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UTILIZATION OF BICARBONATE BY APPLE FRUIT PHOSPHOENOLPYRUVATE CARBOXYLASE

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Abstract—Phosphoenolpyruvate carboxylase (PEPC), was partially purified from apple fruit cv Golden Delicious. Kinetic values for PEP and HCO_3^- suggest a capacity for efficient carbon dioxide refixation. PEPC activity was maximal between 5–10 mM carbonate (HCO_3^-) and inhibition was observed above 10 mM HCO_3^-. In conditions of PEP-saturation, HCO_3^- inhibition of apple fruit PEPC activity appeared non-competitive with respect to PEP and was partially reversible.

INTRODUCTION

In a preliminary study [1] of the photosynthetic character of apple (*Malus domestica* Borkh) fruit, phosphoenolpyruvate carboxylase (PEPC) (E.C. 4.1.1.31) was identified in the fruit tissue. Phan [2] suggested C_4 photosynthesis in apple fruit was based on the presence and activity of C_4 organic acids and enzymes, interpretations of chloroplast morphology and the existence of a carbon dioxide concentrating mechanism. Apple fruit PEPC and chloroplasts have been found to differ in type from those found in C_4 photosynthetic tissue [1, 3]. In an attempt to clarify the photosynthetic position of apple fruit we studied PEPC particularly in relation to kinetic constants for HCO_3^-, relating these to the known physiological concentrations. In fruit, but not in leaf tissue, internal carbon dioxide concentration may reach 1–5% [4–6]; physiological HCO_3^- at these concentrations may become relevant for inhibition of PEPC activity.

RESULTS AND DISCUSSION

The kinetics of apple fruit PEPC with respect to K_m (PEP, HCO_3^-) and K_i (HCO_3^-) are shown in Table 1. Apple fruit PEPC appears to be an efficient carboxylating enzyme with low K_m (PEP) and a large capability for refixing respired carbon dioxide. The kinetic experiments were done with the aid of the coupled malate dehydrogenase (E.C. 1.1.1.31) (MDH) reaction (see Experimental), which was found to be insensitive to the HCO_3^- concentration used.

The kinetic constants (Table 1) were measured in conditions when the enzyme was saturated for PEP, i.e. $20 \times K_m$. However, at subsaturating PEP concentrations, apple fruit PEPC showed substrate competition between PEP and HCO_3^- at K_m (PEP) concentration and a non-competitive behaviour above a concentration of 0.2 mM PEP (Fig. 1). This effect is similar to that previously reported with PEPC from potato tubers [7].

Maximum PEPC activity of apple fruit was obtained at 5–10 mM HCO_3^- (Fig. 2) and inhibition of activity started above 10 mM, a concentration which corresponds to *ca* 5% CO_2 at pH 7.8 which is the optimum pH for this tissue [8]. In apple fruit, internal carbon dioxide rises to 1–5% at maturity [4–6] which is below the starting point for inhibition of PEPC. Consequently under physiological conditions, inhibition by HCO_3^- would occur only rarely even in tissue with excess carbon dioxide. The comparative HCO_3^- inhibition data essentially confirm observations with single HCO_3^- concentrations, which were reported to be inhibitory, such as 3–5% carbon dioxide [9] and 30 mM HCO_3^- [10] with CAM tissue.

Partially purified apple fruit PEPC was assayed prior to and after addition of HCO_3^- at a concentration which caused 50% inhibition of activity. Excess HCO_3^- was then removed from the extracts by gel filtration. Relative to the initial activity under optimum conditions (5 mM HCO_3^-), large HCO_3^- concentrations (250 mM) depressed the PEPC activity to 55% and this was restored to 88% by removal of the HCO_3^- (activity expressed/mg protein), demonstrating the reversibility of the inhibition.

Table 1. Kinetics of apple fruit PEPC

Tissue	K_m(PEP) (mM)	K_m(HCO_3^-) (mM)	K_i(HCO_3^-) (mM)	V_{max} (nkat /g fr.wt)	V_{max} (nkat /mg protein)
Fruit	0.09	0.20	106	1.8	4.2
Seeds	0.09	0.20	115	5.4	10.8

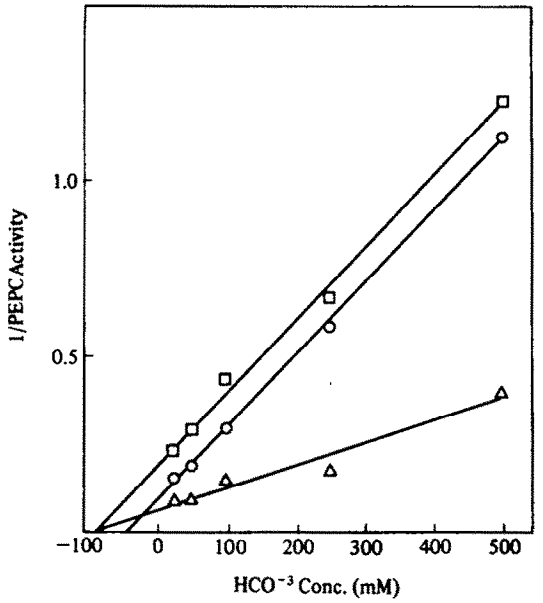

Fig. 1. PEPC kinetics. Interaction of the inhibition of apple fruit PEPC activity by large bicarbonate concentrations at various PEP substrate levels. PEP $1 \times K_m$ (□), $2 \times K_m$ (○), $20 \times K_m$ (△).

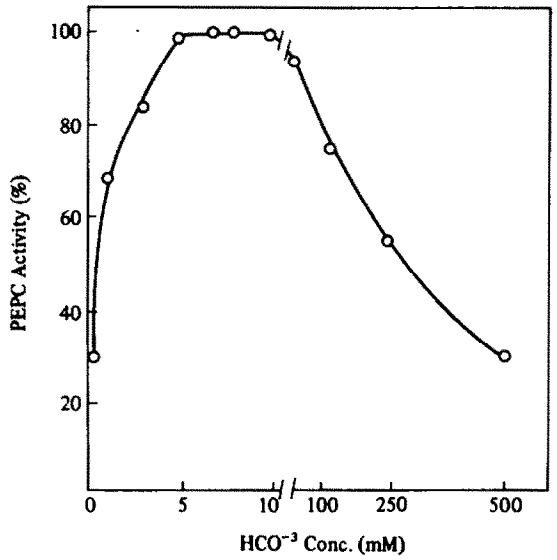

Fig. 2. The response of PEPC activity to bicarbonate concentration.

EXPERIMENTAL

Plant material. Apple fruit cv Golden Delicious were field grown at Long Ashton on 8 year old EMLA 106 rootstock.

Preparation of extracts and enzyme assays. PEPC was partially purified as previously reported [1]. Activity was measured spectrophotometrically at 340 nm by coupling the reaction to the oxidation of NADH from MDH. The assay medium contained 50 mM Tris–HCl pH 7.8, 5 mM $MgCl_2$, 0.25 mM EDTA, 2 mM DTT, 10 units of MDH, 0.1 mM NADH and variable amounts of HCO_3^- (5 mM in the standard assay). The reaction was started by addition of PEP.

Evaluation of kinetic constants. Initial rate measurements, using the above assay, were recorded using a double beam spectrophotometer linked to a microcomputer. K_m and V_{max} values were calculated using a computer programme (Hucklesby, D. P. unpublished) based on a least squares analysis of data after Hanes transformation of s/v against s [11]. Estimates were also made from the same data and computer programme by direct linear plot [12] selecting the median values for K_m and V_{max} [12]. The two methods gave good agreement. K_i was measured using a graphical method [11] as $1/v$ against i. Values given are derived from at least two repetitions of two extracts with at least six substrate concns. With PEPC, HCO_3^- serves as a substrate at small and an inhibitor at large concns. For each expt, the HCO_3^- concn giving max PEPC activity was evaluated to define the range of HCO_3^- concns subsequently used in the kinetic assays. All chemicals were made up in Tris buffer adjusted to pH 7.8 at room temp. at which the calculated endogenous HCO_3^- concn, following Hasselbach–Henderson equations [8], was 0.3 mM HCO_3^-, this value was integrated into all data presented in this paper.

MDH activity was measured spectrophotometrically at 340 nm in the direction of oxaloacetic acid reduction using a standard procedure [13]. *Protein* was measured using a protein assay reagent (BioRad) according to the manufacturer's instructions with ovalbumin as a standard.

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