MALIC ENZYME ISOZYMES IN POME FRUITS*

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1. Introduction

The activity of malic enzyme [1, 2] (L-malate:NADP oxidoreductase (decarboxylating), EC 1.1.1.40) increases significantly during ripening of pome fruits in association with several other biochemical changes, including an increase in the respiratory quotient of intact fruits, increase in NADP and NADPH content of the fruit cortex [3], increased capacity of fruit tissue slices to decarboxylate malate [4] and the decline in malic acid content of the fruit [5]. The increase of malic enzyme activity during ripening was shown [6] to be partially due to de novo synthesis, as measured by incorporation of [^{14}C]phenylalanine into the electrophoretically purified enzyme. Although there is considerable evidence to indicate that the enzyme participates in the ripening process, the rationale for enhanced synthesis is lacking since the enzyme quantity in mature unripe fruit does not appear to be rate limiting. It occurred to us that synthesis of a malic enzyme isozyme possessing different kinetic properties or located in a distinct subparticle of the cell could provide a rationale for the enhanced synthesis of the enzyme during ripening.

2. Materials and methods

Malic enzyme was purified from pear (*Pyrus communis* L cv. Bartlett) and apple fruits (*Malus sylvestris*, Miller, cv. Wealthy) as previously described [7]. The crude protein extract was adjusted to pH 7.3 and fractionated with ammonium sulfate. The protein fraction that precipitated between 2.2 and 2.6 M ammonium sulfate was further purified either on calcium phosphate gel or by polyacrylamide slab gel electrophoresis before being subjected to isoelectric focusing fractionation in a sucrose gradient column [8].

The relative change in malic enzyme isozymes during pome fruit ontogeny was measured by isoelectric focusing fractionation in acrylamide disc gel as supporting medium. The fractionation in acrylamide gel was carried out according to Dale and Latner [9] with one major modification; protein fractions were not polymerized into the acrylamide matrix but rather applied in 10% sucrose between the gel and cathodial electrolyte (ethanolamine). This modification was necessary since malic enzyme was found to be immobilized when polymerized into the acrylamide gel.

Purification of two malic enzyme isozymes was accomplished by manipulation of pH and ionic strength. Malic enzyme was precipitated between 2.2 and 2.6 M ammonium sulfate at pH of the solution between 5.5 and 9.5. When the precipitation was performed at pH 4.5, 1.8 M ammonium sulfate precipitated the enzyme. Accordingly, the pH of a crude protein extract was adjusted to 5.5 and the enzyme that precipitated between 1.0 and 1.8 M ammonium sulfate was collected by centrifugation. The pH of the supernatant solution was adjusted to 4.5 and a second precipitate containing malic enzyme activity was collected. A portion of the crude protein extract was also fractionated with ammonium sulfate at pH 7.3 to collect both isozymes in one fraction.

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3. Results and discussion

Isoelectric focusing fractionation of malic enzyme revealed two peaks of activity having Ip of 4.55 and 5.45 (fig. 1). Identical results of fractionation were obtained when the enzyme was previously purified on calcium phosphate gel or by acrylamide gel electrophoresis. Likewise, isoelectric focusing of highly purified preparations of the enzyme, containing between 0.5 and 20.0 mg protein, in 110 ml volume gradients resulted in identical separation. Electrophoresis of the two isozymes separated by isoelectric focusing showed that the two isozymes had equal rates of electrophoretic mobility on 7.5% polyacrylamide in Tris-glycine buffer at pH 8.5. It seems therefore that previous measurements of [^{14}C]phenylalanine incorporation [6] represented total incorporation into both isozymes.

Amino acid analyses (table 2) were performed on a highly purified preparation that contained both isozymes and on the Ip 4.55 isozyme obtained from this mixture by isoelectric focusing. The Ip 4.55 isozyme

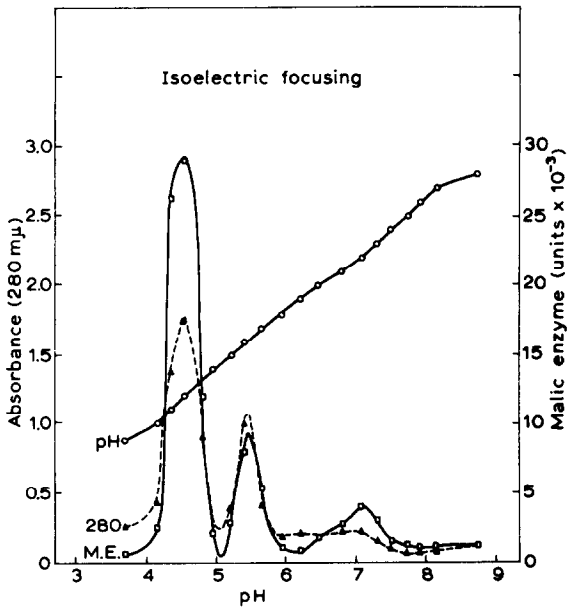

Fig. 1. Isoelectric focusing fractionation of malic enzyme in a sucrose density gradient. 19.8 mg protein was fractionated for 42 hr at 700 V potential. The presence of a peak at pH 7.1 was due to absorption and tailing of the enzyme along the glass column.

Table 1
Amino acid composition of Ip 4.55 and 5.45 enzymes of Bartlett pear fruit malic enzymes.

Amino acid	Micromoles per milligram protein	
	Ip 4.55 + Ip 5.45[1]	Ip 4.55[2]
Aspartic acid	1.540	1.150
Threonine[3]	0.5 (est.)	0
Serine	0.439	0.451
Glutamic acid	1.092	1.066
Proline	0	0
Glycine	0.707	0.708
Alanine	0.756	0.716
Valine	0.776	0.719
Cysteine	trace	trace
Methionine	0.091	0.112
Isoleucine	0.469	0.433
Leucine	0.870	0.916
Tyrosine	0.310	0.291
Phenylalanine	0.315	0.314
Lysine	0.686	0.572
Histidine	0.148	0.139
Arginine	0.375	0.368

[1] Enzyme from sucrose gradient electrophoresis (1.51 mg protein hydrolyzate loaded on column).
[2] Enzyme from isoelectric focusing (0.619 mg protein hydrolyzate loaded on column).
[3] Threonine was present but was insufficiently separated from aspartic acid to obtain a reliable measurement of quantity. Estimated to be about equal to that of serine.

was devoid of threonine and contained less aspartic acid and lysine than the combined Ip 4.55 and 5.45 isozymes (table 1). The absence of threonine in the Ip 4.55 isozyme may account for its lower isoelectric point.

Isoelectric focusing fractionation of Wealthy apple malic enzyme obtained from fruits at successive stages of development revealed (fig. 2) that both isozymes were present even at an early stage of development. A probable shift in the relative quantities of the two isozymes occurs during fruit ripening.

The two isozymes obtained by ammonium sulfate fractionation at their respective Ip were inhibited differentially with NADP when the oxaloacetate decarboxylase activity of the enzyme was tested (table 2). Concentration of 1.6×10^{-5} M NADP caused 82% inhibition of the Ip 4.55 isozyme. Con-

Table 2

NADP inhibition of oxaloacetate decarboxylase activity of pear malic enzyme fractionated by ammonium sulfate at different pH.

NADP (M)	2.2–2.6 M, pH 7.3		1.0–1.8 M, pH 5.5		1.0–1.8 M, pH 4.5	
	μl CO_2 per min	inhibition (%)	μl CO_2 per min	inhibition (%)	μl CO_2 per min	inhibition (%)
0	9.1		5.5		7.6	
4.7×10^{-6}	5.9	37	5.1	7	3.7	50
1.6×10^{-5}	3.3	65	4.6	18	1.4	82
4.7×10^{-5}	1.5	85	3.5	36	1.2	84

Assay was carried out in the Warburg respirometer. Rate of CO_2 release between 10 and 20 min corrected for spontaneous oxaloacetate decarboxylation in the presence of Mn^{2+} is presented.

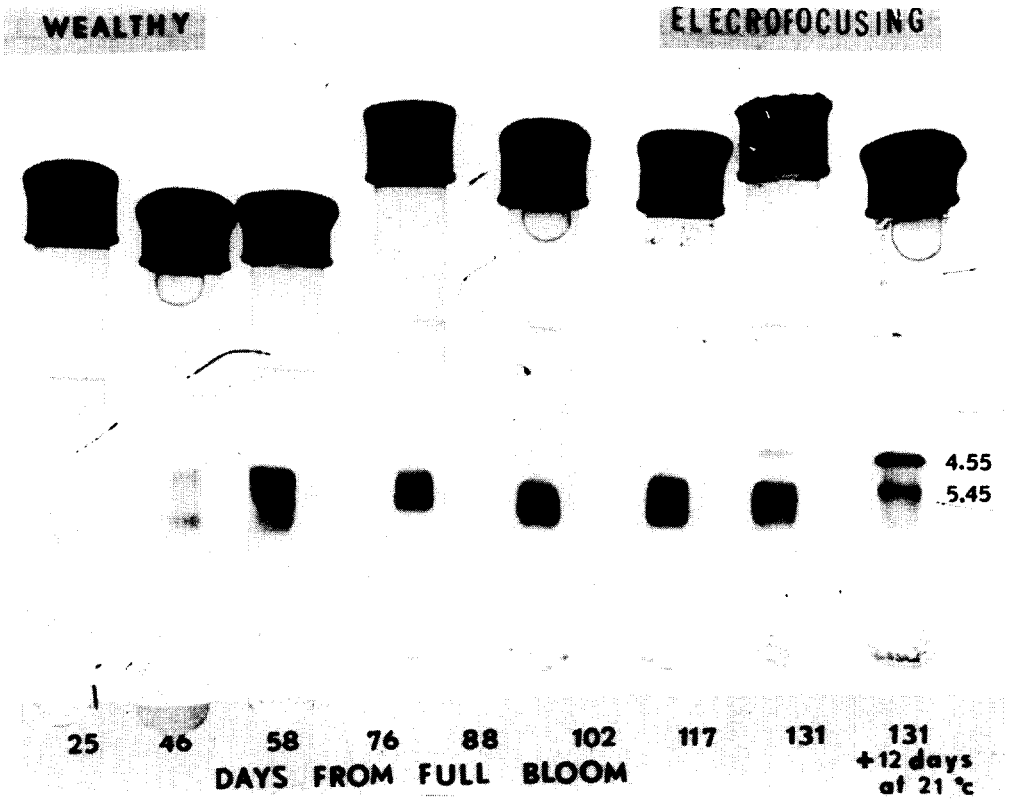

Fig. 2. Isoelectric focusing in polyacrylamide disc gel of Wealthy apple malic enzyme at successive stages of development. Electrofocusing was carried out for 14 hr in 5% gels. Cathode and anode were located at the top and bottom of the gel, respectively.

siderably less inhibition was found with the 5.45 isozyme at any of the concentrations tested. It is of interest to note that the concentration of NADP that causes 50% inhibition with the Ip 4.55 isozyme was found to exist in pome fruits [3].

Our results demonstrate the existence and changes in the relative quantities of two malic enzyme isozymes in pome fruits and the differential inhibition of the oxaloacetate decarboxylase activity of the two isozymes by NADP. The synthesis of a malic enzyme isozyme that can decarboxylate oxaloacetate in the presence of excess NADP might therefore play a role in the physiology of the fruit, particularly in cases where accumulation of oxaloacetate can cause inhibition of mitochondrial respiration [10].

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