[Δ]-Aminolevulinic acid dehydratase activity during tomato fruit development and ripening

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δ-Aminolevulinic acid dehydratase activity during tomato fruit development and ripening

by

Marios Costa Kyriacou

A Thesis Submitted to the Graduate Faculty in Partial Fulfillment of the Requirements for the Degree of MASTER OF SCIENCE

Department: Horticulture Interdepartmental Major: Plant Physiology

Signatures have been redacted for privacy

Iowa State University
Ames, Iowa
1994
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Figure 2. Effect of ALA concentration on ALAD activity at pH 8.7. Enzyme was extracted from fruit 10 days postanthesis. Each point is a mean of eight replicates (Fisher's LSD, \( P = 0.05 \)).

Figure 3. ALAD activity (●) and soluble protein content (▲) during tomato fruit development and ripening. Each point is a mean of eight replicates (Fisher's LSD, \( P = 0.05 \)).
1. GENERAL INTRODUCTION

Tomato fruit ripening spans the last 15 to 20 days of fruit development, and it is characterized by several physiological events of postharvest significance. Chlorophyll content in the fruit decreases, and there is a concomitant increase in lycopene content (the major carotenoid pigment in tomato fruit). These changes result in the gradual transition in fruit pigmentation from green to red that is manifest shortly after physiological maturity. Genetic and biochemical control of the ripening process in tomato fruit is of great interest to the agricultural and food industries because it may lead to greater utilization of produced fruit.

The purpose of this research was to study the mechanism of developmental regulation of chlorophyll biosynthesis during tomato fruit development and ripening. In particular, we studied the enzymatic activity of δ-aminolevulinic acid (ALA) dehydratase (ALAD), the first committed enzyme in the chlorophyll/heme pathway, during fruit development and ripening and in different organs of the tomato plant.

Thesis Organization

This thesis was organized by using journal manuscript format. In addition to the included paper, a general review of literature and conclusions were included. A list of references cited in the general review of literature follows the summary and conclusions chapter.
2. GENERAL REVIEW OF LITERATURE

Chlorophyll and heme biosyntheses occur via a common pathway from δ-aminolevulinic acid (ALA) through the monopyrrole porphobilinogen (PBG) to the asymmetric, cyclized tetapyrrole protoporphyrin IX (Smith and Griffiths, 1993). ALA is the first committed tetapyrrole precursor, and it is formed by one of two distinct pathways found in photosynthetic organisms. In plants, algae, and some photosynthetic bacteria, ALA is formed from glutamic acid by a five-carbon pathway that involves three enzymatic reactions and tRNA^Glu (Houen et al., 1983). In other photosynthetic bacteria, as in yeast and animals, ALA is formed by ALA synthase in a condensation reaction that involves glycine and succinyl coenzyme A (Beale and Castelfranco, 1974). Although the presence and activity of ALA synthase in higher plant tissues has not been excluded categorically, the preferential and specific contribution of ^14C label to ALA by glutamate and α-ketoglutarate has been demonstrated widely (Beale and Castelfranco, 1974; Castelfranco and Jones, 1975), and the five-carbon pathway generally is accepted as the synthetic route for ALA that is destined for all tetapyrroles in plants.

ALA dehydratase [(porphobilinogen synthase) ALAD] is the first committed enzyme in the biosynthetic pathway of chlorophyll and heme. It catalyzes the dimerization of ALA to PBG in a condensation reaction (Jordan and Seehra, 1980). The first ALA molecule forms a Schiff base with the enzyme (Nandi et al., 1968) and contributes the propionic acid side chain of the product. The second ALA molecule completes the PBG molecule and contributes the acetate side chain (Jordan and Seehra, 1980). Formation of the aromatic pyrrole ring of PBG is facilitated by the stereospecific removal of one of the two C-5 hydrogens of the first ALA molecule by the enzyme (Abboud and Akhtar, 1976).
ALAD has been isolated and characterized partially from a number of plant sources such as spinach leaves (Liedgens et al., 1980), radish seedlings (Hualt et al., 1987), pea leaves and *Arum* spadices (Smith, 1988), tobacco leaves (Schneider, 1970), and wheat leaves (Nandi and Waygood, 1967). Plant ALAD is a hexameric protein that consists of six identical subunits each with a molecular weight of 50 kDa (Liedgens et al., 1980). It has a highly conserved active site domain centered on a catalytically active lysine residue that participates in Schiff base formation with the substrate, and a metal ligand-binding domain based on conserved aspartate, tyrosine, and histidine residues (Boese et al., 1991). Maximum enzymatic activity requires Mg$^{2+}$ or Mn$^{2+}$, thought to function in either catalysis and/or enzyme stabilization, although residual activity of about 30% is retained in their absence (Nandi and Waygood, 1967; Liedgens et al., 1980). ALAD is inhibited by EDTA and by divalent heavy metal ions (Scaproni and Perucci, 1984). Substrate analog levulinic acid and succinyl acetone exert competitive inhibition on the enzyme (Brumm and Friedman, 1981). Essential sulfhydryl groups on ALAD account for the enzyme’s high sensitivity to oxygen and thiol reagents. Rapid loss of enzymatic activity upon exposure of the native enzyme to O$_2$ is associated with the oxidation of two highly reactive SH groups to form a disulfide link (Smith and Griffiths, 1993). Full catalytic activity can be restored by treatment of the enzyme with thiol reagents. The reactive sulfhydryl groups seem to be involved in the binding of the metal ion to the enzyme (Boese et al., 1991). The pH optima reported for ALAD from various plant sources range from 7.4 in tobacco leaves (Shetty and Miller, 1969) to 9.5 in tobacco callus (Kaul and Sabharwal, 1974).

ALAD and PBG deaminase (PBGD) (the second enzyme in the biosynthetic pathway of chlorophyll, which catalyzes the polymerization of four PBG molecules to
form the first tetrapyrrole of the pathway), are confined to the plastids, presumably in the stroma. Such is the case in pea and even in the spadix tissues of *Arum* where synthesis of nonplastid heme is predominant (Smith, 1988). Therefore, plastid ALAD is involved also in the synthesis of nonplastid heme and a tetrapyrrole compound must be exported from the plastid. In greening radish cotyledons, most of the ALAD activity seems to be associated with the plastid stroma and partly with the thylakoid membranes (Narsi et al., 1988). Yet, it has been indicated that the portion of the relatively hydrophobic amino-terminal region of ALAD, with characteristics of a membrane-spanning domain, might comprise part of the transit peptide for chloroplast import (Boese et al., 1991).

Previous studies on several plants have shown that the content of ALAD protein and its enzymatic activity depend upon the tissue and stage of development during light-induced greening. Kasemir and Masoner (1975) showed that ALAD activity increased during light-induced development of mustard seedling cotyledons, and they established the involvement of phytochrome in ALAD light induction. Similar results by Huault et al. (1987) indicated an increase in ALAD activity during chloroplast differentiation in etiolated radish cotyledons. Immunoquantification of ALAD during differentiation in etiolated radish cotyledons (Huault et al., 1984; Huault et al., 1987) demonstrated a parallel increase between ALAD protein content and enzyme activity, and the researchers proposed *de novo* synthesis as the main cause for the light-induced increase in ALAD activity. In contrast to the increase in ALAD activity, steady state levels of ALAD mRNA exhibited little or no change during light-induced greening of etiolated pea leaf tissues, and this suggested that light regulation of ALAD occurs postranscriptionally, either at the level of translation or enzyme activation (Boese et al., 1991). Accumulation of ALAD
mRNA was highest in photosynthetically active leaves and stems, and only marginally detectable in root tissues (Boese et al., 1991). Developmentally, ALAD activity was shown to decrease continuously with age of primary leaves of bean seedlings (Naito et al., 1980).

The regulation of ALAD activity in senescing plant tissues and degreening fruits, and the role of the enzyme in the developmental regulation of chlorophyll biosynthesis require further research. The incentive for this research was the study of ALAD behavior during the ontogeny of the tomato fruit in view of the potential control of the degreening process through regulation of ALAD activity.
3. δ-AMINOLEVULINIC ACID DEHYDRATASE ACTIVITY DECLINES DURING TOMATO FRUIT DEVELOPMENT AND RIPENING

A paper to be submitted to the Journal of the American Society for Horticultural Science

Marios C. Kyriacou, David J. Hannapel¹, and Richard J. Gladon¹

Abstract. δ-Aminolevulinic acid (ALA) dehydratase [ALAD, (EC 4.2.1.24)] activity and soluble protein content were determined in tomato (Lycopersicon esculentum Mill. 'Rutgers') fruit pericarp extracts during development and ripening. Analyses were conducted at 5-day intervals between days 10 and 60 postanthesis. The decline in ALAD activity was most pronounced between days 10 and 25. At the mature green stage (day 40), and before carotenoid revelation (day 45), activity of ALAD had declined to a minimum. ALAD activity remained detectable at residual levels throughout ripening (days 40 to 60). The declines in ALAD activity and soluble protein content were coincidental, but decline in protein content was less rapid. Furthermore, ALAD activity was greatest in extracts of chlorophyllous organs (stems, leaves, immature fruit), where both heme and chlorophyll biosynthesis take place. ALAD activity was only marginally detectable in extracts of nonchlorophyllous organs (roots, overripe fruit) where heme synthesis is predominant. The pH optimum and Km for tomato fruit ALAD was similar to that of ALAD enzymes isolated from other sources.

¹Associate Professors
Introduction

δ-Aminolevulinic acid (ALA) dehydratase [ALAD, (EC 4.2.1.24)] is the first committed enzyme in the chlorophyll biosynthetic pathway. It catalyzes the dimerization of two ALA molecules into the monopyrrole porphobilinogen (PBG) (Jordan and Seehra, 1980). Light regulation of several chlorophyll biosynthesis enzymes, including ALAD, is well documented (Castelfranco and Beale, 1983; Harel, 1978). Previous studies with several plant species have shown that ALAD protein content and its enzymatic activity depend upon the type of tissue and the stage of development during light-induced greening. ALAD activity increased during light-induced development of cotyledons of mustard (Sinapis alba L.) seedlings, and phytochrome involvement in ALAD light induction was established (Kasemir and Masoner, 1975). Huault et al. (1987) found parallel increases in ALAD protein content and enzyme activity during chloroplast differentiation in etiolated cotyledons of radish (Raphanus sativus L.). In etiolated leaf tissues of pea (Pisum sativum L.), steady-state levels of ALAD mRNA exhibited little or no change during light-induced greening, and this suggested posttranscriptional light regulation of ALAD activity, either at the level of translation or enzyme activation (Boese et al., 1991).

Little research has been conducted on ALAD activity in senescing tissues, but Naito et al. (1980) showed that ALAD activity decreased continuously with age of primary leaves of bean (Phaseolus vulgaris L.). The purpose of this research was to measure changes in ALAD activity during tomato fruit development and ripening. The level of ALAD activity in extracts of various organs of tomato plants was measured, and some characteristics of the ALAD enzyme also were determined.
Materials and Methods

Chemicals. ALA was purchased from Porphyrin Products (Logan, Utah). Polyvinylpolypyrrolidone, Tris buffer, bovine serum albumin, dithiothreitol, trichloroacetic acid, mercuric chloride, and p-dimethylaminobenzaldehyde (Ehrlich’s reagent) were obtained from Sigma (St. Louis, Mo.). All other chemicals purchased from commercial sources were reagent grade.

Plant materials. 'Rutgers' tomato plants were grown in an environmentally controlled greenhouse (20°C night, 24°C day). Supplementary irradiance of ≈300 μmol s⁻¹ m⁻² from high pressure sodium lamps was provided from 0700 to 1900 daily. Uniform transplants were produced in a rockwool-based hydroponic system. Four weeks after sowing, seedlings were transplanted into pots of 3 soil: 4 peat: 3 perlite (by vol.) for the remainder of one fruiting cycle (single truss production) (McAvoy et al., 1989). Seedlings were maintained with 150 mg N/L of Cal-Mag Peters Excel (15-5-15) nutrient solution from Grace-Sierra (Milpitas, Calif.). Plants were trained to a single stem, and the stem apex was removed above the first leaf that followed the first inflorescence. Flowers were pollinated and tagged at anthesis (Lyons and Pratt, 1964), and three fruits were retained on each plant. Stages of fruit development were as follows: (i) cell division from 0 to 20 days, (ii) cell expansion from 20 to 40 days, (iii) mature green at ≈40 days, (iv) breaker to turning (carotenoid revelation) at ≈45 days, (v) light pink at ≈50 days, (vi) pink at ≈55 days, (vii) ripe at ≈60 days, and (viii) overripe at ≈65 days (Martin et al., 1979). Fruit was harvested at each of 12 physiological classes ranging from 10 to 65 days postanthesis at 5-day intervals. Root, stem, and leaf tissues were obtained from seedlings that were 4 weeks old.

Enzyme extraction. A core of tissue (1 cm in diameter) was removed from the axis
of each fruit with a stainless steel cork borer, and 1.0 g of tissue from the blossom end of the core (including the epidermis) was used for extraction. Similarly, 1.0 g of root, stem (basal part of epicotyl), or leaf (laminae of first leaf pair) tissues was used. The tissue was ground to a fine powder under liquid N2 in a mortar. Immediately after grinding, 1.0 g of polyvinylpolypyrrolidone was mixed with the powdered tissue to prevent phenolic oxidation. After addition of 5.0 ml of 25 mM Tris-HCl buffer (pH 7.6) that contained 4 mM dithiothreitol, the homogenate was filtered through four layers of cheesecloth and centrifuged at 10,000 x g for 10 min. The pellet was discarded, and the supernatant was used as the enzyme source. Protein content of the extract was determined by the Bradford (1976) method.

Enzyme assay. ALAD activity was measured according to the method of Smith and Griffiths (1993) modified for use on tomato tissues. The standard reaction mixture was prepared in a microcentrifuge tube on ice. It had a total volume of 300 µl that consisted of 113.25 µl of extract, 50 mM Tris-HCl buffer (pH 8.7, unless otherwise mentioned), 10 mM ALA (unless otherwise mentioned), and 6 mM MgCl2. The reaction was initiated by addition of the extract at time zero. After 1 h of incubation at 37C with gentle shaking (150 rpm), the reaction was stopped by addition of 300 µl of a solution that contained 10% trichloroacetic acid and 2.7% HgCl2 (W/V). The mixtures were microcentrifuged at 14,000 rpm for 8 min to precipitate proteins, and 500 µl of supernatant was mixed 1:1 with modified Ehrlich’s reagent (Mauzerall and Granick, 1956). After 15 min, absorbance of the mixture at 553 nm was determined against the zero blank. PBG concentration was calculated by using a molar extinction coefficient of 6.2 x 10^4 M^-1cm^-1 (Kaul and Sabharwal, 1974). The effect of pH on ALAD activity was determined by varying the pH of the Tris-HCl reaction buffer. Initial velocity at
increasing substrate concentrations was determined, and $V_{\text{max}}$ and $K_m$ values were obtained from an Eadie-Hofstee plot.

**Experimental design.** Replicates of fruit of the same physiological class were obtained from the entire plant population. ALAD activity and protein content determinations during fruit development and ripening were done in a randomized block design of eight blocks. Each block consisted of 11 fruits with one fruit for each physiological class from 0 to 60 days. Determination of organ-specific ALAD activity was done in a completely randomized design with eight replications. Replicates were single fruits or single samples of root, stem, or leaf tissues. The effects of pH and ALA concentration on ALAD activity were determined according to randomized block designs with eight blocks. Extract from one fruit was used in each block. Fruits 10 days postanthesis were used for studying the effect of ALA concentration, and fruits 15 days postanthesis were used for studying the effect of pH. The test for presence of an enzyme inhibitor was done by using a randomized block design with four blocks. Each block included one fruit at 20 and 65 days postanthesis and their combined (1:1) extracts. For statistical analysis, we used software package SAS Release 6.06 from SAS Institute (Cary, N.C.).

**Results**

ALAD activity in several buffer systems (sodium phosphate, HEPES, carbonate-bicarbonate, borate-sodium borate) was determined, and the greatest activity was observed in Tris-HCl ($pK'_{a}$ at 20°C=8.3) (data not presented). ALAD activity, with Tris-HCl buffer, increased sharply between pH 7.6 and 8.3 and reached a plateau near pH 8.7 (Fig. 1). Therefore, all further assays were conducted at pH 8.7.
ALAD exhibited typical Michaelis-Menten kinetics (Fig. 2). A subsequent $K_m$ of 0.74 mM for ALA and a $V_{max}$ of 49.4 nmol PBG/mg protein/h were obtained from an Eadie-Hofstee plot of $V$ against $V/S$. The saturating ALA concentration of 10 mM was used in all subsequent analyses. During time-course studies of ALAD activity, the rate of reaction was linear for up to 80 min (data not presented). Therefore, the incubation period was restricted to 1 h.

Between days 10 and 40 postanthesis, both ALAD activity and soluble protein content dropped sharply (Fig. 3). During ripening, days 40 to 60 postanthesis, protein content declined slightly and ALAD activity remained detectable at residual levels.

In a separate analysis from that of Fig. 3, ALAD activity from fruit 20 days postanthesis was $44.8 \pm 3.6$ nmol PBG/ml extract/h, whereas activity from fruit 65 days postanthesis was $1.8 \pm 0.7$ nmol PBG/ml extract/h. When extracts from each of these ages of fruit were mixed in equal volumes, the resultant activity was 92.3% ($21.5 \pm 2.4$ nmol PBG/ml extract/h) of the expected additive activity.

Extracts from roots and from fruit 65 days postanthesis both yielded residual levels of activity (Table 1). The highest ALAD activity was observed in leaf extracts, whereas fruit 15 days postanthesis yielded an ALAD activity almost twice that found in stem extracts.

Discussion

ALAD enzyme from crude extracts of 'Rutgers' tomato fruit exhibits a broad pH optimum between 8.7 and 9.2 in 50 mM Tris-HCL buffer (Fig. 1). Typical Michaelis-Menten kinetics were observed, and a $K_m$ of 0.74 mM for ALA was derived (Fig. 2). Similar results have been reported by other researchers. Schneider (1970) found a broad
L.), and a pH optimum of 8.2 for the highly purified enzyme. A broad pH optimum of 9.2 to 9.5 has been reported for ALAD from crude extracts of green callus of tobacco (Nicotiana tabacum L.) (Kaul and Sabharwal, 1974). Nandi and Waygood (1967) found a $K_m$ of 1.0 mM for ALA in leaves of wheat (Triticum aestivum L.), and Kaul and Sabharwal (1974) reported a $K_m$ of 0.46 mM for ALA.

Our results show that ALAD activity decreases rapidly between days 10 and 25 of fruit development (Fig. 3). This three-fold decline in activity indicates that developmental regulation of ALAD activity and chlorophyll biosynthesis takes place at the initial stages of fruit development, during the period of cell division and the beginning of cell enlargement. Inactivation of many photosynthesis-specific genes in tomato pericarp tissue takes place in the presence of light within 2 weeks after pollination (Piechulla et al., 1987). Naito et al. (1980) showed that chloroplast replication and enlargement coincide with chlorophyll synthesis (expressed as ALA synthesis capacity and ALAD activity) in intact bean leaves during ageing. ALAD activity decreased continuously with leaf age but was still detectable at the onset of senescence. These findings support our data that show ALAD activity decreases to a minimum (≈day 40) before carotenoid revelation (≈day 45) (Figure 3). By contrast, the activities of other ripening-related enzymes, such as polygalacturonase (Grierson and Tucker, 1983) and acid invertase (Sato et al., 1993), are marked by great increases after the initiation of ripening. Our results indicate a decline in chlorophyll synthesis ability, not just increased chlorophyll breakdown, precedes the visual transition in fruit pigmentation.

The residual ALAD activity that was maintained after fruit maturation (days 40 to 60) (Fig. 3) and into senescence (day 65) (Table 1), might be explained by the constant demand for pyrrolic compounds, particularly heme for respiratory cytochromes,
It seems unlikely that the decline in ALAD activity in older fruit is due to the presence of a soluble enzyme inhibitor. Activity in a 1:1 mixture of extracts from fruit 20 and 65 days postanthesis was nearly additive. Presence of a soluble enzyme inhibitor in ripe fruit would have resulted in low ALAD activity in mixed extracts, and this was not the case. Presence of a bound inhibitor, nevertheless, which could have been removed with cellular debris during extraction, cannot be excluded on the basis of our results. The marked decline observed in ALAD activity, probably would not have been caused by an inhibitor of this type, particularly because ALAD does not seem to be a membrane-associated enzyme (Smith, 1988; Boese et al., 1991).

The coincidental declines in soluble protein content and ALAD activity during tomato fruit development and ripening suggest that the decrease in ALAD activity may have been caused by a decrease in the amount of enzyme in the pericarp tissue. A decline in ALAD protein seems likely, particularly because it has been shown that steady-state levels of ALAD mRNA remain stable during development and ripening of 'Rutgers' tomato fruit (G. Polking, personal communication). These findings suggest postranscriptional regulation of ALAD activity, either at the level of translation or enzyme activation. Such developmental regulation for ALAD also has been proposed by Boese et al. (1991) who found expression of ALAD is constitutive during light-induced greening of etiolated leaf tissues of pea. Similarly, Hualt et al. (1987), proposed de novo synthesis as the main cause of the light-induced increase in ALAD activity during chloroplast differentiation in etiolated cotyledons of radish.

ALAD activity was higher in chlorophyllous organs (stems, leaves, immature fruit) than in nonchlorophyllous organs (roots, ripe fruit) (Table 1). This reflects the predominance of the heme branch of the porphyrin pathway in roots and ripe fruit
fruit) than in nonchlorophyllous organs (roots, ripe fruit) (Table 1). This reflects the predominance of the heme branch of the porphyrin pathway in roots and ripe fruit whereas both the heme and chlorophyll branches of the pathway are fully active in stems, leaves, and immature fruit. As expected, ALAD activity was greatest in photosynthetically active tissues. ALAD activity in stem extracts was about half of that in young fruit (15 days), in which photosynthetic activity reportedly is important despite the influx of photosynthates from leaves (Piechulla et al., 1987). The minimal ALAD activity in root extracts, which is comparable to that in overripe fruit (65 days), is in contrast to the relatively high levels of steady-state ALAD mRNA found in root tissues of 'Rutgers' tomato seedlings (G. Polking, personal communication). These results also suggest that regulation of ALAD activity in nonchlorophyllous organs occurs postranscriptionally.

Suppression of ALAD activity during tomato fruit development and ripening in the presence of light indicates that, in addition to regulation by light (Kasemir and Masoner, 1975; Huault et al., 1987; Boese et al., 1991), a developmental regulation mechanism is superimposed on chlorophyll biosynthesis enzymes. Such regulation of chlorophyll biosynthesis seems to take place at the initial stages of tomato fruit development and before the visual transition in fruit pigmentation.

**Literature Cited**


Table 1. ALAD activity in extracts of root, stem, and leaf tissues from seedlings 4 weeks old, and pericarp tissues from immature fruit (15 days) and overripe fruit (65 days).^

<table>
<thead>
<tr>
<th>Enzyme source</th>
<th>ALAD activity (nmol PBG/mL extract/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Root</td>
<td>9.8 d</td>
</tr>
<tr>
<td>Stem</td>
<td>32.9 c</td>
</tr>
<tr>
<td>Leaf</td>
<td>137.6 a</td>
</tr>
<tr>
<td>Fruit (15 days)</td>
<td>61.9 b</td>
</tr>
<tr>
<td>Fruit (65 days)</td>
<td>7.7 d</td>
</tr>
</tbody>
</table>

*Means followed by different letters are different according to Fisher’s LSD (P = 0.05).

Each mean represents eight replications.
Fig. 1. Effect of pH on ALAD activity. Enzyme was extracted from fruit 15 days postanthesis and 50 mM Tris-HCl buffer and 10 mM ALA were used. Each point is a mean of eight replicates (Fisher's LSD, $P = 0.05$).

Fig. 2. Effect of ALA concentration on ALAD activity at pH 8.7. Enzyme was extracted from fruit 10 days postanthesis. Each point is a mean of eight replicates (Fisher's LSD, $P = 0.05$).

Fig. 3. ALAD activity (●) and soluble protein content (▲) during tomato fruit development and ripening. Each point is a mean of eight replicates (Fisher's LSD, $P = 0.05$).
Fig. 1

ALAD activity (nmol PGB/mg protein/h) vs pH

LSD = 2.3 (0.05)
Fig. 3

ALAD activity (nmol PBG/ml extract/h)

Protein content (mg protein/ml extract)

Time postanthesis (days)

LSD (0.05) = 8.7

LSD (0.05) = 0.08
4. SUMMARY AND CONCLUSIONS

A procedure for assaying the activity of δ-aminolevulinic acid (ALA) dehydratase (ALAD) was adopted and modified for use on tomato tissues. ALAD enzyme from crude extracts of 'Rutgers' tomato fruit exhibited a broad pH optimum between 8.7 and 9.2 in 50 mM Tris-HCl buffer. Typical Michaelis-Menten kinetics were observed, and a $K_m$ of 0.74 mM for ALA was derived. Similar results from other plant sources have been reported by several researchers.

ALAD activity and soluble protein content were determined in tomato fruit pericarp extracts during development and ripening. Analyses were conducted at 5-day intervals between days 10 and 60 postanthesis. The activity of ALAD declined throughout development and ripening. The decline was most pronounced between days 10 and 25 postanthesis. At the mature green stage (day 40), and before carotenoid revelation (day 45), ALAD activity had declined to a minimum but remained detectable at residual levels throughout ripening (days 40 to 60). ALAD activity and soluble protein content declined coincidentally, but the decline in protein content was less rapid. The decline in ALAD activity was probably not due to presence of a soluble enzyme inhibitor because the activity in a 1:1 mixture of extracts from fruit 20 and 65 days postanthesis was nearly additive. The residual ALAD activity that was maintained after fruit maturation (days 40 to 60) and into senescence (day 65) might be explained by the constant demand for pyrrolic (heme) compounds.

The coincidental declines in soluble protein content and ALAD activity during tomato fruit development and ripening suggest that the decrease in ALAD activity may have been caused by a decrease in the amount of enzyme in the pericarp tissue. A decline in ALAD protein seems likely, particularly because it has been shown that steady-
state levels of ALAD mRNA remain stable during development and ripening of 'Rutgers' tomato fruit. These findings suggest postranscriptional regulation of ALAD activity, either at the level of translation or enzyme activation. Such developmental regulation for ALAD also has been proposed by other researchers.

ALAD activity was higher in chlorophyllous organs (stems, leaves, immature fruit) than in nonchlorophyllous organs (roots, ripe fruit). This reflects the predominance of the heme branch of the porphyrin pathway in roots and ripe fruit whereas both the heme and chlorophyll branches of the pathway are fully active in stems, leaves, and immature fruit. As expected, ALAD activity was highest in photosynthetically active tissues. ALAD activity in stem extracts was about half of that in young fruit (15 days), in which photosynthetic activity reportedly is important despite the influx of photosynthates from leaves. The minimal ALAD activity in root extracts, which is comparable to that in overripe fruit (65 days), is in contrast to the relatively high levels of steady-state ALAD mRNA found by collaborators in root tissues of 'Rutgers' tomato seedlings. These results also suggest that regulation of ALAD activity in nonchlorophyllous organs occurs postranscriptionally.

Suppression of ALAD activity during tomato fruit development and ripening in the presence of light indicates that in addition to regulation by light, already established by other researchers, a developmental regulation mechanism is superimposed on chlorophyll biosynthesis enzymes. Such regulation of chlorophyll biosynthesis seems to take place at the initial stages of tomato fruit development and before the visual transition in fruit pigmentation.
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