SOME ASPECTS OF CARBOHYDRATE METABOLISM IN RIPENING MANGOES

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Summary : The enzymes, glucose-6-phosphate dehydrogenase, 6-phosphogluconic dehydrogenase and malic enzyme, associated with the production of NADPH needed for some of the anabolic reactions have been found to be competitively inhibited by β carotene and fatty acids. This feed back control in turn appears to regulate sugar breakdown in the ripe mango.

Metabolic regulation in mammalian tissue is a well studied phenomenon. Fatty acids have been implicated in the production of more glucose in perfused mammalian tissues (1-6) and inhibition of the enzymes of glycolysis and HMP-shunt in liver (7) and in Arthrobacter crystallopoietes (8).

Earlier (9) it has been shown that in mango fruit, fatty acids activate the process of citrate break-down to liberate acetyl CoA and oxaloacetate for synthetic reactions. The availability of sugars for high respiration (10) and synthetic reactions is limited by the fact that sugars accumulate (11) in the ripening mangoes thus suggesting the involvement of a regulatory mechanism for sugar accumulation. The present investigation indicates that fatty acids and β-carotene are also responsible for the regulation of carbohydrate metabolism in ripe mango.

MATERIALS AND METHODS

Plant material : Mangoes (Mangifera indica L.ev.Alphonse)

used for this study were purchased from local farm; immediately after picking the mature unripe mangoes were kept for ripening at 20°-25°C. Ripe fruits having a golden yellow pulp were used for the investigation.

Enzyme preparation : All the procedures were carried out at 0°-5°C. The fruits were peeled and a known weight of the pulp was manually ground for 20 minutes in a mortar with 0.1 M tris-HCl buffer pH7.4 to make a 30 % extract. During grinding, when necessary, the pH was maintained by careful, dropwise addition of 0.1 N NaOH. The homogenate was centrifuged at 18,000 x g for 15 minutes and the supernants were assayed for the individual enzymes. The protein in the cell-free extract was estimated by the method of Lowry et al.(12).

Assay of enzyme activity : Unless otherwise stated the assay system contained, in micromoles. Malic enzyme was estimated by the method of Ochoa (13). Tris-HCl buffer, (pH 7.4) 200; $MnCl_2$, 5; L-malate, 20; NADP, 1.5 and an appropriate concentration of the enzyme in a final volume of 3 ml. Glucose-6-phosphate and 6-phosphogluconic acid dehydrogenases were determined by the method of Kornberg and Horecker (14). The test system contained glycyl glycine buffer, (pH 7.4) 200; $MgCl_2$, 5; glucose 6-phosphate, 6; NADP, 1.5 and an appropriate concentration of mango extract in a final volume of 3.0 ml. For the estimation of 6-phosphogluconic acid dehydrogenase $MgCl_2$ was omitted from the test system and 6-phosphogluconate was added. Isocitrate dehydrogenase was estimated by the method of Kornberg (15). The assay system contained phosphate buffer, (pH 7.0) 100; NADP, 1.5; $MgCl_2$, 5; DL-isocitrate, 6 and the enzyme extract in a total volume of 3.0 ml. In all the above cases the increase in optical density was measured at 340 mμ against the respective substrate blanks.

Invertase activity was determined by measuring quantitatively the hexose formed by the method of Nelson (16). The assay system contained sodium acetate buffer (pH 5.0) 100; sucrose, 25; and enzyme extract in a total volume of 2 ml. Blank tubes contained boiled enzyme. Incubation was carried out at 37°C for one hour and the reaction was stopped by adding 1 ml of 1.5 M dibasic sodium phosphate and heating in a boiling water bath for 2 minutes. Precipitated protein was filtered and aliquots were anasysed for reducing sugar. A unit of invertase activity is that amount which liberates one micromole of reducing sugar under the assay condition. For amylase the assay system contained 0.5 ml of 1 % soluble starch tris-HCl buffer (pH 7.0) 100; and an appropriate concentration of the enzyme extract in 2.0 ml. Incubation was carried out at 37°C for 1 hour. Blank tubes contained boiled enzyme. Reducing groups liberated were measured by the reduction of 3,5 dinitro-salicylic acid. β-carotene and fatty acids were disolved in acetone and added to the assay system. Acetone blanks were run along with the experimental.

RESULTS AND DISCUSSION

Using mango fruit Mattoo et al (10) have found that the activities of the enzymes glucose-6-phosphate dehydrogenase, 6-phosphogluconic dehydrogenase and malic enzyme increases by about 4 fold during ripening and also that sugars, β carotene and fatty acids accumulate in the ripe fruit (11). Attempts were therefore made to study the effect of β-carotene and fatty acids, on some of the enzymes involved in glucose catabolism.

Table I

Effect of 0.10 μM oleic acid, 0.27 μM Caprylic acid and 0.08 μM β Carotene on some of the enzymes in carbohydrate metabolism

Enzymes tested	Specific activity (units*/mg protein) Enzyme activity in presence of			
	Control	Oleic acid(δ)	Caprylic acid	β-Carotene
Glucose-6-phosphate dehydrogenase	15.0	-	5.0 (66)	5.0 (66)
6-Phosphogluconic* acid dehydrogenase	13.0	5 (61)	6.6 (49)	3.3 (77)
Malic enzyme*	60.0	-	21 (65)	42 (30)
Isocitrate* dehydrogenase (NADP)	5.0	-	5.0 (0)	5.0 (0)
Invertase a) Mango	0.162	0.162 (0)	0.162(0)	0.162 (0)
b) Yeast**	2.66	2.66 (0)	2.66 (0)	2.66 (0)
Amylase a) Mango	1.00	1.0 (0)	1.0 (0)	1.0 (0)
b) Banana	1.40	1.40 (0)	1.40 (0)	1.40 (0)
c) Aspergillus oryzae**	1.22	1.22 (0)	1.22 (0)	1.22 (0)

* Units are 0.001 change in O.D per one minute at 340 mu.

δ Oleic acid was omitted in inhibition studies where Mg^{++} or Mn^{++} ions were used.

** Purified enzymes from Sigma Chemical Co. were used.

Values in parenthesis indicate percentage inhibition.

The results summarised in Table I show that the initial enzymes of hexose monophosphate shunt (namely glucose-6-phosphate dehydrogenase and 6-phosphogluconic dehydrogenase) and malic enzyme are inhibited by fatty acid and β-carotene; where as invertase and amylase from mango and different sources

Table II

Effect of increasing concentrations of fatty acid and β carotene on glucose-6-phosphate dehydrogenase, 6-phosphogluconic dehydrogenase and malic enzyme

Concentration In μM	Enzyme units/mg protein		
	Glucose-6-phosphate dehydrogenase*	6-phosphagluconic dehydrogenase	Malic enzyme*
Control	15	13	60
a) Oleic acid			
0.025	-	8.3	-
0.050	-	6.6	-
0.10	-	5.0	-
b) Caprylic acid			
0.060	10.0	10.0	48.0
0.130	7.0	8.3	36.0
0.210	5.0	6.6	21.0
ii β-carotene			
0.020	8.0	10.0	58.0
0.040	5.0	6.6	53.0
0.080	3.0	3.3	42.0

and isocitrate dehydrogenase from mango are not inhibited. The inhibition of the enzymic activities was found to be progressive with increasing concentrations of the inhibitors (Table II). The inhibition of the enzymes, was found to be of the competitive type (Fig.1,2 and 3). Km values with respect to the substrate was found to be 1.5×10^{-5}, 1.1×10^{-5} and 5×10^{-4} M for glucose-6-phosphate dehydrogenase, 6-phospho

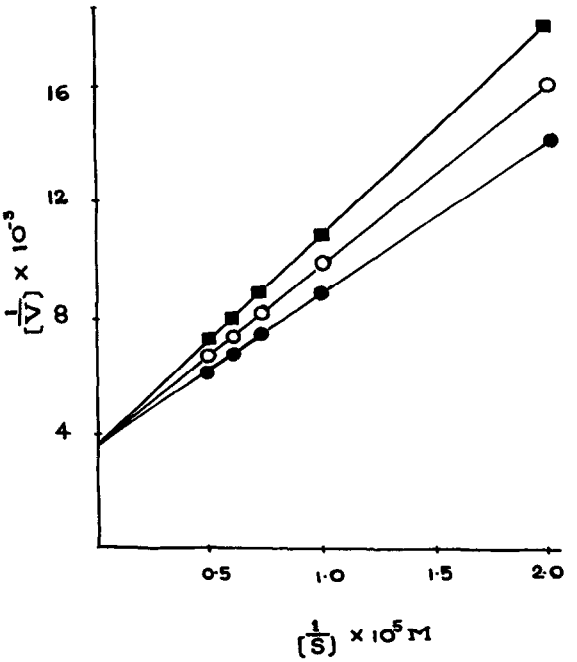

Fig. 1 :- Line-weaver Burk plot for glucose-6-phosphate dehydrogenase (●); in the presence of Caprylic acid (■) and β-carotene (o), at the inhibitor concentrations of 0.27 and 0.08 μM respectively.

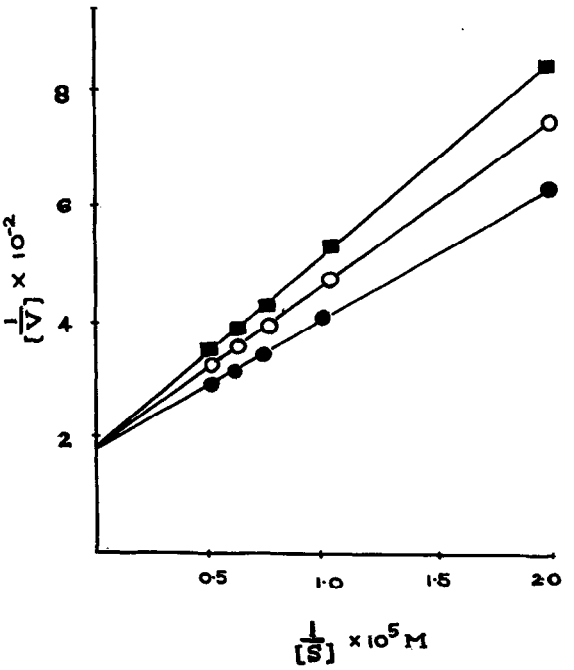

Fig. 2 :- Line-weaver Burk plot for 6-phospho-gluconic dehydrogenase (●); in the presence of oleic acid (■) and β-carotene (o) at the concentrations of 0.10 and 0.08 μM respectively.

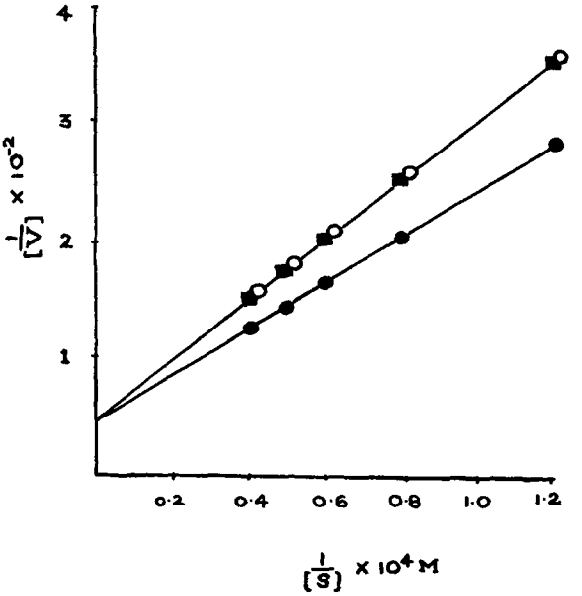

Fig. 3 :- Line-weaver Burk plot for malic enzyme (●), in the presence of caprylic acid (■) and β-carotene (o) at the concentrations of 0.27 and 0.08 μM respectively.

gluconic dehydrogenase and malic enzyme respectively. With β-carotene the Ki of these enzymes were 1.7×10^{-5}, 1.8×10^{-5} and 7.1×10^{-4}M, and in presence of fatty acid the inhibition constants were 2.2×10^{-5}, 2.5×10^{-5}, 7.1×10^{-4}M. No significant difference was detected when the enzymes were pre-incubated with fatty acids and β-carotene for 10 minutes before the initiation of the enzyme reaction. It was further found that bovine serum albumin (1 mg/ml) when added to the system had no effect on the inhibitory activity of fatty acids and β-carotene. These results suggest that fatty acid and β-carotene control the utilization of sugars via the hexose monophosphate shunt and thus sugars accumulate in the ripe fruit. Crude fatty acids prepared from mangoes also show an inhibitory action towards these enzymes indicating the physiological significance.

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REFERENCES

1. Struck E, Ashmore, J., and Wieland, O., Advances in enzyme regulation, 4, 219 (1966).
2. Williamson J.R., Edward T.B., and Chalz R.S., J. Biol. Chem. 244, 4607 (1969).
3. Williamson J.R., Kreisberg R.A., and Felts, P.W., Proc. Nat. Acad.Sci. U.S.A. 56, 247 (1966)
4. Haynes C.R. (Jr.), Advances in enzyme regulation 3, 111, (1965).
5. Krebs, H.A., Sneake R.N., and Hen S.R., Biochem. J. 94, 712 (1965)
6. Weinhouses E., Fridman, B., Goodman E.H., Fed. Proc. 25, 347 (1966)
7. Weber G., Hirdconvery, H.J., Science 154, 1357 (1966)
8. Ferdinandus J., and Clark J.B., J. Bacteriol 98, 1109 (1969)
9. Mattoo A.K. and Modi V.V., Biochem. Biophys. Res.Comm. 39, 895 (1970)
10. Mattoo, A.K., Modi V.V. and Reddy V.V.R., Indian J. Biochem. 5, 111 (1968)
11. Modi V.V. and Reddy V.V.R., Indian J. Exp. Biol. 5, 233 (1967)
12. Lowry O.H., Rosebrough, J.J.,Farr, A.L. and Randall R.J., J. Biol. Chem. 193, 265 (1951)
13. Ochoa S., Cited in Methods in Enzymology, (edited by S.P. Colowick and N.O. Kaplan Academic Press Inc. New York) 1, 739 (1955)
14. Kornberg A., and Horecker, B.L., Cited in Methods in Enzymology, (edited by S.P. Colowick and N.O. Kaplan Academic Press Inc. New York) 1, 323, (1955)
15. Kornberg, A., J. Biol. Chem. 189, 123 (1951)
16. Ashwell G., Cited in Methods in Enzymology, (edited by S.P. Colowick and N.O. Kaplan Academic Press Inc. New York) 3, 85 (1955)