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PH REGULATION OF PHOTOSYNTHETIC PEPC FROM AVOCADO FRUIT

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Abstract

Phosphoenolpyruvate carboxylase, PEPC, assimilates or recycles $C0_2$ within the fruit and appears to be a key enzyme in avocado fruit photosynthesis. PEPC was extracted from cv. Fuerte avocado fruit and was purified by ammonium sulfate precipitation, gel filtration and hydroxylapatite chromatography and found to consist of two isoenzymes. Regulation of enzyme activity in the avocado fruit was primarily by activation of the glyolytic intermediate glucose-6-phosphate and end-product inhibition by L-malate in a strongly pH-dependent manner.

1. Introduction

The large energy requirement of the avocado fruit, mostly for its oil synthesis, is partially accounted for by a high respiration rate relative to other fruits (Blanke1991).

Phosphoenolpyruvate carboxylase [PEPC, EC 4.1.1.31] catalyses the carboxylation of phosphoenolpyruvate (PEP) with bicarbonate (HCO_3) (Notton and Blanke, 1992 and 1993). PEPC is a key enzyme in fruit photosynthesis (Blanke and Lenz, 1989) where it (re-) assimilates $C0_2/HCO_3$ within the fruit and has not yet been purified from any fruit. Regulation of PEPC activity in avocado fruit is by two effectors, inhibition by L-malate and stimulation by glucose-6-phosphate (Blanke and Notton, 1991; Notton and Blanke, 1993).

The objective of the present work is to investigate the pH-dependence of this regulation and purify the enzyme from avocado fruit.

2. Materials and methods

2.1 - Enzyme extraction

Pericarp of ripe avocado cv. Fuerte was diced and extracted by grinding in a pre-cooled pestle and mortar in sand using 50 mM Tricine-NaOH at pH 7.8, containing 5 mM $MgSO_4$, 5 mM $NaHCO_3$, 15 mM DTT, 20 mM ascorbate and 5 % (w/w) PVP. The homogenate was filtered through muslin, centrifuged at 20,000 g for 30 mints and floating oils removed.

2.2 - Purification of PEPC

PEPC was precipitated between 30% and 50% saturated ammonium-sulfate. The resultant pellet was dissolved in 50 mM MOPS buffer pH 7.0, containing 5mM DTT, and the

solution desalted on a Sephadex G-25 column and applied to spheroidal hydroxylapatite. Unbound protein was removed by washing the column with the MOPS buffer and PEPC eluted by a stepwise inorganic phosphate gradient from 0 to 150 mM Pi. The 50mM Pi in MOPS fraction contained 70-80% of the PEPC and was made 50% saturated with ammonium sulfate to precipitate the PEPC, the pellet redissolved in MOPS buffer and the solution stored at -20ºC. This procedure resulted in a 25-fold enrichment of the PEPC with a recovery of 50% of the initial activity.

2.3 - PEPC assay

The initial rate of PEPC was measured as described by Notton and Blanke (1993) by coupling the reaction to the oxidation of NADH in the presence of MDH, and adding malate, and glucose-6-phosphate as required for the particular experiment.

3. Results and Discussion

3.1 - Purification and isoforms-of PEPC

On the hydroxylapatite column, 70-80% of the bound PEPC was removed with MOPS buffer containing 5OmM Pi, the remainder of the PEPC was removed by MOPS buffer containing 100mM Pi. MOPS buffer containing 150mM Pi removed more protein but no PEPC (figure2). This suggests the presence of two isoforms of PEPC in avocado fruit which confirms reports of PEPCs from leaves (Notton and Blanke, 1993).

The 5OmM Pi in MOPS fraction was made 50% saturated with ammonium sulfate to precipitate the PEPC, the pellet redissolved in MOPS buffer and the solution stored at -20C. This procedure resulted in a 25-fold enrichment of the PEPC with a recovery of 50% of the initial activity. To our knowledge, this is the first purification of PEPC from a fruit.

3.2 Effect of pH on stimulation of PEPC by glucose-6-phosphate

The response of avocado PEPC to 5mM glucose-6-phosphate, over the pH range 6-9 showed that stimulation of activity occurred only in the region of pH 7. An examination of this effect over the narrow range of pH 6.3-7.5 showed that stimulation of PEPC occurred only between pH 6.5 and 7.0 with a maximum at pH 6.8 and no glucose-6-phosphate stimulation at pH 7.8.

3.3 Effect of PEP concentration on stimulation of PEPC

Increasing concentrations of PEP from 0. 11 mM to 0.66 mM caused, at pH 6.8 only a small decrease in the activation factor, from 3.2 to 2.8 (figure 1).

3.4 -Effect of pH on inhibition of PEPC by malate

We have previously found that malate inhibition of avocado fruit PEPC was extremely sensitive to pH, 50% inhibition of activity occurring at 46 MM at pH 8.0 and 0.15 mM at pH 7.0, equivalent to a factor of 300fold. At the pH of maximal PEPC stimulation by glucose-6-phosphate (pH 6.8), 50% inhibition occurred with less than 10 /μM malate, equivalent to a factor of 4,600. This appears to be the largest pH sensitivity reported for PEPC inhibition and may reflect the fruit source of the enzyme.

3.5 -Reversal of malate inhibition by glucose-6-phosphate at pH 6.8

5mM glucose-6-phosphate partially reversed the inhibition caused by malate at pH 6.8; only to 70% of the activity observed in the absence of malate.

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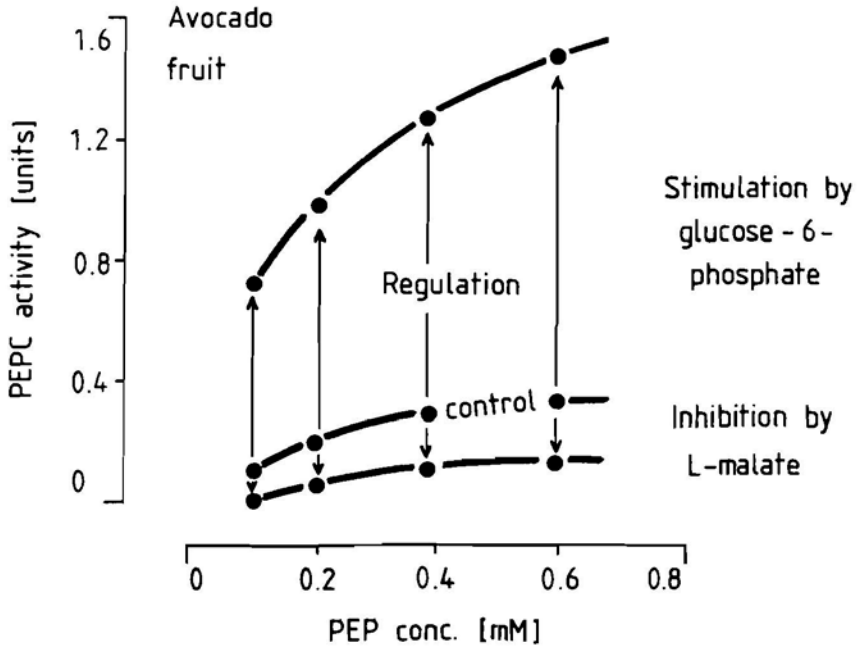

Figure 1. Regulation of avocado fruit PEPC by pH, stimulation by glucose-6-phosphate (5 mM) and inhibition by L-malate (5 μM)

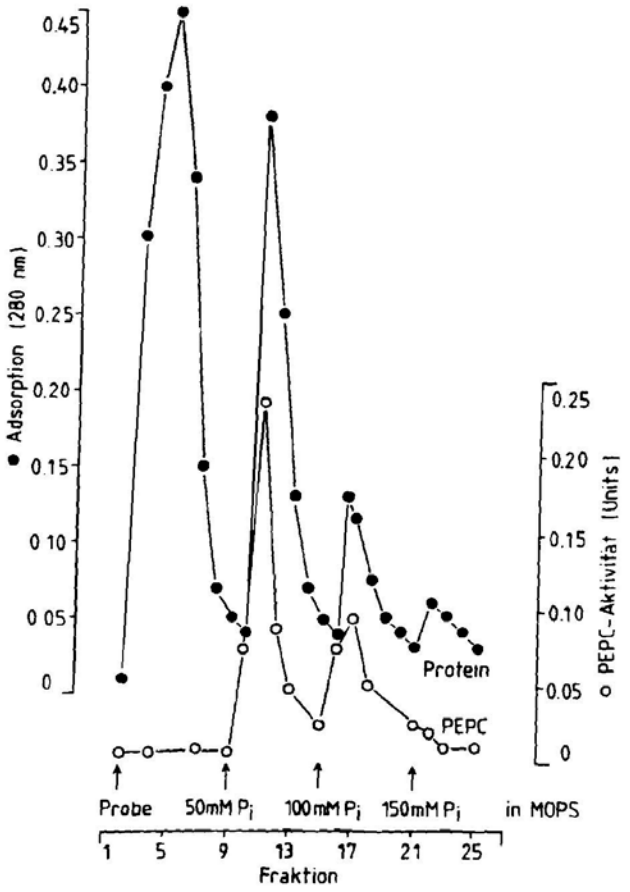

Figure 2. Chromatography of avocado fruit PEPC on spheroidal hydroxylapatite. Stepwise elution of PEPC using 50, 100 and 150 mM Pi in 50 mM MOPS at pH 7.0.