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QUANTITATIVE CHANGES IN *t*RNA DURING ETHYLENE INDUCED RIPENING (AGEING) OF TOMATO FRUITS

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Key Word Index—*Lycopersicon esculentum*; Solonaceae; tomato; *t*RNA; ageing; fruit ripening; ethylene.

Abstract—Iso-accepting forms of $tRNA^{met}$, $tRNA^{leu}$, $tRNA^{lys}$, and $tRNA^{tyr}$ were isolated from combined walls and septa of tomato fruits at 5 consecutive stages of ethylene induced ripening. Changes in the relative amount of some $tRNA^{leu}$ and $tRNA^{lys}$ were discerned 10 hr after exposure to ethylene. Individual patterns of change for each of several iso-acceptor *t*RNAs were evident throughout the ripening sequence. Maximal changes were: $tRNA^{lys}$, −66·3%; $tRNA^{leu}$, −24·8%; and $tRNA^{met}$, +26·7%.

INTRODUCTION

The possibility that ageing [1,2] as well as protein synthesis [3,4] may be regulated at the translational step has led to the discovery of changes in the *t*RNA of ageing mammalian, microbial and plant tissues [5]. The studies with plant tissues, however, have been confined to soybean cotyledons [2,6] and wheat leaves [7], and have not included fruits whose orderly ripening sequence has been especially well suited to the study of ageing [8,9]. More specifically, ageing (ripening) fruit tissues undergo changes in RNA and protein metabolism [10,11] and a decline in the ability to respond to stress [12,13]. The latter phenomenon is frequently observed in various ageing tissues and may, according to some theories of ageing [14], result from alterations in *t*RNA and malfunctions at the translation level.

In the preceding paper [15] methods were described for the isolation of functional *t*RNA from fruit tissues. In the present study we examined the status of $tRNA^{met}$, $tRNA^{leu}$, $tRNA^{lys}$, and $tRNA^{tyr}$ in ripening tomato (*Lycopersicon esculentum*, cv Ace) fruits.

RESULTS AND DISCUSSIONS

Uniformly green tomatoes, sufficiently mature to ripen with added ethylene, were used in the experiment. The application of ethylene to the tomatoes increased their respiratory rate by 50% within 10 hr (Fig. 1). Pink colour became evident after 50 hr of treatment, and at 194 hr the fruits were completely ripe and beginning to show signs of breakdown. Transfer RNA was extracted from a representative sample of fruits at 0, 10, 30, 70, and 194 hr after onset of the ethylene treatment.

It is known that the acylation or "charging" reactions for the various *t*RNAs have different optimal concentrations of Mg^{2+} and ATP [16,17]. Tomato $tRNA^{met}$ and $tRNA^{leu}$ charged maximally with 20 mM $Mg(OAc)_2$ and 2 mM ATP; $tRNA^{tyr}$ and $tRNA^{lys}$ charged best with 12 mM $Mg(OAc)_2$ and 1 mM ATP. The charging of tomato *t*RNA species exhibited first order kinetics with a linear rate for the first 10 min. The reactions were completed after 20 min, and there was no decrease in the amount of acylated *t*RNA with incubations of up to 60 min indicating that the *t*RNA and synthetase were free of ribonuclease [2,15,18].

Dual labelling and co-chromatography were employed for a direct comparison of the *t*RNA from fruits at each of the 4 stages of ethylene induced ripening with *t*RNA from the 0-hr control. All *t*RNAs were acylated utilizing the same synthetase from 0-hr tissue thus avoiding preferential acylation of iso-acceptor *t*RNAs by different synthetases. A series of 16 co-chromatographic separations were required to monitor ripening induced changes in each of the 4 acylated *t*RNA species examined. The results of 6 of these separations are shown in Fig. 2. To facilitate quantitative comparison, the ordinate scales are adjusted so that peak height of the first eluting iso-acceptor (peak 1 or $tRNA_1$) is the same height for each co-chromatographed sample. Quantitative shifts in each iso-acceptor *t*RNA relative to peak 1 were calculated according to Vold and Sypherd [19] (see experimental) and values thus obtained are indicated in Figs. 3 and 4.

After 10 hr of ethylene treatment the chromatographic patterns of the methionyl-*t*RNAs remained virtually coincident (Fig. 2). However, as the fruits ripened $tRNA_2^{met}$

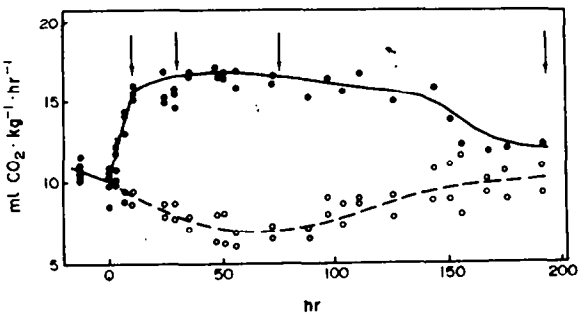

Fig. 1. Respiration of tomato fruits with (●——●) and without (○---○) continuous exposure to 500 ppm C_2H_4. Arrows indicate times when samples are gaken for the extraction of *t*RNA.

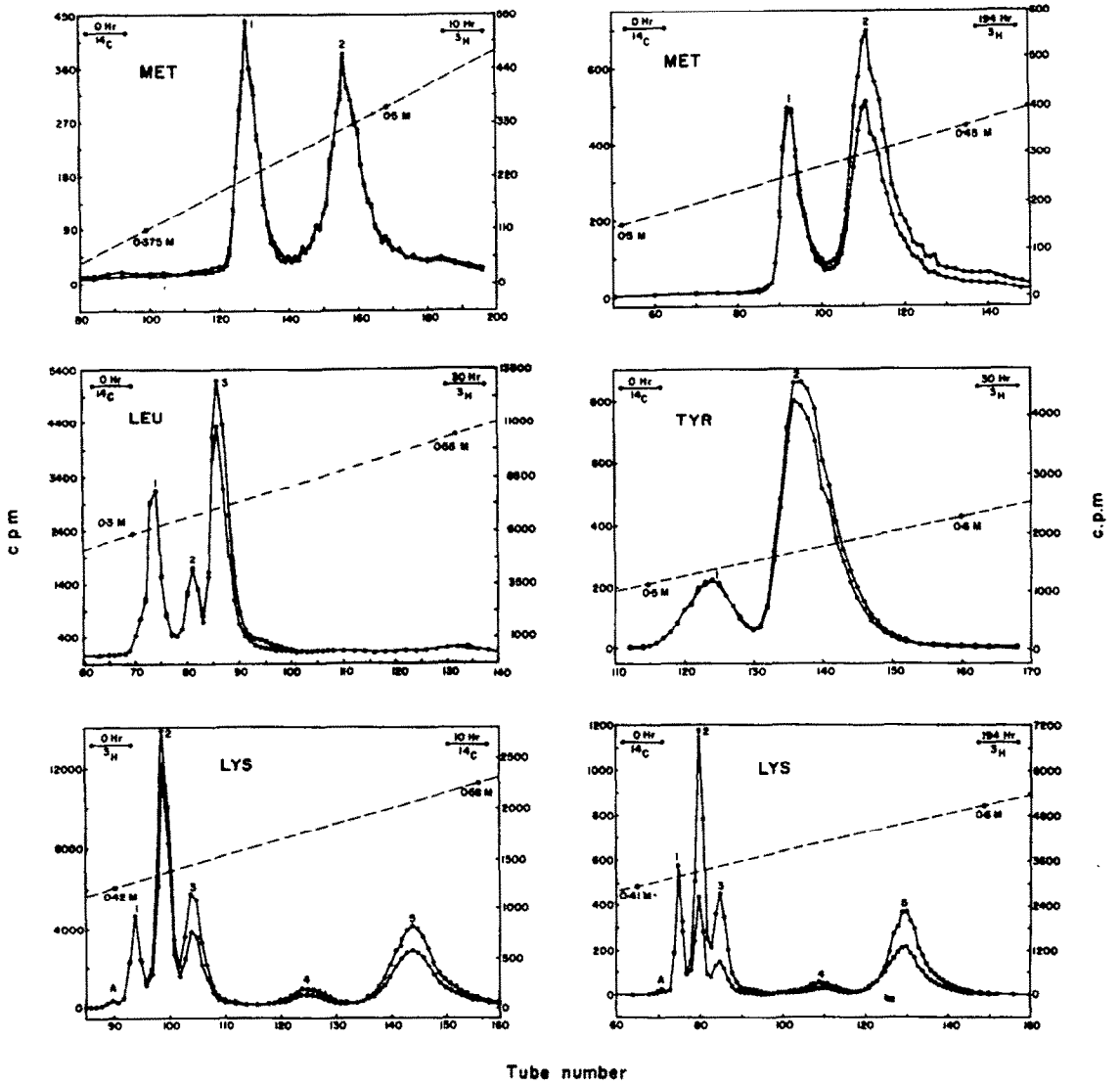

Fig. 2. Co-chromatographic profiles of tomato *t*RNA species isolated at 0 hr (●——●) and after specified number of hr (○——○) of exposure to 500 ppm C_2H_4. Shown are only those portions of each chromatographic profile in which the *t*RNA appeared. Each RPC-6 column was eluted with 500 ml of buffer (see ref. [15]) and salt gradient (— — —) as follows. 0·3 M → 0·75 M NaCl for leucyl-*t*RNA, 0·25 M → 0·7 M NaCl for methionyl-*t*RNA, and 0·25 M → 0·85 M NaCl for lysyl- and tyrosyl-*t*RNA. Flow rate was 0·2 ml/min and 2 ml fractions were collected per tube. Chromatography was at room temp.

continued to increase relative to $tRNA_1^{met}$ resulting in a 26·7% shift in peak ratios after 194 hr (Figs. 2 and 3). As in the case of $tRNA_2^{met}$, there was no apparent change in the relative amount of $tRNA_2^{leu}$ after the first 10 hr. However, there was a discernible 9·2% decrease in the third iso-acceptor, $tRNA_3^{leu}$, during the same period (Fig. 3). Thereafter, $tRNA_3^{leu}$ continued to decrease resulting in a relative loss of 24·8% at 30 hr. This was followed by a slight reversal as the tissue continued to age (Fig. 3).

Although RPC-6 chromatography revealed only three distince $tRNA^{leu}$ peaks (Fig. 2), at least two others were marginally charged. However, they were not of sufficient magnitude for meaningful evaluation. Anderson and Cherry [18] have reported that soybean hypocotyl amino-acyl synthetase will not charge late eluting $tRNA^{leu}$ species 5 and 6 whereas synthetase isolated from the cotyledons will. A similar differential charging may be operative in the synthetase preparation from green tomatoes.

Transfer RNA^{tyr} separated into two peaks (Fig. 2) which underwent only modest quantitative change with ripening, i.e. a 12 and 20% decline in $tRNA_2^{tyr}$ at 30 and 194 hr respectively. On the other hand, each of the five detected $tRNA^{lys}$ species (Fig. 2) exhibited a distinct pattern of change with ripening and progressive ageing of the tissues (Fig. 4). Lys-$tRNA_4$, discerned at low but reproducible levels, increased 25·6% in 10 hr and then declined rapidly to values of −9·1, −34·2, and −43·0% at 30, 74 and 194 hr respectively. Iso-acceptors $tRNA_{2,3,5}^{lys}$ all decreased with ethylene induced ageing, but at characteristically different rates.

The early eluting minor fraction, $tRNA_A^{lys}$, (Figs. 2 and 4) is somewhat unusual and has many of the character-

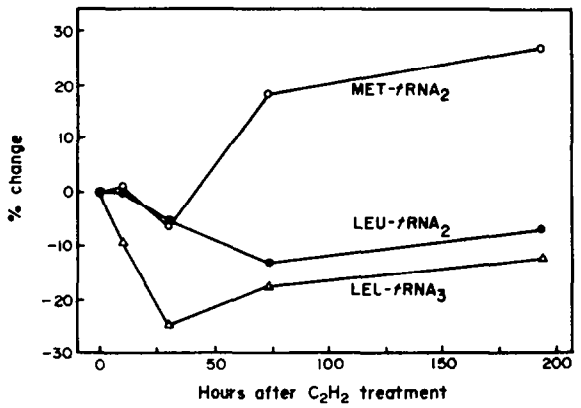

Fig. 3. Patterns of change in relative amounts of $tRNA_2^{met}$ and $tRNA_{2,3}^{leu}$ associated with C_2H_4 induced ripening of tomato fruits.

istics of an early eluting iso-acceptor $tRNA^{leu}$ species ($tRNA_L^{lys}$) discussed by Babcock and Morris [20]. They concluded that $tRNA_L^{leu}$ was formed via a highly specific cleavage of $tRNA_6^{leu}$ by a factor present in the synthetase preparation.

Although the synthesis of unique tRNA species has been postulated as a means of evoking the production of different proteins [4], it has been observed that in most cases of ageing and senescence there is simply a decline in the amount or chargeability of various iso-accepting tRNA species [6,21–24]. As exemplified by data on $tRNA^{leu}$ (Table 1) this would also appear to be the case in ageing tomato tissue. With the possible exception of $tRNA_1^{lys}$, a decline in absolute level of chargeability of the tRNA species tested accompanied the ripening of tomato fruit. Nonetheless, the individual patterns of decline of the several iso-acceptor $tRNA^{lys}$ species and the reversals in the ralative levels of $tRNA_2^{met}$, $tRNA_{2,3}^{leu}$ and $rRNA_4^{lys}$ (Figs. 3 and 4) each occurring at a characteristic time, are strong indicators of specific and true, age-related, quantitative changes in iso-acceptor tRNAs. Some of the changes could be attributed to fluctuations in chloroplastic or mitochondrial tRNA.

Recent evidence by Hobson [25] on the appearance of new isoenzymes with the onset of ripening of tomatoes implies that synthesis may be a determinative factor. Moreover, it has been observed that protein synthesis may be limited by the levels of tRNA [26]. In

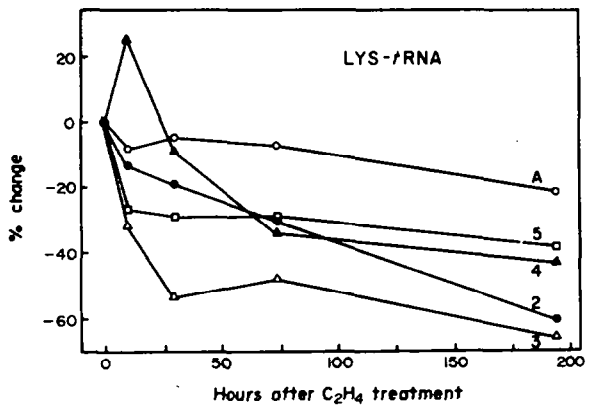

Fig. 4. Patterns of change in $tRNA_{A,2,3,4,5}^{lys}$ associated with the C_2H_4 induced ripening of tomato fruits.

Table 1. Decline in chargeability of $tRNA^{leu}$ with C_2H_4 induced ripening of tomato fruit

Hr of C_2H_4 treatment	cpm/μg tRNA
0	124
10	118
30	110
74	100
194	88

this context, the quantitative changes in tomato iso-accepting tRNAs observed in this study could well influence the translation of mRNA and the progress of ethylene induced ripening (ageing) and senescence.

EXPERIMENTAL

Respiration and ethylene treatment. Tomato fruits were placed at 20° in gallon jars and subjected to a measured flow of H_2O-saturated air. After a period of equilibriation, C_2H_4 was also metered into the jars to produce 500 ppm C_2H_4. Rates of CO_2 evolution were measured with the method of ref. [27]. At the indicated intervals a uniform sample of 6 or 7 tomatoes were chilled, and the seeds and core removed. The remaining fruit wall and septal tissues were placed in liquid N_2, crushed, and stored at −78° until used.

Extraction of tRNA and synthetases. Detailed procedures for the extraction and purification of tRNA from 100 g samples of frozen tomato tissue have been described elsewhere [15]. Acylating enzymes were extracted from tomato fruit by the method of ref. [28] modified for use with acidic plant tissues [15].

Acylation and separation of iso-acceptor tRNAs. tRNA was acylated with the designated amino acid labelled with either ^3H or ^{14}C. The acylation reaction was stopped with the addition of phOH. At this point, acylated tRNA from one of the C_2H_4 treated samples (10, 30, 70, or 194 hr) was combined with 0-hr, control tRNA newly charged with opposite label. The combined dual-labelled tRNAs were collected by alcoholic precipitation and then separated by co-chromatography on a 5 × 120 mm RPC-6 column prepared as described in ref. [29]. The procedure was repeated for each of the remaining samples of tRNA. Details regarding elution, collection and radioactive counting are given elsewhere [15]. Hewlett-Packard Model 9810A computer was programmed to normalize all elution profiles counts on peak 1. The method of ref. [19] was used to analyze the shift in relative amount of each iso-acceptor tRNA. In this procedure a peak ratio is first calculated to compare the area under each recorded iso-acceptor peak

$$\text{peak ratio} = \frac{\text{Total cpm in peak 1}}{\text{Total cpm in peak } n}$$

The difference between the ratios for 0-hr and ethylene treated amino-acyl-tRNA in each peak is then expressed as percent change:

$$\% \text{ change} = \frac{(\text{peak ratio 0-hr} - \text{peak ratio X-hr})}{\text{highest peak ratio}} \times 100.$$

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