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THE DISTRIBUTION AND PROPERTIES OF NADP MALIC ENZYME IN FLOWERING PLANTS

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Key Word Index—Angiospermae; malic enzyme; allosteric properties; distribution; flood tolerance.

Abstract—Malic enzyme is shown to be widely distributed in higher plants and contrary to earlier reports is present in the roots of flood tolerant species. Excluding members of the Gramineae, the malic enzyme from 27 out of 28 species examined was shown to exhibit allosteric properties. On the other hand the malic enzyme present in members of the Gramineae shows little or no allosteric properties.

INTRODUCTION

MALIC enzyme [L-malate-NADP oxidoreductase (decarboxylating) E.C. 1.1.1.40] has been isolated from a number of plants, but has been reported absent from potato tubers[1] and from the roots of flood tolerant plants.[2] The malic enzyme from potato tubers has recently been purified and shown to exhibit allosteric properties.[3] Data presented by Dilley[4] for the malic enzyme of apples can also be interpreted in terms of allosteric properties. However the malic enzyme from wheat[5] *Bryophyllum crenata*,[6] maize,[7] *Pennisetum purpureum*[8] and *Bryophyllum tubiflorum*[9] does not appear to show allosteric properties.

The present investigation was undertaken to determine the distribution of malic enzyme in plants, paying particular attention to those cases where its absence has been recorded. Additionally, we have studied the occurrence of allosteric properties.

RESULTS

Distribution of malic enzyme

We have used crude extracts, ammonium sulphate precipitates and fractions purified on DEAE-cellulose to survey plants for the presence of malic enzyme. Because of the reported absence of malic enzyme in the roots of flood tolerant species we have examined the four species which have been reported by McManmon and Crawford[2] to lack malic enzyme. Malic enzyme activity was demonstrated in the roots of all four species—*Mentha aquatica*, *Myosotis scorpiodes*, *Senecio aquaticus* and *Glyceria maxima* and also in *Pulicaria dysenterica* grown under flooded conditions. The results of this survey of some 38 species are pre-

[1] CLEGG, C. J. and WHITTINGHAM, C. P. (1970) *Phytochemistry* **9**, 279.
[2] MCMANMON, M. and CRAWFORD, R. M. M. (1971) *New Phytologist* **70**, 299.
[3] DAVIES, D. D. and PATIL, K. D. (1974) *Biochem. J.* **137**, 45.
[4] DILLEY, D. R. (1966) *Plant Physiol.* **41**, 214.
[5] HARARY, I., KOREY, S. R. and OCHOA, S. (1953) *J. Biol. Chem.* **203**, 595.
[6] WALKER, D. A. (1960) *Biochem. J.* **74**, 216.
[7] JOHNSON, H. S. and HATCH, M. D. (1970) *Biochem. J.* **119**, 273.
[8] COOMBS, J., BALDRY, C. W. and BUCKE, C. (1973) *Planta* **110**, 109.
[9] BRANDON, P. C. and VAN BOEKEL-MOL, T. N. (1973) *European J. Biochem.* **35**, 62.

sented in Tables 1 and 2. It should be noted that these results are not all directly comparable since plants were examined from widely differing environments and at various stages of development.

TABLE 1. ACTIVITY OF MALIC ENZYME IN VARIOUS SPECIES OF DICOTYLEDONS

Family	Species	Organ	Malate conc (mM)	Activity* (ΔE_{340}/min/g fr. tissue) pH 7·0 (Mn)	pH 7·0 (Mg)	pH 7·6 (Mn)	pH 7·6 (Mg)
Ranunculaceae	*Delphinium bella-donna*	Leaf	0·17	0·085	—	0·018	—
			0·82	0·38	0·015	0·26	0·003
	Pulsatilla vulgaris	Leaf	0·17	0·06	—	0·035	—
			0·82	0·11	0·015	0·088	0·002
Cruciferae	*Brassica oleracea*	Leaf	0·17	—	—	—	0·048
			0·33	—	0·09	—	—
Malvaceae	*Malva sylvestris*	Leaf	0·27	0·025	—	0·015	—
			0·53	—	0·012	—	0·002
Leguminosae	*Pisum sativum*	Epicotyl	0·33	0·1	0·012	0·043	0·018
		Leaf	0·33	0·37	0·045	0·09	0·001
		Root	0·33	0·06	0·02	0·025	0·01
Crassulaceae	*Bryophyllum blossfeldiana*	Root	0·33	0·014	0·005	0·005	—
			0·66	—	0·007	—	0·004
Cucurbitaceae	*Cucumis sativus*	Fruit	0·17	—	0·008	—	0·002
Urticaceae	*Urtica dioica*	Leaf	0·5	—	—	0·001	—
Boraginaceae	*Myosotis scorpioides*	Root	0·33	0·025	0·003	—	0·002
Solanaceae	*Solanum tuberosum*	Tuber	0·33	—	0·21	—	0·042
	Solanum lycopersicum	Fruit	0·33	—	—	0·002	—
			0·82	—	—	—	0·004
Scrophulariaceae	*Verbascum thapsus*	Leaf	0·27	0·19	—	0·028	—
			0·53	—	0·005	—	0·004
	Antirrhinum majus	Leaf	0·27	0·069	—	0·025	—
			0·53	—	0·002	—	0·008
Labiatae	*Mentha aquatica*	Root	0·33	0·1	0·024	0·025	0·001
		Leaf	0·33	—	0·02	—	0·008
	Lamium purpureum	Leaf	0·33	0·09	0·013	0·03	0·001
	Lamium album	Leaf	0·33	0·08	0·018	0·004	0·008
	Stachys lanata	Leaf	0·27	0·004	—	0·001	—
			0·53	0·005	0·002	0·003	—
Plantaginaceae	*Plantago major*	Leaf	0·5	—	—	0·05	0·004
Compositae	*Senecio aquaticus*	Root	0·33	0·08	0·006	—	—
	Pulicaria dysenterica	Root	0·33	0·06	0·014	0·003	—
	Echinops ritro	Leaf	0·17	0·04	—	0·014	—
			0·82	0·094	0·004	0·062	0·01
	Lactuca sativa	Leaf	0·33	—	—	0·042	—

* Assay system: NADP (0·4 mg); MOPS buffer (0·1 M pH as indicated); malate as indicated; $MnCl_2$ or $MgCl_2$ (1·66 mM) and enzyme in a total volume of 3 ml.

Effect of succinate on the activity of malic enzyme from various species

It has been shown that succinate is a positive effector for potato malic enzyme[3] and we have used this response as a diagnostic test for the presence of allosteric properties in various preparations of malic enzyme. The response of the preparations to succinate has been examined at two pH's—7·0 and 7·6 and with two activating cations Mn and Mg. The results are presented in Tables 3 and 4.

TABLE 2. ACTIVITY OF MALIC ENZYME IN VARIOUS SPECIES OF MONOCOTYLEDONS

Family	Species	Organ	Malate conc (mM)	Activity* (ΔE_{340}/min/g fr. wt) pH 7·0 (Mn)	pH 7·0 (Mg)	pH 7·6 (Mn)	pH 7·6 (Mg)
Lilaceae	*Tulipa gesnerana*	Leaf	0·82	0·10	0·004	0·056	0·002
Juncaceae	*Juncus effusus*	Stem	0·33	0·07	0·017	0·064	0·006
Iridaceae	*Iris germanica*	Leaf	0·33	0·08	0·006	0·05	0·001
Orchidaceae	*Odontoglossum* sp.	Leaf	0·33	0·20	—	—	—
			0·82	—	0·027	0·005	0·005
Cyperaceae	*Carex riparia*	Leaf	0·33	0·05	—	0·018	0·02
	Luzula campestris	Leaf	0·33	0·015	—	0·006	—
			0·82	—	0·005	—	0·001
Commelinaceae	*Tradescantia* sp.	Leaf	0·33	0·025	0·009	—	—
			0·82	—	—	0·008	0·001
Gramineae	*Oryza sativa*	Leaf	0·33	0·25	0·16	0·15	0·07
	Phragmites communis	Leaf	0·33	0·04	0·018	0·005	0·007
	Glyceria maxima	Root	0·33	0·13	0·043	0·067	0·031
	Triticum vulgare	Leaf	0·33	0·2	0·04	0·06	0·07
	Holcus lanatus	Leaf	0·33	0·03	0·016	0·02	—
			0·66	—	0·01	—	0·006
	Zea mays	Leaf	0·33	0·3	0·18	0·29	0·23
	Arundmaria japonica	Leaf	0·33	—	0·025	—	—
			0·82	0·025	—	—	—
	Saccharum officinarum	Leaf	0·33	0·025	—	0·058	—
			0·66	—	0·013	—	0·047
Musaceae	*Musa paradisiaca*	Leaf	0·33	0·028	—	0·008	—
			0·82	—	0·006	—	0·005

* Assay conditions as in Table 1.

TABLE 3. EFFECT OF SUCCINATE ON THE ACTIVITY OF MALIC ENZYME FROM VARIOUS SPECIES OF THE DICOTYLEDONS

Family	Species	Organ	Preparation	Malate conc (mM)	% Activity* pH 7·0 (Mn)	pH 7·0 (Mg)	pH 7·6 (Mn)	pH 7·6 (Mg)
Ranunculaceae	*Delphinium bella-donna*	Leaf	$(NH_4)_2SO_4$	0·82	75	811	101	1700
	Pulsatilla vulgaris	Leaf	$(NH_4)_2SO_4$	0·82	72	286	85	1300
Cruciferae	*Brassica oleracea*	Leaf	$(NH_4)_2SO_4$	0·17	—	—	—	258
Malvaceae	*Malva sylvestris*	Leaf	G-25	0·27	80	—	107	—
				0·53	—	113	—	533
Leguminosae	*Pisum sativum*	Epicotyl	DE-52	0·33	80	252	155	234
		Leaf	DE-52	0·33	72	106	117	467
Crassulaceae	*Bryophyllum blossfeldiana*	Root	G-25	0·33	96	120	146	—
				0·66	—	107	114	188
Cucurbitaceae	*Cucumis sativus*	Fruit	$(NH_4)_2SO_4$	0·17	—	2200	—	525
Boraginaceae	*Myosotis scorpiodes*	Root	DE-52	0·66	200	157	210	200
Solanaceae	*Solanum lycopersicum*	Fruit	$(NH_4)_2SO_4$	0·33	—	—	900	—
				0·82	—	—	—	250
Scrophulariaceae	*Verbascum thapsus*	Leaf	G-25	0·27	94	—	309	—
				0·53	—	300	—	150
	Antirrhinum majus	Leaf	G-25	0·53	—	600	—	83
Labiatae	*Mentha aquatica*	Root	DE-52	0·33	79	110	65	—
				0·66	82	55	63	74
		Leaf	G-25	0·33	—	80	—	100
	Lamium purpureum	Leaf	G-25	0·53	98	138	152	170
	Lamium album	Leaf	G-25	0·53	—	75	750	300
	Stachys lanata	Leaf	G-25	0·53	40	107	83	266
Compositae	*Senecio aquaticus*	Root	DE-52	0·66	200	157	210	200
	Pulicaria dysenterica	Root	DE-52	0·33	142	219	389	—
		Leaf	$(NH_4)_2SO_4$	0·82	64	212	126	200
	Lactuca sativa	Leaf	$(NH_4)_2SO_4$	0·33	—	—	160	—

* Assay system as in Table 1. Succinate 1·33 mM. Results are expressed as: (activity in presence of succinate)/(activity in absence of succinate) × 100.

TABLE 4. EFFECT OF SUCCINATE ON THE ACTIVITY OF MALIC ENZYME FROM VARIOUS SPECIES OF THE MONOCOTYLEDONS

Family	Species	Organ	Preparation	Malate conc (mM)	% Activity* pH 7·0 (Mn)	pH 7·0 (Mg)	pH 7·6 (Mn)	pH 7·6 (Mg)
Liliaceae	*Tulipa gesnerana*	Leaf	$(NH_4)_2SO_4$	0·82	77	750	2800	125
Juncaceae	*Juncus effusus*	Leaf	DE-52	0·66	–	42	158	196
Iridaceae	*Iris germanica*	Leaf†	DE-52 (E_1)	0·66	–	118	104	510
		Leaf†	DE-52 (E_2)	0·33	122	–	309	–
				0·66	–	395	–	216
Orchidaceae	*Odontoglossum* sp.	Leaf	$(NH_4)_2SO_4$	0·82	–	190	1100	33
Cyperaceae	*Carex riparia*	Leaf	DE-52	0·13	130	–	–	–
				1·7	–	–	118	167
	Luzula campestris	Leaf	$(NH_4)_2SO_4$	0·33	75	–	160	–
				0·82	–	350	–	300
Gramineae	*Oryza sativa*	Leaf	DE-52	0·66	84	98	84	94
	Phragmites communis	Leaf	DE-52	0·33	102	107	104	–
	Glyceria maxima	Leaf	DE-52	0·66	91	86	84	98
	Triticum vulgare	Leaf E_1†	DE-52	3·3	107	100	120	104
		E_2†	DE-52	3·3	104	120	108	120
	Holcus lanatus	Leaf	G-25	0·33	46	–	88	–
	Saccharum officinarum	Leaf	G-25	0·33	66	–	62	–
				0·66	–	52	–	71
	Zea mays	Leaf	DE-52	0·33	67	31	68	53
	Arundmaria japonica	Leaf	$(NH_4)_2SO_4$	0·33	91	–	–	–
				0·82	–	73	–	–
Musaceae	*Musa paradiseaea*	Leaf	$(NH_4)_2SO_4$	0·33	–	–	250	–
				0·82	–	170	–	40
Commelinaceae	*Tradescantia* sp.	Leaf	$(NH_4)_2SO_4$	0·33	100	–	275	–
				0·82	–	155	–	400

* Conditions as in Table 3.
† Two enzymes, see text and Fig. 1.

Kinetic properties of purified malic enzyme from various species

To determine enzyme kinetic constants it is necessary to obtain enzyme preparations free from interfering reactions. Accordingly, we have purified malic enzyme from 15 species of higher plants. In general ion exchange chromatography on DEAE cellulose gave a single peak with malic enzyme activity but in the case of *Iris* and *Triticium* two peaks were observed. Some examples of the fractionation obtained by DEAE cellulose chromatography are shown in Fig. 1.

Using these purified preparations we have examined the effect of varying the malate concentration on the rate of malate decarboxylation. Rate versus malate concentration plots were made at 2 pH values—7·0 and 7·6—with two activating cations Mn and Mg and in the presence and absence of succinate. The results are presented in Table 5 as S (0·5) values; that is, the concentration of substrate giving half maximum velocity. In the case of enzymes showing normal Michaelis Menten kinetics K_m = S (0·5). If the enzyme exhibits allosteric properties and responds positively to succinate, the S (0·5) value will decrease on the addition of succinate.

A number of other kinetic constants can be used to define the allosteric properties of an enzyme but the wide range of response observed with different enzyme preparations makes comparison difficult and as an alternative we present the data for a number of examples in graphical form (Figs. 2 and 3).

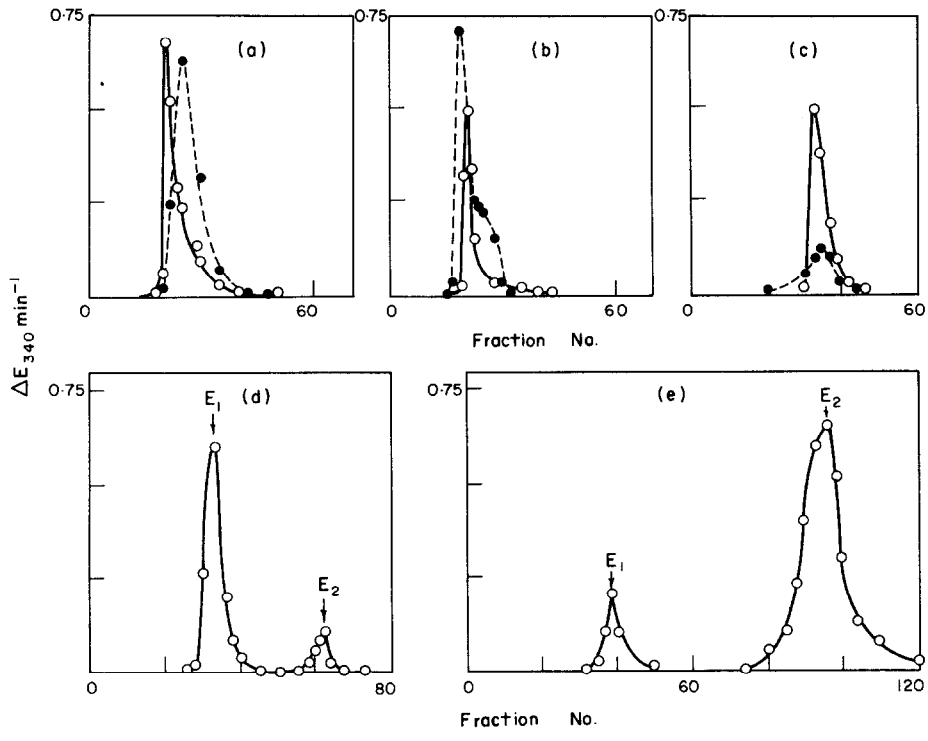

FIG. 1. PURIFICATION OF MALIC ENZYME FROM VARIOUS SPECIES BY CHROMATOGRAPHY ON DE-52 CELLULOSE.

(a)–pea (epicotyl); (b)–pea (leaf); (c)–pea (root); (d)–*iris germanica* (leaf); (e)–wheat (leaf). Assay conditions as described in text. Gradient for elution 0·05 M Tris (pH 8·6) to 0·5 M Tris (pH 7·4). Fractions 4 ml. Malic enzyme was assayed as described in the Experimental section. Malic dehydrogenase was assayed by measuring the decrease in E_{340} in cuvettes containing oxaloacetate (0·1 mM) NADH (0·4 mg) MOPS buffer (pH 7·0, 0·1 M) and enzyme in 3 ml. The activity of malic dehydrogenase is 10 × the scale value. ○ Malic enzyme; ● malic dehydrogenase.

Effect of Mn and Mg on the activity of malic enzyme

The purified preparations of malic enzyme show an absolute requirement for a divalent cation. We have compared Mn and Mg under various conditions and the results are presented in Tables 1–5 and in Figs. 2 and 3.

DISCUSSION

Malic enzyme in relation to flood tolerance

The demonstration that malic enzyme is present and highly active in the roots of all four flood tolerant species reported by McManmon and Crawford[2] to lack malic enzyme, weakens the evidence for the metabolic theory of flood tolerance proposed by Crawford.[10] This attractive theory suggests that in flood tolerant species, anaerobiosis leads to the accumulation of malate rather than ethanol. It appears to us that this proposal is somewhat

[10] CRAWFORD, R. M. M. (1969) *Ber D. Bot. Ges.* **82**, 111.

TABLE 5. EFFECT OF SUCCINATE ON THE S (0·5) VALUES FOR MALATE IN THE OXIDATIVE DECARBOXYLATION OF MALATE CATALYSED BY MALIC ENZYME FROM VARIOUS SOURCES

			S (0·5) mM*							
			pH 7·0				pH 7·6			
			(Mn)		(Mg)		(Mn)		(Mg)	
Family	Species	Organ	Control	+ Succ	Control	+ Succ	Control	+ Succ	Control	+ Succ
Leguminosae	*Pisum sativum*	Leaf	0·12	0·1	0·35	0·15	0·8	0·7	2·5	1·1
		Epicotyl	0·15	0·12			2·0	1·5	4·4	2·0
		Roots	0·1	0·2	3·0	2·6	2·0	1·6	3·5	2·0
Boraginaceae	*Myosotis scorpioides*	Roots	1·0	0·5	0·9	0·6	3·0	1·4	1·2	0·8
Compositae	*Senecio aquaticus*	Roots	0·7	0·5	2·2	0·7	7·0	0·5		
	Pulicaria dysenterica	Roots	0·4	0·1	1·9	1·0	13·0	1·3	N.D.	N.D.
Labiatae	*Mentha aquatica*	Roots	0·25	0·35	1·1	1·3	1·5	1·7	2·3	2·5
	Lamium album	Leaves	0·14	0·12	0·8	0·6	0·6	0·6	2·5	2·0
	Lamium purpureum	Leaves	0·25	0·25	1·5	0·3	2·3	1·5	4·5	1·9
Iridaceae	*Iris germanica*	Leaves (E_1)†	0·4	0·3	3·1	0·4	1·6	0·7	5·0	0·7
		(E_2)	4·5	3·0	3·5	0·5	1·5	1·0	7·5	2·0
Juncaceae	*Juncus effusus*	Stem	0·4	0·4	2·1	1·3	0·6	0·5	1·6	0·6
Cyperaceae	*Carex riparia*	Leaves	0·5	0·5	1·3	1·2	2·8	2·1	2·7	1·2
Gramineae	*Phragmites communis*	Leaves	0·4	0·4	1·0	1·0	2·9	2·4	4·5	3·5
	Zea mays	Leaves	0·12	0·12	0·25	0·25	0·15	0·25	0·3	0·5
		Roots	0·25	0·25	0·4	0·4	0·3	0·3	0·45	0·45
	Triticum vulgare	Leaves (E_1)†	2·1	2·0	7·0	7·0	2·0	2·0	2·5	2·5
		(E_2)	1·0	1·0	1·3	1·3	5·0	5·0	10·0	10·0
	Oryza sativa	Leaves	0·6	0·6	1·1	1·1	1·2	1·2	2·2	2·2
	Glycetia maxima	Leaves	0·1	0·1	0·5	0·5	0·3	0·3	1·0	1·0

* Concentration of substrate giving half maximal velocity (in enzymes showing normal kinetics — km).
† See Table 4.

unlikely on energetic grounds; thus glycolysis leading to the accumulation of ethanol yields 2 mol of ATP/mol of glucose metabolized, whereas glycolysis leading to the accumulation of malate according to the reaction scheme proposed by Crawford gives no net gain of ATP. Since the biological significance of glycolysis is presumably to provide ATP, the metabolic route proposed by Crawford is self defeating.

Nevertheless, as shown by Crawford and Tyler,[11] malic acid does accumulate in flood tolerant species on flooding. Three possibilities suggest themselves: (i) phosphoenolpyruvate carboxykinase rather than phosphoenolpyruvate carboxylase is involved in the carboxylation of phosphoenolpyruvate thereby allowing a net gain of ATP during malate accumulation; (ii) flood tolerant plants may possess a particularly active system for the transport of malate into the vacuole; and (iii) the malic enzyme may be inhibited. In this connection we wish to report the presence of a powerful inhibitor of malic enzyme in potato tubers. This inhibitor has been purified and its properties, as well as its distribution in the plant kingdom are being examined.

The allosteric properties of malic enzyme from higher plants

The data presented in this paper establishes the association of allosteric properties with malic enzyme from a wide range of higher plants. However the malic enzyme found in a number of species belonging to the *Graminae* does not exhibit allosteric properties—succinate acts as an inhibitor rather than as an activator. It should be noted that in cases where malic enzyme exhibits allosteric properties succinate activates the enzyme when the malate concentration is low, but inhibits the enzyme when the malate concentration is high. The presence or absence of allosteric properties associated with malic enzyme is of limited taxonomic significance. Excluding members of the *Graminae*, we have examined

[11] CRAWFORD, R. M. M. and TYLER, P. D. (1969) *J. Ecology* **57**, 235.

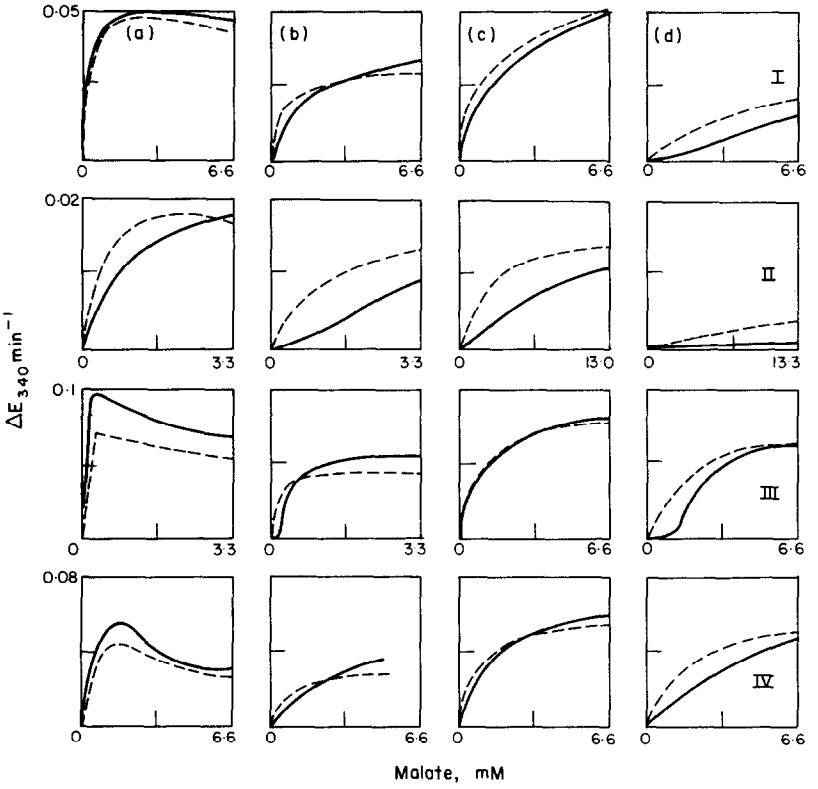

FIG. 2. EFFECT OF SUCCINATE ON THE PLOT OF RATE VS. MALATE CONCENTRATION FOR MALIC ENZYME FROM VARIOUS SPECIES OF DICOTYLEDONS UNDER VARIOUS CONDITIONS.
Column (a) MOPS buffer pH 7·0, 0·1 M; $MnCl_2$ (1·66 mM); column (b) MOPS buffer pH 7·0, 0·1 M; $MgCl_2$ (1·66 mM); column (c) MOPS buffer pH 7·6, 0·1 M; $MnCl_2$ (1·66 mM); column (d) MOPS buffer pH 7·6, 0·1 M; $MgCl_2$ (1·66 mM). The dotted line is in the presence of succinate (1·33 mM). Assay conditions: NADP (0·4 mg), enzyme (0·1 ml), buffer, $MnCl_2$ or $MgCl_2$ and malate as indicated in the final volume of 3 ml. Line I—Lamium purpureum (leaf); Line II—Senecio aquatica (root); Line III—Pisum sativum (leaf); Line IV—Pisum sativum (epicotyl).

the malic enzyme of 28 species of higher plants and 27 were found to exhibit allosteric properties. The single exception, *Mentha aquatica* is a member of the Labiatae and three other members of the family examined by us possess a malic enzyme with allosteric properties. Leaves of *Bryophyllum crenata*[6] and *Bryophyllum tubiflorum* have been reported to possess a malic enzyme lacking allosteric properties, whereas we have found that malic enzyme prepared from the roots of *Bryophyllum blossfeldiana* exhibits allosteric properties. The other side of the coin is that of the eight members of the *Graminae* examined by us, allosteric properties were completely absent from 6 species but in the case of wheat a small activating effect by succinate is observed but there is no evidence for sigmoid kinetics. In the case of *Phragmites communis* there are small but reproducible changes on addition of succinate and the plot of rate versus malate concentration shows significant sigmoidicity. Thus it seems unlikely that the presence or absence of allosteric properties can be usefully applied to problems of taxonomy.

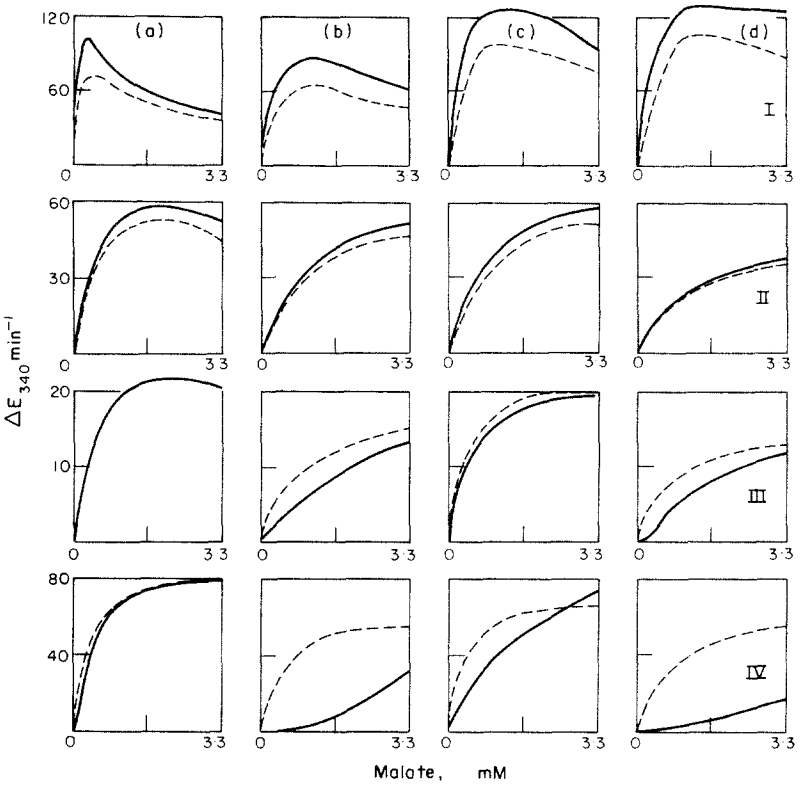

FIG. 3. EFFECT OF SUCCINATE ON THE PLOT OF RATE VS. MALATE CONCENTRATION FOR MALIC ENZYME FROM VARIOUS SPECIES OF MONOCOTYLEDONS UNDER VARIOUS CONDITIONS.
Column (a) MOPS buffer pH 7·0, 0·1 M; $MnCl_2$ (1·66 mM); column (b) MOPS buffer pH 7·0, 0·1 M; $MgCl_2$ (1·66 mM); column (c) MOPS buffer pH 7·6, 0·1 M; $MnCl_2$ (1·66 mM); column (d) MOPS buffer pH 7·6, 0·1 M; $MgCl_2$ (1·66 mM). The dotted line is in the presence of succinate (1·33 mM). Assay conditions: NADP (0·4 mg), enzyme (0·1 ml) buffer, $MnCl_2$ or $MgCl_2$, and malate as indicated in a final volume of 3 ml. Line I—Zea mays (leaf); Line II—Oryza sativa (leaf); Line III—Juncus effusus (stem); Line IV—Iris germanica (leaf) [E_1].

The absence of allosteric properties in an enzyme preparation may represent changes associated with isolation. Allosteric properties are frequently labile, e.g. cold labile, and it is difficult to prove that the absence of allosteric properties represents the *in vivo* situation. Nevertheless, it seems likely that the malic enzyme present in various members of the *Graminae* is devoid of allosteric properties and this observation is significant in relation to the suggestion that malic enzyme has a role in a metabolic pH-stat.[12] However, the proposed pH-stat could function without allosteric effectors—the sensitivity of the pH-stat being determined by the slope of the activity versus pH curve.

Requirements for Mn or Mg

Malic enzyme has an absolute requirement for a divalent cation, but the enzyme responds differently to Mn or Mg. In general the sigmoidicity is greatest when Mg is the activating cation—in one sense Mn and succinate exert similar effects—appearing to put the enzyme into its more active state. Unfortunately we do not know whether Mg or Mn functions as the activating cation *in vivo*.

EXPERIMENTAL

Plant material. Wild plants were collected locally and we are grateful to Dr. E. Ellis of Surlingham, Norfolk, for providing some of the marsh plants. Greenhouse plants were provided by the John Innes Institute, Colney Lane, Norwich. Roots of marsh plants were grown in water culture in the University greenhouse. Plants were identified by reference to standard specimens.

Chemicals. All general chemicals were of the highest purity available commercially. NADP, L-malate, Tris (under the trade name Trizma) and MOPS (Morpholinopropane sulphonic acid) were purchased from Sigma Chemical Co. London. Sephadex G-25 was obtained from Pharmacia, Uppsala, Sweden and DE52 DEAE-cellulose was from W. & R. Balston Ltd., Maidstone, Kent, U.K.

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 MOPS buffer (pH 7·0, 0·1 M), $MnCl_2$ (1·66 mM), $NADP^+$ (0·4 mg) and sodium malate (3·3 mM) in a volume of 3 ml.

Enzyme purification. A homogenate was prepared by grinding the plant material in a chilled mortar with acid washed sand (2/10 g plant material). Polyclar (0·5/10 g plant material) and Tris–HCl buffer (2 ml/g; pH 7·4, 0·05 M) containing 2-mercaptoethanol (5 mM). The extract was squeezed through 3 layers of muslin and centrifuged at 15,000 *g* for 10 min. The clear supernatant was stirred while $(NH_4)_2SO_4$ (42 g/100 ml extract) was slowly added. After stirring for 10 min the extract was centrifuged at 10000 *g* for 15 min. The precipitate was dissolved in Tris–HCl buffer (pH 7·4, 0·05 M) and in a number of cases this $(NH_4)SO_4$ fraction was used with further purification. Further purification was achieved by passage through a column (15 × 2·5 cm) of Sephadex G-25 previously equilibrated with Tris–HCl buffer (pH 8·6, 50 mM). The active fraction was in some cases used for kinetic studies; in other cases it was applied to a column (42 × 2·5 cm) packed with DE-52 DEAE-cellulose previously equilibrated with Tris–HCl buffer (pH 8·6, 50 mM). The column was eluted by applying a linear concentration gradient obtained by placing 250 ml of Tris–HCl buffer (pH 8·6, 50 mM) in the mixing cylinder and an eq. vol. of Tris–HCl buffer (pH 7·4, 0·5 M) in the reservoir. Fractions (4 ml) were collected for assay and the peak fractions combined. In most cases malic enzyme emerged as a single peak, but in the case of *Iris germanica* and wheat two peaks emerged—the first peak is referred to as E_1 and the second as E_2.

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