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Regulation of 'Malic' Enzyme of *Solanum tuberosum* by Metabolites

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A purification of 'malic' enzyme from potato is described. The purified enzyme is specific for NADP and requires a bivalent cation for activity. At pH values below 7 the plot of rate versus malate concentration approximates to normal Michaelis–Menten kinetics. At pH values above 7 the plot of rate versus malate concentration is sigmoid. A number of dicarboxylic acids activate the enzyme and remove the sigmoidicity. The enzyme is inhibited by phosphate, triose phosphates and AMP. In general, effectors of the oxidative decarboxylation of malate behave in the same manner in the reductive carboxylation of pyruvate. The response of the enzyme to energy charge is reported and the physiological significance of the response to metabolites is discussed in relation to the proposed role of the enzyme in the control of pH.

One way in which the pH of plant cytoplasm may be regulated is by balancing carboxylating and decarboxylating reactions so as to control the number of carboxyl groups. If the carboxylating enzyme(s) have an alkaline pH optimum and the decarboxylating enzyme(s) an acid pH optimum, then the system will behave as a pH-stat. It has been proposed (Davies, 1973) that phosphoenolpyruvate carboxylase, malate dehydrogenase and 'malic' enzyme [L-malate–NADP oxidoreductase (decarboxylating), EC 1.1.1.40] may function as a pH-stat system, and the properties of phosphoenolpyruvate carboxylase have been shown to be consistent with such a role for the enzyme (Wong & Davies, 1973). 'Malic' enzyme has been reported to be absent from potato tubers (Clegg & Whittingham, 1970); however, in the present paper we report its purification from this source. The response of the enzyme to various metabolites is reported and the general kinetic properties of the enzyme are examined in relation to the proposed role in a metabolic pH-stat.

Experimental

Materials

Buffers. All buffers were prepared with glass-distilled water. Tris was obtained under the trade name Trizma from Sigma (London) Chemical Co., London S.W.6, U.K. It was dissolved in water at 25°C and adjusted to the required pH with HCl (A.R. grade). Mes [2-(*N*-morpholino)ethanesulphonic acid] and Tes [*N*-tris(hydroxymethyl)methyl-2-aminoethane sulphonic acid] buffers (Good *et al.*, 1966) were obtained from Hopkin and Williams Ltd., Chadwell Heath, Essex, U.K. The buffers were dissolved in water at 25°C and adjusted to the required pH with freshly prepared KOH.

Other chemicals. $NADP^+$, NADPH, ATP, ADP, AMP, various sugar phosphates and L-malate were obtained from Sigma (London) Chemical Co., DE-52 DEAE-cellulose was from W. and R. Balston Ltd., Maidstone, Kent, U.K., and Sephadex G-25 and G-200 were from Pharmacia, Uppsala, Sweden. Other chemicals used were obtained from Hopkin and Williams Ltd.

Methods

Protein measurement. Protein was determined by the spectrophotometric method of Warburg & Christian (1942).

Enzyme assay. The standard assay was carried out at pH 7.0 by measuring the increase in E_{340} associated with $NADP^+$ reduction. The assay mixture contained Tes buffer (pH 7.0, 0.1 M), $MgCl_2$ (3.3 mM), $NADP^+$ (0.4 mg) and sodium malate (3.3 mM) in a volume of 3 ml. The reaction was started by the addition of 0.1 ml of enzyme, containing less than 0.1 unit. Under these conditions, the relation between reaction rate and enzyme concentration was linear. Assays were carried out at 30°C in a Unicam SP. 500 spectrophotometer equipped with a Vitatron recorder.

Unit of enzyme activity. A unit of enzyme activity is defined as the formation of 1 μmol of NADPH/min, which is equivalent to an increase in E_{340} of 2.07/min. Specific activity is defined as the units of activity/mg of protein.

Enzyme purification. Potato tubers (*Solanum tuberosum*) were peeled, cut into segments and rinsed in ice-cold water. Subsequently all steps were carried out at 2°C. Protein was extracted from the tissue by passing potato segments (750 g) and 750 ml of a solution containing Tris–HCl buffer (pH 7.4, 0.05 M) and 2-mercaptoethanol (5 mM) through a domestic Kenwood centrifuge juice extractor. The centrifuge basket was lined with Miracloth (Calbiochem) to retain cell debris and starch grains. The clear extract

(1 litre) was stirred while $(NH_4)_2SO_4$ (250g) was added. After stirring for 10min, the extract was centrifuged at 10000*g* for 15min. The precipitate, which contained 8% of the 'malic' enzyme present in the extract, was discarded and the supernatant treated with a further 70g of $(NH_4)_2SO_4$. The second precipitate, collected by centrifuging at 10000*g* for 15min, was dissolved in about 70ml of Tris–HCl buffer (pH7.4, 0.05M) and assayed for 'malic' enzyme and lactate dehydrogenase (Davies & Davies, 1972). The supernatant was treated with a further 100g of $(NH_4)_2SO_4$ and this precipitate was collected and assayed as for the previous fraction. The final $(NH_4)_2SO_4$ fraction, which was relatively uncontaminated with lactate dehydrogenase, was desalted on a column (15cm×2.5cm) of Sephadex G-25 previously equilibrated with Tris–HCl buffer (pH8.4, 50mM). The active fraction was applied to a column (42cm×2.5cm) packed with DE-52 DEAE-cellulose previously equilibrated with Tris–HCl buffer (pH8.4, 50mM). The column was eluted by applying a linear concentration gradient obtained by placing 250ml of Tris–HCl buffer (pH8.4, 50mM) in the mixing cylinder and an equal volume of Tris–HCl buffer (pH7.4, 0.5M) in the reservoir. Fractions (45 drops) were collected and assayed for activity. Fractions 75–87 contained most of the activity, with a flat peak between fractions 79 and 81. A smaller peak, with less than 15% of the activity found in the main peak, was observed between tubes 91 and 102, but was not further studied. Fractions 75–87 were combined and 40ml was subjected to electrofocusing in the LKB electrofocusing system 8100-10 by using the 110ml column by the method described by Haglund (1972). The results of this purification scheme are shown in Table 1.

Results

Oxidative decarboxylation of malate

(i) *pH optimum.* The pH optimum of the enzyme varies with the concentration of malate. Under the conditions of the standard assay, except that the pH and the concentration of malate were varied, malate concentrations of 8.3, 2.8, 1.4 and 0.7mM gave pH optima of 7.4, 7.2, 6.7 and 6.4 respectively.

(ii) *Requirement for a bivalent cation.* The enzyme showed some activity in the absence of a bivalent cation. However, the activity was greatly enhanced by the addition of Mg^{2+} or Mn^{2+}. At pH7.6, with saturating concentrations of Mg^{2+} and Mn^{2+}, the activity with Mg^{2+} was twice that with Mn^{2+}. However, the affinity of the enzyme for Mn^{2+} was much greater than for Mg^{2+}; the concentrations producing half-maximum velocity were 5μM and 0.8mM respectively.

(iii) *Effect of concentration of substrates.* Previous work on 'malic' enzyme purified from higher plants has shown normal Michaelis–Menten kinetics (Harary *et al.*, 1953; Walker, 1960; Dilley, 1966; Johnson & Hatch, 1970; Coombs *et al.*, 1973; Brandon & van Boekel-Mol, 1973). Since allosteric properties are frequently lost during purification, the kinetic properties of the enzyme were examined at all stages of purification. An inhibitor present in the crude extract and in the $(NH_4)_2SO_4$ fraction was partially removed by passage through a column of Sephadex G-25.

(iv) *Effect of $NADP^+$ concentration.* The plot of rate versus $NADP^+$ concentration showed normal Michaelis–Menten kinetics. Under the conditions of the standard assay, except that the $NADP^+$ concentration was varied, the apparent K_m was 45μM. The enzyme showed no detectable activity with NAD^+.

(v) *Effect of malate concentration.* The plot of rate versus malate concentration approximates to normal Michaelis–Menten kinetics at pH values below 7. Under the conditions of the standard assay, except that the malate concentration was varied and the pH was 6.8, the apparent K_m for malate was 0.67mM. At pH values above 7 the enzyme exhibited sigmoid kinetics. When results were plotted in the form of the Hill equation, the Hill coefficient (n_H) was 1 at pH6.8, but at pH values above 7 n_H was greater than 2. Numerous determinations with enzyme preparations at various stages of purification have given values of n_H from 2 to 3. The effect of pH on the plot of rate versus malate concentration is shown in Fig. 1.

Table 1. *Purification of 'malic' enzyme from potato tubers*

For details see the text.

Fraction	Volume (ml)	Protein (mg/ml)	Enzyme activity (units/ml)	Specific activity (units/mg)	Purification
Extract	1000	12.7	0.15	0.012	—
0–250g of $(NH_4)_2SO_4$	80	14.3	0.15	0.01	—
250–320g of $(NH_4)_2SO_4$	75	11.3	1.1	0.16	13
320–420g of $(NH_4)_2SO_4$	70	8.8	1.8	0.2	17
Sephadex G-25	75	9.0	4.4	0.5	41
DE-52 (peak)	3.0	0.15	0.6	4.0	330
Electrofocusing	2.0	0.01	0.3	30.0	2500

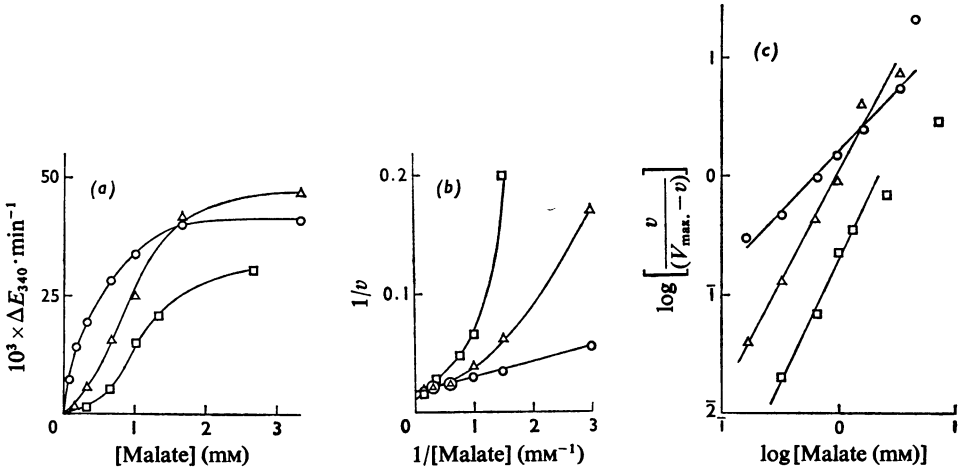


Fig. 1. *Effect of pH and malate concentration on the rate of malate decarboxylation catalysed by partially purified 'malic' enzyme*

(*a*) Direct plot of rate versus malate concentration; (*b*) double-reciprocal plot; (*c*) Hill plot. Enzyme activity was determined as described in the text except that the malate concentration and pH were varied as indicated. Velocity is expressed as the change in E_{340}/min. ○, pH6.9 ($n_H = 1$); △, pH7.3 ($n_H = 2.0$); □, pH7.6 ($n_H = 2.1$).

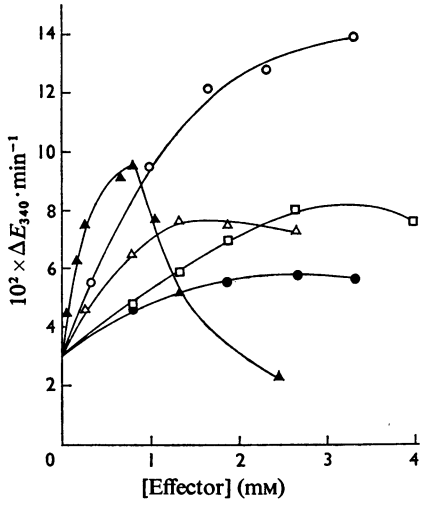


Fig. 2. *Effect of various compounds on the rate of decarboxylation of malate catalysed by partially purified 'malic' enzyme*

Enzyme activity was assayed as described in the text except that the malate concentration was 0.5mM and the pH was 7.6. ▲, Oxaloacetate; ○, fumarate; △, L(+)-tartrate; □, phosphoenolpyruvate; ●, 2-oxoglutarate.

(vi) *Activators of malate decarboxylation.* (*a*) Dicarboxylic acids. A number of dicarboxylic acids which gave no reaction in the absence of malate, were found to activate the enzyme when assayed at pH7 and above (Fig. 2). There is an optimum concentration at which the dicarboxylic acids stimulate malate decarboxylation. In most cases high concentrations produce only slight inhibition, but with oxaloacetate there is pronounced inhibition (Fig. 2). The dicarboxylic acids eliminate the sigmoidicity in the plot of rate versus malate concentration when Mg^{2+} is the activating cation (Fig. 3). The percentage stimulation produced by the dicarboxylic acids is greatest when the malate concentration is low and when the pH is alkaline. At high concentrations of malate the dicarboxylic acids behave as inhibitors (Fig. 3).

(*b*) Phosphoenolpyruvate. The enzyme is activated by phosphoenolpyruvate (Fig. 2).

(vii) *Inhibitors of malate decarboxylation.* Crude preparations of the enzyme contain an inhibitor which is partially removed by gel filtration on Sephadex G-25. The inhibitor appears to be heat-stable but has not been further characterized. The presence of the inhibitor explains the apparent increase in total activity which occurs during purification and may partially explain the failure of Clegg & Whittingham (1970) to obtain active preparations of 'malic' enzyme from potatoes.

(*a*) Phosphate. At pH values below 7 phosphate inhibits the enzyme, changing the approximately Michaelis–Menten kinetics into sigmoid kinetics (Fig. 4). At pH values above 7.2, where the plot of rate versus malate concentration is clearly sigmoid, phosphate inhibits the enzyme and increases the sigmoidicity. Thus at pH7.6, $s_{(0.5)}$ for malate was 2.7mM,

but in the presence of 13.3mM-phosphate, $s_{(0.5)}$ was 4mM. However, plotting the data according to the Hill equation showed that the Hill coefficient (n_H) was not significantly changed by the addition of phosphate (Fig. 5). The inhibition produced by phosphate is partially reversed by the addition of succinate (Fig. 5).

(*b*) Sulphate. Sulphate inhibits in a similar manner to phosphate, but the strength of inhibition was less than that observed for phosphate.

(*c*) Phosphate esters. A number of phosphate esters were examined and found to inhibit malate decarboxylation. Hexose phosphates are relatively poor inhibitors, but the phosphate esters of glyceric acid are strong inhibitors (Fig. 6).

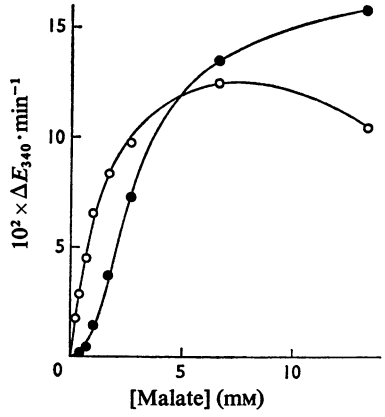


Fig. 3. *Effect of succinate on the oxidative decarboxylation of malate catalysed by partially purified 'malic' enzyme*

Enzyme activity was assayed as described in the text except that the malate concentration was varied, the pH was 7.6 and succinate was present in some assays. ●, Control (no succinate); ○, succinate (1.3mM).

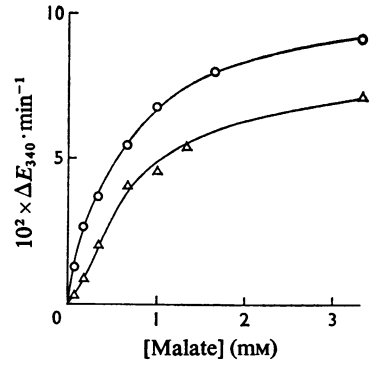


Fig. 4. *Effect of phosphate on the oxidative decarboxylation of malate catalysed by partially purified 'malic' enzyme*

Enzyme activity was assayed as described in the text except that the malate concentration was varied, the pH was 6.9 and phosphate was present in some assays. ○, Control (no phosphate); △, phosphate (13.3mM).

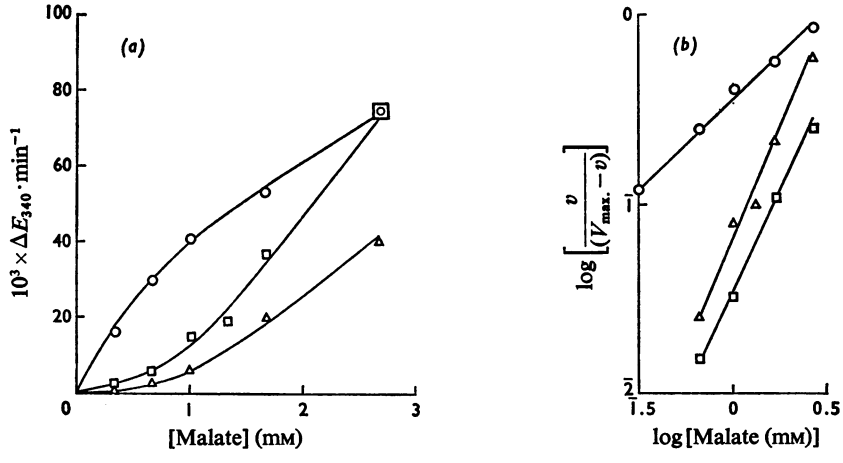


Fig. 5. *Effect of phosphate on the oxidative decarboxylation of malate catalysed by partially purified 'malic' enzyme in the presence and absence of succinate*

(*a*) Direct plot of rate versus malate concentration; (*b*) Hill plot. Enzyme activity was assayed as described in the text, except that the malate concentration was varied, the pH was 7.6 and phosphate and succinate were present in some assays. Velocity is expressed as the change in E_{340}/min. □, Control (no phosphate, no succinate) ($n_H = 2.2$); △, phosphate (8.3mM) ($n_H = 2.3$); ○, phosphate (8.3mM)+succinate (1.3mM) ($n_H = 1$).

(*d*) Organic and amino acids. As previously noted, a number of organic acids activate the enzyme when present in low concentrations (Fig. 2). However, a number of compounds (citrate, malonate and aspartate) inhibit the enzyme at all concentrations tested (0–3.3mM). At a concentration of 3.3mM and at pH7, these compounds produced the following percentage inhibitions; citrate 90, malonate 30, aspartate 10 (Davies, 1973).

(*e*) Nucleotides. Adenine nucleotides inhibit the enzyme, but the relative inhibition produced by ATP, ADP and AMP varies with concentration and pH. At pH7, ADP is the most effective inhibitor (Fig. 7*a*), but at pH6.5 and at concentrations below 300μM, AMP is most effective, whereas ATP is the most effective at higher concentrations (Fig. 7*b*). The inhibition produced by adenine nucleotides, particularly at high concentrations, may be due to chelation of Mg^{2+}. The effect of ADP on the plot of rate versus $NADP^+$ concentration was examined and the results (Fig. 8) show that ADP introduces sigmoidicity into the plots.

(viii) *Energy charge.* The effect of nucleotides on the activity of the enzyme has been examined in relation to Atkinson's (1968) concept of energy charge. The energy charge is defined as

$$\frac{[ATP]+0.5[ADP]}{[ATP]+[ADP]+[AMP]}$$

being fully charged (1.0) when only ATP is present and being completely discharged (0) when only AMP is present. The results are shown in Fig. 9.

Reductive carboxylation of pyruvate

(i) *pH optimum.* The effect of pH on the activity of the enzyme was measured at two concentrations of pyruvate (1.67 and 16.7mM). The pH optimum (6.8) was not affected by the pyruvate concentration or by the presence of succinate, which activates the carboxylation (Fig. 10).

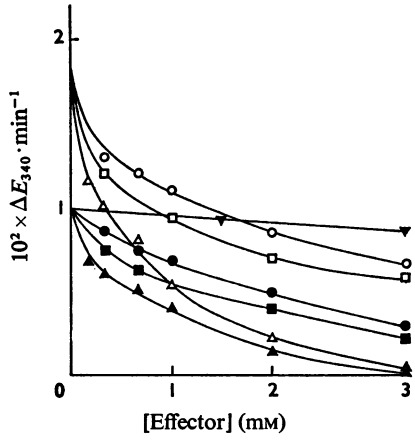


Fig. 6. *Effect of various compounds on the rate of oxidative decarboxylation of malate in the presence and absence of succinate*

Enzyme activity was assayed as described in the text except that the malate concentration was 0.167mM. ●, 3-Phosphoglycerate; ○, 3-phosphoglycerate+succinate (1.33mM); ■, 2,3-diphosphoglycerate; □, 2,3-diphosphoglycerate+succinate (1.33mM); ▲, 2-phosphoglycerate; △, 2-phosphoglycerate+succinate (1.33mM); ▼, fructose 1,6-diphosphate.

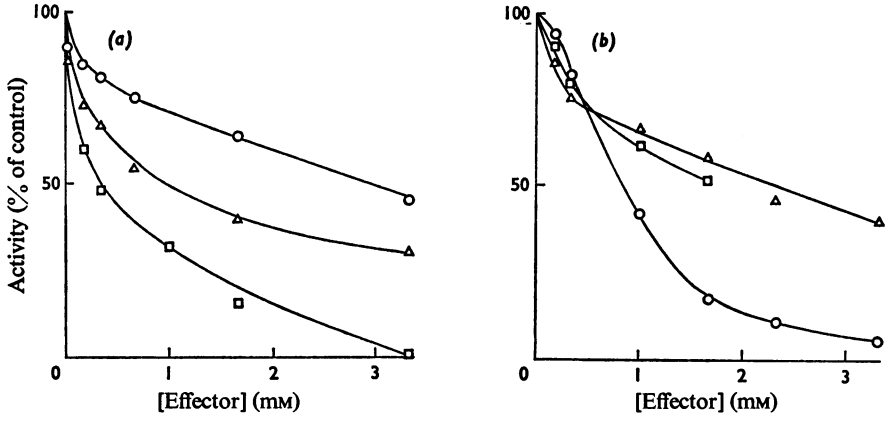


Fig. 7. *Effect of adenine nucleotides on the rate of oxidative decarboxylation of malate*

Enzyme activity was determined as described in the text except that the malate concentration was 0.13mM. In (*a*) the pH was 7.4 and in (*b*) the pH was 6.5. ○, ATP; □, ADP; △, AMP.

(ii) *Requirement for a bivalent cation.* At pH7.6, the enzyme showed little or no activity in the absence of a bivalent cation. Mg^{2+} had little effect on enzyme activity, but Mn^{2+} activated the enzyme. The concentration of Mn^{2+} producing half-maximum velocity was 55μM. In the presence of 1.3mM-succinate, the concentration of Mn^{2+} producing half-maximum velocity was 6μM (Fig. 12*c*). At pH7.0, the enzyme showed significant activity in the absence of a bivalent cation. The concentration of Mn^{2+} producing half maximum velocity was 5μM.

(iii) *Effect of pyruvate concentration.* At pH7.0 in the presence of NADPH (160μM), Mn^{2+} (1mM) and $NaHCO_3$ (10mM) the plot of rate against pyruvate concentration showed normal Michaelis–Menten kinetics and the apparent K_m for pyruvate was 3.3mM. Under the same conditions, except that the pH was 7.6, the plot of rate against pyruvate concentration was sigmoid and the $s_{(0.5)}$ value was 16mM (Fig. 11). When plotted in the form of the Hill equation, the Hill coefficient (n_H) was 1.9.

(iv) *Effect of NADPH concentration.* At pH7.0, in the presence of Mn^{2+} (1mM), $NaHCO_3$ (10mM) and pyruvate (16.6mM), the plot of rate against NADPH concentration showed normal Michaelis–Menten kinetics and an apparent K_m for NADPH of 13μM. At pH7.6, normal Michaelis–Menten kinetics were again observed and the K_m for NADPH was 102μM.

(v) *Effect of $NaHCO_3$ concentration.* Dalziel & Londesborough (1968) have shown for wheat-germ 'malic' enzyme that CO_2 rather than HCO_3^- is the reactive species for carboxylation. We have not

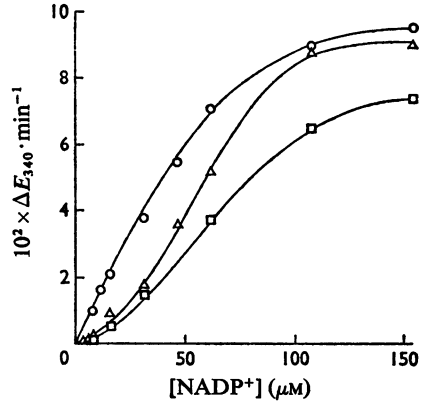


Fig. 8. *Effect of ADP on the oxidative decarboxylation of malate*

Enzyme activity was measured as described in the text except that the malate concentration was 1.66mM and $NADP^+$ was varied as indicated. ○, Control (no ADP); △, ADP (0.6mM); □, ADP (3.3mM).

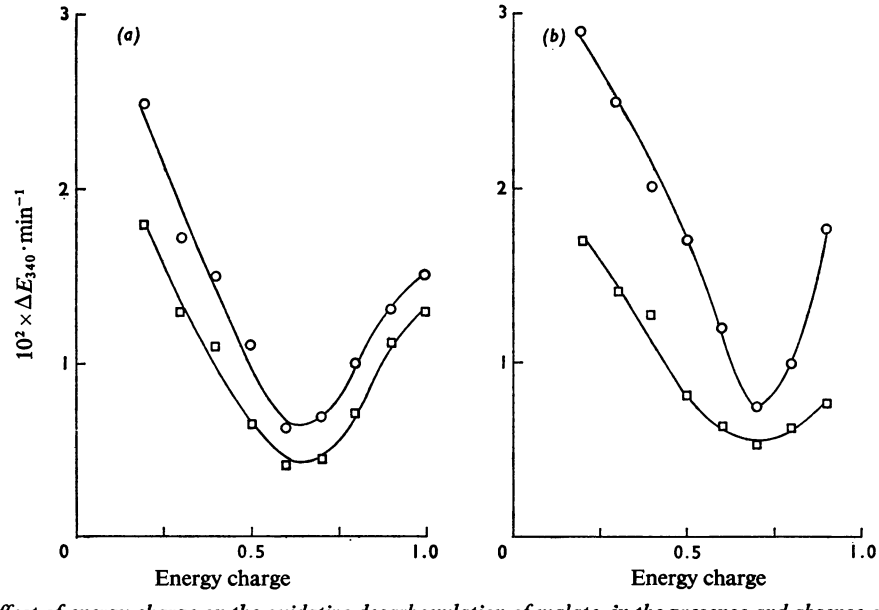


Fig. 9. *Effect of energy charge on the oxidative decarboxylation of malate, in the presence and absence of succinate*

Enzyme activity was determined as described in the text except that the malate concentration was (*a*) 67μM and (*b*) 133μM. The equilibrium constant for the adenylate kinase reaction was taken as 0.8 and the energy charge was calculated as described by Atkinson (1968). The concentration of the adenine nucleotide pool (ATP+ADP+AMP) was 0.033mM. □, Control (no succinate); ○, succinate (670μM).

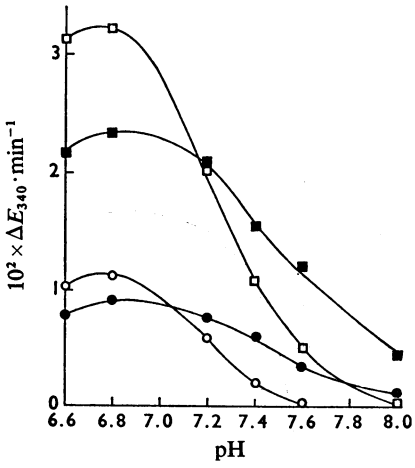


Fig. 10. *Effect of pH on the reductive carboxylation of pyruvate in the presence and absence of succinate*

Enzyme activity was assayed at 340nm in cells of 1cm light-path containing Mes buffer (0.1M) for pH6.6 and 6.8 or Tes buffer (0.1M) for pH7.2–8.0. Mn^{2+} (1mM), $NaHCO_3$ (10mM), NADPH (0.4mg), pyruvate and enzyme were present in a final volume of 3ml. □, Pyruvate (16.7mM); ■, pyruvate (16.7mM) and succinate (1.3mM); ○, pyruvate (1.67mM); ●, pyruvate (1.67mM) and succinate (1.3mM).

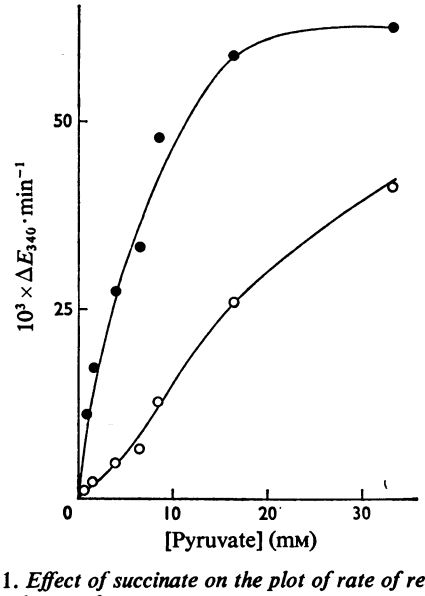


Fig. 11. *Effect of succinate on the plot of rate of reductive carboxylation of pyruvate versus pyruvate concentration at pH7.6*

Enzyme activity was assayed at 340nm under the conditions given in Fig. 10. ○, Control (no succinate); ●, succinate (1.3mM). The scale of activity with succinate is 3× the scale for the control.

Table 2. *Effect of various compounds on the reductive carboxylation of pyruvate catalysed by 'malic' enzyme*

Enzyme activity was assayed at 340nm, in Tes buffer, pH8.0 (0.1M), $MnCl_2$ (1.66mM), $NaHCO_3$ (10mM), NADPH (0.4mg), pyruvate (5mM) and enzyme (0.1ml) in a final volume of 3ml.

Effector	Concentration (mM)	Activation (%)
Succinate	1.3	1075
Fumarate	3.3	540
2-Oxoglutarate	2.7	190
Oxaloacetate	0.5	190
Phosphoenolpyruvate	2.7	390
L(+)-Tartrate	2.7	200
D(−)-Tartrate	2.7	280

studied the requirement for CO_2 but have determined the effect of the $NaHCO_3$ concentration on the rate of carboxylation. At pH7.0 in the presence of Mn^{2+} (1mM), pyruvate (16.6mM) and NADPH (160μM) the plot of rate against $NaHCO_3$ concentration approximated to Michaelis–Menten kinetics and the K_m for $NaHCO_3$ was 2.7mM. Under the same conditions except that the pH was 7.6, normal Michaelis–Menten kinetics were observed and the K_m for $NaHCO_3$ was 11mM.

(vi) *Activators of pyruvate carboxylation.* A number of compounds were examined for their effect on the rate of pyruvate carboxylation. The results are shown in Table 2. Succinate, which activates the carboxylation reaction at pH values above 7.2, inhibits the reaction at pH values of 7 and below (Fig. 10). At pH7.6 the plot of rate against pyruvate concentration is sigmoid in the absence of succinate, but in the presence of succinate Michaelis–Menten kinetics are observed (Fig. 11). At pH7.6 the plot of rate against $NaHCO_3$ shows Michaelis–Menten kinetics and the effect of succinate approximates to the 'V' system of Monod *et al.* (1965) (Fig. 12*a*). The plot of rate against NADPH concentration shows Michaelis–Menten kinetics, but the activation by succinate appears to involve an increase in $V_{max.}$ and a decrease in the K_m for NADPH (Fig. 12*b*).

Discussion

The response of 'malic' enzyme to metabolites is generally compatible with its proposed role in a metabolic pH-stat (Davies, 1973). Thus, as shown in Table 3, 'malic' enzyme responds to a number of effectors in a manner opposite to that reported for phosphoenolpyruvate carboxylase (Wong & Davies, 1973). The comparison between the two enzymes can be extended to their specificity towards effector molecules. Thus both enzymes respond to SO_4^{2-} and HPO_4^{2-}, suggesting the possibility that they respond to two

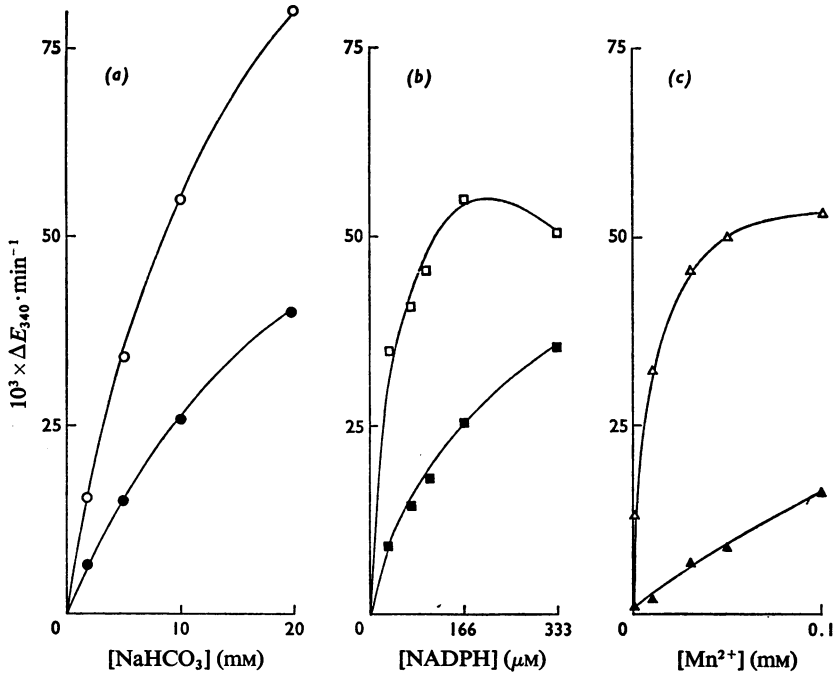


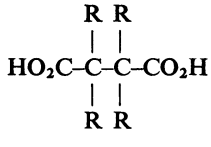
Fig. 12. *Effect of succinate on the plot of rate of reductive carboxylation of pyruvate against (a) $NaHCO_3$ concentration, (b) NADPH concentration and (c) Mn^{2+} concentration*

Enzyme activity was assayed at 340nm under the conditions given in Fig. 10 except that $NaHCO_3$, NADPH and Mn^{2+} concentrations were varied as indicated. Control (no succinate), closed symbols; succinate (1.3mM), open symbols. In (*b*) the scale of activity with succinate is 3× the scale for the control.

Table 3. *Comparison of the responses of 'malic' enzyme and phosphoenolpyruvate carboxylase to various effectors*

	Effect on	
Effector	Potato 'malic' enzyme (this paper)	Maize phosphoenolpyruvate carboxylase (Wong & Davies, 1973)
Phosphate	Inhibits	Activates
Sulphate	Inhibits	Activates
Phosphoglyceric acids	Inhibit	Activate
Dicarboxylic acids	Activate	Inhibit
Aspartate	Slight inhibition	Inhibits
AMP	Inhibits	Activates

closely positioned negative charges. Both enzymes respond to dicarboxylic acids in a manner implying a relatively unspecific site; both enzymes respond to compounds of the general formula



where any R can be H or OH.

There is a temptation to suggest that all the responses to metabolites have physiological significance; thus the activation of 'malic' enzyme by the product of phosphoenolpyruvate carboxylase and the inhibition of phosphoenolpyruvate carboxylase by the organic acid substrate of 'malic' enzyme makes teleological sense. It is noteworthy that 2-phosphoglycerate (the substrate of enolase) inhibits 'malic' enzyme, whereas phosphoenolpyruvate (the product of enolase) activates the enzyme, but the observation may have no physiological significance.

The response of 'malic' enzyme to energy charge deserves further comment. The response is complex, being affected by pH and by the concentration of nucleotides. Under one set of defined conditions (Fig. 9) the plot of rate versus energy charge does not conform to either the R or U type proposed by Atkinson (1968) but instead shows a minimum rate at an energy charge of approx. 0.5. This reflects the fact that the concentration of ADP is maximal at an energy charge of 0.5, and ADP is the most inhibitory of the adenine nucleotides under the conditions specified in Fig. 9. Atkinson (1968) has emphasized the importance of the constancy of the energy charge between 0.75 and 0.9; thus Chapman *et al.* (1971) suggest that growth of *Escherichia coli* can only occur at energy charges above 0.8. In some plants energy-charge values close to 0.8 have been reported (Weissman, 1972), but in a number of other cases the energy charge lies between 0.6 and 0.8 (Bomsel & Pradet, 1968; Pacold & Anderson, 1973). In this range the responses of 'malic' enzyme and phosphoenolpyruvate carboxylase to energy charge intersect. This is consistent with the view that there is a metabolic interlock between the control of energy charge and the control of pH. On the other hand the results reported here, like those of Purich & Fromm (1973), counsel caution in the interpretation of enzyme responses to energy charge.

The properties of 'malic' enzyme have been investigated in both directions, carboxylation and decarboxylation. The proposal that the enzymes function in a metabolic pH-stat assumes that the physiological function of the enzyme lies in the decarboxylation of malate. However, it should be noted that at pH7.6 and with Mn^{2+} as a cofactor and succinate as activator, the ratio $V_{max.}$ (decarboxylation)/$V_{max.}$ (carboxylation) is about 4. This raises the possibility that the enzyme functions in the direction of carboxylation. However, it seems likely that, at physiological concentrations of the reactants, the ratio, rate of decarboxylation/rate of carboxylation, will greatly exceed 4.

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