removed endogenous malate (and probably substrates of other NADP dehydrogenases), resulting in enzyme preparations that did not reduce NADP in the absence

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respectively. Thus, NADP-ME could be directly involved in acid metabolism in ripening fruit as suggested earlier (Goodenough et al., 1985). NADP-ME acting alone would lead to accumulation of pyruvate and, if the TCA cycle becomes inoperative (Goodenough et al., 1985), some other pathway of pyruvate use seems necessary. More likely, action of NADP-ME provides a source of pyruvate to sustain the TCA cycle; this would decouple the cycle from glycolysis and its associated metabolic controls and could, therefore, account for the climacteric rise in respiration. NADP-ME could also participate in the metabolism of citrate, since the TCA cycle could only metabolize this substrate as far as oxaloacetate; the action of NADP-ME could provide a bypass to generate pyruvate from malate and allow the cycle to continue.

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