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GLUTAMATE OXALOACETATE TRANSAMINASE ACTIVITY IN DEVELOPING *LYCOPERSICON ESCULENTUM* FRUIT

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Abstract—Glutamate oxaloacetate transaminase (GOT) occurs in both the mitochondrial and cytoplasmic fractions from tomato fruit tissue. Changes in activity of the enzyme from fruit at selected developmental stages have been followed. The combined activity fell from the immature green stage to the full red condition whilst the proportion in the mitochondria reached a peak in green-orange fruit. The activity of cytoplasmic, but not mitochondrial, GOT was stimulated by the addition of pyridoxal-5-phosphate. In the green areas of fruit showing blotchy ripening, the combined activity was equivalent to that in normal immature green fruit but with a much higher proportion of the activity in the mitochondria. Mitochondrial GOT could constitute a system in ripening tomato fruit whereby the accumulation of inhibitory concentrations of oxaloacetate affecting the oxidation of succinate and malate might be controlled.

INTRODUCTION

ISOLATED mitochondria from sweet potato,[1] avocado,[2] apple[3] and tomato[4–6] readily metabolize succinate and malate. However, during the oxidation of either substrate various forms of inhibition have been observed, probably connected with the build-up of quantities of oxaloacetic acid (OAA) which is known to affect succinate[7] and malate dehydrogenases.[8] One of the more effective methods used to counteract the reduced rate of malate oxidation with avocado[9] and tomato[5] mitochondria preparations is by the introduction of a small quantity of glutamate which, in the presence of glutamate oxaloacetate transaminase (L-aspartate: 2-oxoglutarate aminotransferase, E.C. 2.6.1.1), reacts with OAA to form 2-oxoglutarate and L-aspartate. Since glutamic acid and glutamine are contained by tomato fruit in appreciable quantities,[10] it was of interest to follow the intracellular location and activity of glutamate oxaloacetate transminase (GOT) during the normal and abnormal ripening of tomato fruit. In this way it might be possible to assess the influence of trans-

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[10] HOBSON, G. E. and DAVIES, J. N. (1971) *The Biochemistry of Fruits and their Products* (HULME, A. C., ed.), Vol. 2, p. 437, Academic Press, London.

amination in controlling the rate of succinate and malate oxidation in the mitochondria through its effect on the levels of OAA.

RESULTS

GOT Activity in Evenly Ripening Tomato Fruit

The potent inhibition of succinate and malate dehydrogenases in intact tomato fruit mitochondria by OAA is illustrated by the values in Table 1 in which the effect of the inclusion of a small amount of the acid with the appropriate substrate is shown. Removal of these inhibitions by the inclusion of glutamate in the medium was effective during succinate oxidation by apple mitochondria[3] and malate oxidation by avocado mitochondria,[2] presumably through the action of GOT. Hence the activity of this enzyme in tomato mitochondria has been followed.

TABLE 1. EFFECT OF OAA ON SUCCINATE AND MALATE OXIDATION BY MITOCHONDRIA FROM MATURE GREEN TOMATO FRUIT

Substrate (17 mM)	Rate of oxidation* (μl O_2/hr/mg N/mg N)	Substrate (17 mM)	Rate of oxidation* (μl O_2/hr/mg N)
Succinate	519	Malate + TPP (133 μM)	269
Succinate + 0·1 mM OAA	301	Malate + TPP + 1mM OAA	208

* In the presence of non-limiting amounts of ADP (state 3).

GOT activity was detected in both mitochondrial and cytoplasmic fractions isolated from developing tomato fruit. Whereas the combined activity fell continuously throughout development, maximal mitochondrial activity was obtained from fruit at the green–orange stage of ripening (Table 2). Calculated as activity per mg mitochondrial nitrogen the activity at the green–orange stage was significantly higher ($P \leqslant 0{\cdot}05$) than that derived from large green fruit and from fruit riper than green–orange. The specific activity of the mitochondrial GOT isolated from fruit at the mature green stage was also significantly higher ($P \leqslant 0{\cdot}05$) than the activity from fruit subsequent to the green–orange stage. It may be noted that the mitochondrial GOT activity during ripening bears a close similarity to the rate of malate oxidation[6] during the same period and, under the assay conditions adopted in this study, GOT is sufficiently active to transaminate all the OAA so produced.

A general fall in the cytoplasmic GOT activity was observed throughout development; the level in large green fruit was significantly higher ($P \leqslant 0{\cdot}05$) than that shown by riper fruit. Similarly, the activity from mature green fruit was also significantly higher ($P \leqslant 0{\cdot}05$) than that from late ripening stages. A relatively wide variation in the activity found in large green fruit was probably caused by the difficulty in selecting samples of a similar physiological age compared with later, more easily recognizable stages.

Maximal activity of the mitochondrial transaminase occurred close to pH 7·4 and at a slightly higher pH for the cytoplasmic enzyme. However, the enzymes from both sources were routinely assayed at pH 7·4.

In no case did the addition of pyridoxal-5-phosphate to the assay medium for mitochondrial transaminase affect the rate of enzyme action; this suggests that the cofactor is not limiting during the ripening phase. By contrast, cytoplasmic transaminase was stimulated by up to 15% by the addition of the cofactor which was, therefore, included routinely in the assay medium. Occasionally, Mn^{2+} ions have been found to stimulate GOT from plants,[12] but in the present study the inclusion of 5 mM $MnSO_4$ inhibited the reaction.

TABLE 2. THE DISTRIBUTION OF GOT ACTIVITY IN TOMATO FRUIT DURING DEVELOPMENT

Source of enzyme	Enzyme activity*/100 g fresh tissue at the following ripening stages: Large green	Mature green	Green–Orange	Green–orange/Orange–green	Orange–green	Red	Standard error of the treatment means
Combined (mitochondrial + cytoplasmic)	26·47[a]	20·84[b]	18·14[bc]	16·50[c]	12·92[d]	12·28[d]	0·83
Mitochondrial	4·80[b]	5·57[a]	5·77[a]	3·77[c]	2·72[d]	2·55[d]	0·22
Specific mitochondrial activity (per mg mitochondrial N)	1·03[bc]	1·19[ab]	1·24[a]	0·94[c]	0·68[d]	0·73[d]	0·05
Cytoplasmic	21·67[a]	15·27[b]	12·37[bc]	12·73[bc]	10·20[c]	9·73[c]	0·80

* The activity is expressed as μmol of OAA reduced per min. Each value is the mean of three determinations. Figures having dissimilar superscripts are significantly different at $P \leqslant 0{\cdot}05$ by the Studentized range test.[11]

GOT Activity in Abnormally Ripening Fruit

In fruit showing symptoms of 'blotchy' ripening,[13] often associated with low potassium status, irregular hard green or yellow patches occur on otherwise soft red fruit. The mitochondrial activities of GOT in the green areas were significantly higher ($P \leqslant 0{\cdot}001$) than

TABLE 3. GOT ACTIVITY IN TOMATO FRUIT SHOWING 'BLOTCHY' RIPENING

Source of enzyme	Enzyme activity*/100 g fresh tissue of: Green area	Red area	Standard error of the treatment means
Combined (mitochondrial + cytoplasmic)	28·08	13·64	0·94
Mitochondrial	7·71	3·54	0·24
Specific mitochondrial activity (per mg mitochondrial N)	1·65	1·04	0·06
Cytoplasmic	20·37	10·10	0·90

* The activity is expressed as μmol OAA reduced per min. Each value is the mean of three determinations.

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[13] BEWLEY, W. F. and WHITE, H. L. (1926) *Ann. Appl. Biol.* **13**, 323.

those in the red areas (Table 3). When compared with evenly ripening fruit (Table 2), the activity in the green regions of blotchy fruit was considerably enhanced, and the red regions also had a somewhat higher activity than might have been anticipated.

Similarly, the cytoplasmic activity was significantly higher ($P \leqslant 0{\cdot}01$) in the green areas of affected fruit than in the red areas. Whereas the value for the red tissue was typical of evenly ripening fruit, the green areas contained an abnormally high activity, equivalent to a stage prior to the mature green point in development.

DISCUSSION

The assay procedure for GOT adopted in this study involved driving the reaction in the opposite direction to its postulated role, i.e. the transamination of OAA by GOT. However, the enzyme reaction is reported to be readily reversible.[14,15] As the glutamic acid content of tomato fruit increases considerably during ripening (from about 2–20 mM),[10] this would tend to favour reaction with OAA.

Values for the combined activity of GOT in tomato fruit are based on the sum of the cytoplasmic and mitochondrial fractions. The efficiency of extraction of mitochondria from tissue is very difficult to assess, especially since steps were taken to obtain particles having good respiratory control and efficient oxidative phosphorylation, and this requires the exclusion of disintegrated or aggregated particles. Values for mitochondrial activity are therefore comparative rather than absolute, but they indicate that the proportion of GOT activity associated with the mitochondria compared with the combined activity rises to a maximum at the green–orange stage of ripening. Malate levels in tomato fruit fall most rapidly up to this stage[16] at the same time as high GOT levels occur. The cytoplasmic GOT activity, which fell significantly from the large green stage of development, may be related to the decreasing utilization of the two amino acid substrates, L-aspartate and L-glutamate, which accumulate during ripening in the outer walls of the fruit [16]

Bone and Fowden[15] indicated that GOT was active in preparations of mung bean mitochondria but they did not rule out the possibility of a soluble component as well. Romani[17] established the presence of GOT in both peel and cortical tissues of pear, and that the activity was considerably higher in the soluble fractions. In apples Hulme *et al.*[18] have shown that the climacteric rise in respiration is accompanied by an increase in the GOT activity of mitochondria isolated from the peel, but activity during the post-climacteric phase was not discussed. A relatively low level of GOT activity compared with that in other plant tissues has been found in green tomatoes.[19] The investigations of Yu *et al.*[20] into the activity of the enzyme in tomato fruit extracts indicated no clear-cut pattern in the activity during maturation, but at a stage of ripeness immediately prior to the red colour there was from 10 to 60 times more activity in the insoluble fraction than in the soluble part depending on the exact conditions of separation.

In blotchy ripened fruit the green areas show a particularly high GOT activity especially in the mitochondrial fraction. The concentration of glutamate in affected fruit is also higher

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[17] ROMANI, R. J. (1962) *Plant Physiol.* **37,** 523.
[18] HULME, A. C., RHODES, M. J. C and WOOLTORTON, L. S. C. (1967) *Phytochemistry* **6,** 1343.
[19] LEONARD, M. J. K. and BURRIS, R. H. (1949) *J. Biol. Chem.* **170,** 701.
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than with even ripening,[16,21] and high rates of transamination are therefore possible. Furthermore, reduced levels of malate have been recorded in these green areas of affected fruit,[16,21] (229 μequiv/10 g fr. tissue compared with 367 found in normal mature green tissue) which might be caused in part by an accelerated rate of malate oxidation through high GOT activity. The enhanced mitochondrial GOT activity in both the green and the red areas of blotchy fruit suggests that fruit showing this ripening disorder are abnormal as a whole and the green areas cannot be regarded as being merely delayed in development.

Tomato fruit mitochondria are able to oxidize malate either through malate dehydrogenase or by an NAD^+– requiring malic enzyme[22] and these are probably localized in different parts of the mitochondrion.[23] The relative activities of the two enzymes could be greatly affected by pH,[24] and at pH 6·8 particles from green fruit show about 40 times more malate dehydrogenase activity than malic enzyme activity.[22] The equilibrium of the malate dehydrogenase reaction greatly favours malate formation from OAA,[7] hence for the continued operation of the malate dehydrogenase pathway a mechanism for the rapid removal of OAA from its site of production is essential. Regulation of malate oxidation by fruit mitochondria could therefore be effected by control of the OAA level through GOT activity.

OAA levels in tomato fruit have been measured by Trudel and Ozbun,[25] At the mature green and green–orange stages approximately 1·4 μmol OAA/g fr. tissue were found, rising to about 2·7 in ripe tissue. Concentrations of OAA comparable to these inhibit the oxidation of succinate and malate by isolated tomato fruit mitochondria, and the OAA levels have an inverse relationship with the combined cytoplasmic and mitochondrial GOT activity throughout ripening. When GOT is allowed to operate in isolated mitochondria, their oxidative rate clearly parallels the respiratory behaviour of the fruit from which they were derived.[26]

EXPERIMENTAL

Freshly picked tomato fruit, grown in a heated glasshouse, of the variety Potella (a green-back free cultivar of variety Potentate) were used throughout this study. The ripening stages were as follows: Large Green, almost fully grown fruit with firm unexpanded locular contents; Mature Green, fruit with expanded locular contents having the seeds embedded in a jelly-like ground substance, without any trace of yellow colour either externally or around the seeds; Green–Orange, predominantly green external colouration but with distinct yellow or yellow–orange tinges at the blossom-end and especially along the line of the septae; Green–Orange/Orange–Green, fruit with external colour *ca.* 75% green, 25% orange; Orange–Green, as nearly as possible half green and half orange; Red, uniformly ripe fruit not yet crimson in colour. Blotchy ripened fruit have been described by Bewley and White.[13]

Washed, thoroughly chilled outer pericarp tissue was used as the source of the enzyme. Intact mitochondria were prepared by the method of Hobson[6] and the final pellet was taken up in about 1 ml of a medium containing 250 mM sucrose, 10 mM potassium phosphate buffer pH 7·2, 10 mM Tris–HCl buffer pH 7·2, 5 mM $MgCl_2$, 0·5 mM EDTA and 1 mg 'fatty acid-poor' bovine serum albumin (Calbiochem). The oxidative ability of the mitochondria was checked polarographically using a Clark oxygen electrode connected to a potentiometric recorder as described by Wiskich *et al.*[2] Prior to GOT assay the mitochondria were disintegrated by a 4 min treatment in a Potter homogenizer with a tightly-fitting Teflon piston.

After centrifugation at 12 000 *g* for 20 min to precipitate initially the mitochondria from the tomato tissue suspension, the remaining supernatant liquid was recentrifuged at 100 000 *g* for 40 min. The resulting supernatant liquid was designated the cytoplasmic fraction.

The method of Romani[17] was used for determining the activity of GOT, except that *N*-Tris(hydroxymethyl)methyl-2-amino-ethanesulphonic acid (TES) was used instead of Tris. The assay medium consisted

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of 80 μmol TES–HCl pH 7·4, 10 μg pyridoxal-5-phosphate, 10 μmol L-aspartate, 0·3 μmol NADH, 25 μg malate dehydrogenase (Boehringer), 10 μl disintegrated mitochondria or 500 μl cytoplasmic fraction, and the reaction was initiated by the addition of 10 μmol 2-oxoglutarate. The final volume was 3 ml. Changes in optical density at 340 nm and 25° were recorded before and after adding the oxoglutarate. A small background 'NADH oxidase' activity was observed prior to the addition of pyridoxal-5-phosphate and 2-oxoglutarate which was independent of the amount of malate dehydrogenase used. Additionally, a slow rate of non-enzymic transamination between L-aspartate and 2-oxoglutarate occurred in the presence of pyridoxal-5-phosphate. Allowances for these activities were made. It was essential to have excess malic dehydrogenase present in the assay medium so that the rate-limiting step was the transamination reaction.

The nitrogen content of the disintegrated mitochondrial suspensions was determined by the method of Thompson and Morrison[27] as modified by Biale *et al.*[28] As the cytoplasmic fraction contained a relatively high proportion of N-containing chemicals compared with the protein content, the GOT activity could not be expressed on a nitrogen basis and was therefore calculated on a fr. wt basis.

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