# COMPARATIVE STUDIES OF NADP-MALIC ENZYME FROM $C_4$- AND $C_3$-PLANTS*

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SUMMARY : Some enzymological properties were studied comparatively for NADP-malic enzyme from various $C_4$- and $C_3$-plants. The enzyme from $C_4$-plants of "malate formers" showed relatively low Km(Mal) values (0.10 - 0.25 mM) and high pH optima (more than pH 7.4 - 7.8). Contractively, the enzyme from the other groups of higher plants including $C_4$-plants of "aspartate formers", $C_3$-plants and CAM-plant showed relatively high Km(Mal) values (0.68 - 1.05 mM) and low pH optima (less than pH 7.4).

It has been well known that NADP-malic enzyme (EC.1.1.1.40) is widely distributed in various living organisms, including higher animals, higher plants and microorganisms. The enzyme from higher plants has attracted the attention of many researchers since Slack and Hatch reported that the content of NADP-malic enzyme in leaves of at least some $C_4$-plants***is about 45-fold higher than that in $C_3$-plants (1) and that the enzyme plays a role to transfer to the bundle sheath chloroplasts of reducing power as well as $CO_2$ (2,3). Some of the properties and regulation of NADP-malic enzyme in $C_4$-plants is described by Johnson and Hatch (4). The comparison of the activity of NADP-malic enzyme in several $C_4$-plants showed that the enzyme activity in some $C_4$-

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*** Abbreviation : $C_4$-plants, the plants having both $C_4$ dicarboxylic acid pathway and reductive pentose phosphate pathway in photosynthesis; $C_3$-plants, the plants having only reductive pentose phosphate pathway in photosynthesis; CAM-plant, succulent plant having crassulacean acid metabolism.

grasses is about 45-50 times that for $C_3$-plants, but some other $C_4$-species contain only low activities of NADP-malic enzyme (3,4, 5).

In this paper, some enzymological properties were studied comparatively for NADP-malic enzyme from various $C_4$- and $C_3$-plants, including high and low malic enzyme species. Results are also comparatively discussed with those obtained with PEP carboxylase (EC. 4.1.1.31.) from $C_4$- and $C_3$-plants, which recently reported by the authors (6).

## MATERIALS AND METHODS

About 10 g of leaf tissue were homogenized vigorously for 10 min. in a motar and pestle at 4 °C with an equal weight of quartz sand and 20-30 ml of 100 mM Tris-HCl buffer(pH 7.4) containing 7 mM 2-mercaptoethanol. The resulting homogenate was passed through four layers of cheese cloth. The supernatant was kept and the cellular debris added back to the motar containing 20 ml of fresh buffer and ground for a further 10 min. The homogenate was again filtered through cheese cloth and the supernatant kept. This process was repeated so that cellular debris were ground for two more periods of 10 min. Thus obtained supernatants were centrifuged separately at 28,500 x g for 10 min. Clear supernatants were assayed for NADP-malic enzyme activity and protein concentration, respectively. These supernatant solutions were combined and fractionated by solid ammonium sulfate. The precipitation from 30-70 % saturation was used usually for NADP-malic enzyme assay.

The activity of NADP-malic enzyme was assayed spectrophotometrically by measuring the formation of $NADPH_2$ at 340 nm with a double-beam Shimazu Double 40R recording spectrophotometer.

The typical reaction mixture contained 100 mM Tris-HCl(pH 7.4), 5 mM L-malate, 1 mM $MnCl_2$, 0.154 mM NADP and the enzyme in a total volume of 2.0 ml. The reaction was carried out at 30 ℃ and initial rates were used for the calculation of all the enzyme activities. One unit of the enzyme was defined as the amount causing an absorption change of 0.01 at 340 nm under these assay conditions, which correspond to 3.2 nmoles per min.

## RESULTS AND DISCUSSIONS

Effect of pH on the activity of NADP-malic enzyme was surveyed from the leaves of Digitaria sanguinalis ($C_4$-plant) and of Nicotiana tabacum ($C_3$-plant). As shown in Fig. 1, pH optimum of the enzyme from Digitaria sanguinalis was 7.2-7.4 in the presence of 0.5 mM L-malate, whereas in 5 mM malate the optimum was 7.8-8.0. Similar effect of the shift of pH optimum by changing malate

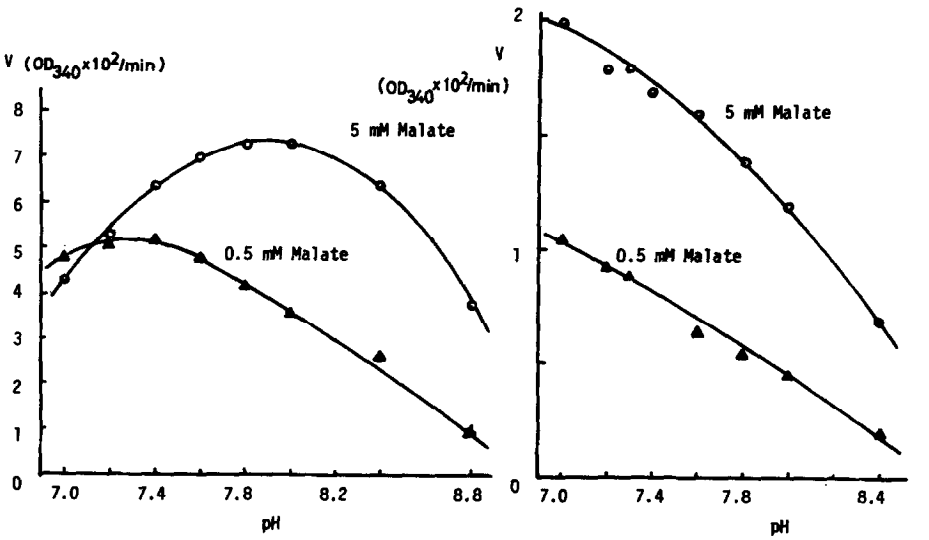


Fig. 1. Effect of L-malate concentration on pH-activity curve of NADP-malic enzyme from Digitaria sanguinalis($C_4$-plant) and Nicotiana tabacum($C_3$-plant). Experimental conditions are the same as those described in MATERIALS AND METHODS except for the pH of the buffer solution and the concentration of L-malate. 6.4 units (Digitaria sanguinalis) and 1.7 units (Nicotiana tabacum) of the enzyme were used.

concentration was observed with other organisms including the original observation with pigeon liver by Rutter and Lardy(7) and with Zea mays by Johnson and Hatch(4). The pH optumum of the enzyme from Nicotiana tabacum, however, was less than 7.0 irrespective of malate concentration used, as shown in Fig. 1.

Malate saturation curves and Km for malate were compared with the enzyme from Zea mays($C_4$-plant) and Oryza sativa($C_3$-plant). As shown in Fig. 2-(a), malate saturation curves showed normal hyperbola in both cases. From the double reciplocal plots

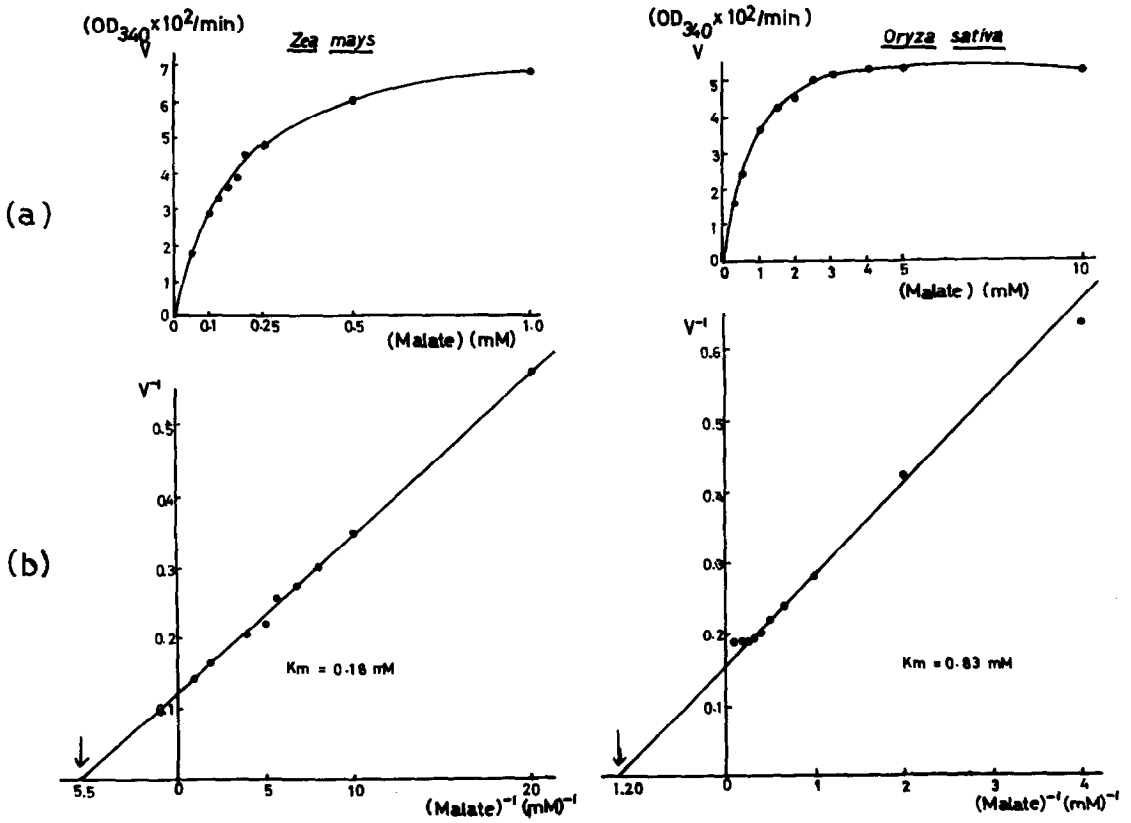


Fig. 2. L-Malate saturation curve and Km for L-malate of NADP-malic enzyme from Zea mays($C_4$-plant) and Oryza sativa ($C_3$-plant).
(a). Initial velocity versus L-malate concentration.
(b). Double reciprocal plots.
Experimental conditions are the same as described in MATERIALS AND METHODS except that the concentration of L-malate were varied as shown. 8 units (Zea mays) and 6.7 units (Oryza sativa) of the enzyme were used.

of the reaction velocity against substrate concentration(Fig. 2-(b)) , Km for malate was 0.18 mM with the enzyme from Zea mays and that from Oryza sativa was 0.83 mM. Km for malate of the enzyme from Oryza sativa was more than 4 times larger than that from Zea mays.

These differences of enzymological properties between some $C_4$- and $C_3$-plant enzymes lead us to the more detailed comparative studies of the pH optimum and Km for malate of NADP-malic enzymes from a variety of plant leaves. The results are shown in Table I as well as the data of PEP carboxylase recently reported by Nishikodo and Takanashi(6). As indicated in Table I, all the enzymes from $C_4$-plants of "malate formers" by Downton(5) or Group 1 by Andrews et al.(8) showed low Km for malate (0.10-o.25 mM) and high pH optima (more than pH 7.4-7.8). Contractively, the enzymes from the other groups of plants including $C_4$-plants of 'aspartate formers"(5), $C_3$-plants and CAM-plant showed relatively high Km for malate (0.68-1.05 mM) and low pH optima (less than pH 7.4), with the exception of Km for malate of Portulaca oleracea and of pH optumum of Amaranthus retroflexus. These results are compartible with those obtained with the enzyme from other sources, for example, Pennisetum purpureum by Coombs et al.(9), Kalanchoë crenata by Walker (10) and Bryophyllum tubiflorum by Brandon and Boekel-Mol (11).

From the above results, it became clear that NADP-malic enzyme from $C_4$-plants of "malate formers" have commonly low Km for malate and high pH optimum compared to those from the other types of higher plants. It is of great interest to compare these data with those of PEP carboxylase, as shown in the right hand of Table I. It is clear that all the PEP carboxylases activated by glycine belong to the group of $C_4$-plants of "malate formers".

TABLE I

Km for L-Malate and pH Optimum of NADP-Malic Enzyme from Various Higher Plants.

| Plant Species | Type of $CO_2$ Fixation | Km for Malate (mM) | pH Optimum | Glycine Activation of PEP Carboxylase |
|---|---|---|---|---|
| Digitaria sanguinalis | $C_4$-Mal. | 0.13 | 7.8 | + |
| Sorghum bicolor | $C_4$-Mal. | 0.10 | 7.8-8.4 | + |
| Zea mays | $C_4$-Mal. | 0.18 | 7.4-7.8 | + |
| Saccharum officinarum | $C_4$-Mal. | 0.18 | 7.8 | + |
| Miscanthus sinensis | $C_4$-Mal. | 0.18 | 7.8-8.4 | + |
| Setaria viridis | $C_4$-Mal. | 0.25 | 7.8-8.4 | + |
| Amaranthus tricolor | $C_4$-Asp. | 0.82 | 7.4 | - |
| Amaranthus retroflexus | $C_4$-Asp. | 0.90 | 7.8 | - |
| Portulaca oleracea | $C_4$-Asp. | 0.26 | 7.4 | - |
| Eleucine indica | $C_4$ | 1.05 | 7.4 | - |
| Oryza sativa | $C_3$ | 0.89 | 7.2 | - |
| Nicotiana tabacum | $C_3$ | 0.99 | < 7.0 | - |
| Sedum spectabile | CAM | 0.68 | < 7.0 | - |

Experimental conditions are the same as described in MATERIALS AND METHODS. 1-10 units of the enzyme was used for each assay.
+ : PEP carboxylase was significantly activated by 5 mM glycine.
- : PEP carboxylase was not activated by 5 mM glycine.

In view of these results, it may be concluded that NADP-malic enzyme and PEP carboxylase from $C_4$-plants of "malate formers" have a common and special enzymological property as compared to those from the other groups of higher plants.

REFERENCES

1. Slack,C.R. and Hatch,M.D., Biochem. J., 103, 660 (1967).
2. Slack,C.R., Hatch,M.D. and Goodchild,D.J., Biochem., J. 114, 489 (1969).
3. Hatch,M.D. and Slack,C.R., In Progress in Phytochemistry, ed, Reinhold,L. and Liwshitz,Y., Interscience, London, 2, 35 (1970).
4. Johnson,H.S. and Hatch,M.D., Biochem. J., 119, 273 (1970).
5. Downton,W.J.S., Canad. J. Bot., 48, 1975 (1970).
6. Nishikido,T. and Takanashi,H., Biochem. Biophys. Res. Commun., 53, 126 (1973).
7. Rutter,W.J. and Lardy,H.A., J. Biol. Chem. 233, 374 (1958).
8. Andrews,T.J., Johnson,H.S. and Slack,C.R., Phytochem. 10, 2005 (1971).
9. Coombs,J., Baldry,C.W. and Bucke,C., Planta(Berl.), 110, 109 (1973).
10. Walker,D.A., Biochem.J., 74, 216 (1960).
11. Brandon,P.C. and van Boekel-Mol,T.N., Europ. J. Biochem., 35, 62 (1973).