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Polyribosomes from Pear Fruit

II. CHANGES OCCURRING IN PULP TISSUES DURING RIPENING AND SENESCENCE

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ABSTRACT

Detailed analyses of polysome profiles from lyophilized pulp tissues of pear fruits (*Pyrus communis* L. cv. Passe-Crassane) at different stages of ripening and senescence, and estimates of the amount of polysomal-associated mRNA, lead to the conclusions that during senescence (ripening), the ripening and the over-ripening processes can clearly be separated and respectively linked to the first and the second increase in the large mRNA species. Ethylene synthesis which occurs at the beginning of a normal ripening at 15°C after a cold storage or an ethephon treatment is related to an increase in mRNA and ribosomal material found only in pulp tissues. Finally, we suggest that in the pear fruit, the sequence of events which occurs during senescence (ripening) is initiated by two systems which regulate ethylene biogenesis, and that the first system is efficient only at low temperatures (from 0 to 4°C).

In earlier papers (2, 4), polysome profiles were examined from lyophilized peel tissue of ripening pear (*Pyrus communis* L. cv. Passe-Crassane), and detailed analyses of polysome distributions were used to measure the amount of polysomal-associated mRNA present in tissues. It has been shown previously (11) that the variety of pear fruit studied in our laboratory required an optimal period of 12 weeks of cold storage before normal ripening at 15°C in which the fruits would exhibit the characteristic climacteric respiratory pattern. Without this prior storage at 0°C, fruits are unable to ripen at 15°C and do not exhibit any climacteric respiration or ethylene synthesis, though an ethylene or ethephon treatment can restore a normal ripening.

In this report, we have examined the polyribosome distribution of pulp tissues of fruits ripened in different conditions and estimated the amount of available mRNA. These results have been compared with those obtained from peel tissues sampled from the same fruits in order to follow changes occurring in peel and pulp tissues during ripening and senescence.

MATERIALS AND METHODS

Fruits. Pear (*Pyrus communis* L. cv. Passe-Crassane) harvested at the beginning of November were stored either at 0°C or at 15°C. Fruits stored at 0°C for 12 weeks were subsequently transferred to 15°C for ripening, while fruits stored initially at 15°C were placed either in air or in air with ethylene (500 μl/l; continuous flow) (11). Other fruits have been treated by ethephon (2-chloroethylphosphonic acid) at 0.2% (v/v) 3 d before the harvest or immediately afterwards and then stored at 15°C.

Measurement of Respiratory Rates and Preparation of Samples. The procedure has been described previously (3). Peel and pulp tissues were frozen separately in liquid N_2, then lyophilized and stored at low air pressure (about 10 torr) in appropriate bottles for subsequent isolation of ribosomes. Under these conditions, tissues can be stored at 20°C for 3 years without noticeable change of polysome profile.

Polysome Extraction Procedure. The procedure, as described previously (3), was conducted at 0 to 4°C on lyophilized material; large polyribosomes sedimenting faster than pentamers were always absent from the fresh tissue profiles obtained with our experimental conditions.

Polysome Profiles Analysis. After density-gradient centrifugation, the contents of the tubes were analyzed. The areas in different regions of the polysomal profiles were measured and divided by the number of ribosomes in polysomes of that size class as described previously (4). Thus, the values represent the relative contents of mRNA strands in each polymeric class (1). The area of absorbance of each size class of unresolved (larger) polyribosomes must be approximated as done by Davies and Larkins (1) except that a 10-mers was used as the average size class. Furthermore, it has been shown previously that RNase activity was eliminated during extraction (3).

RESULTS

We have obtained representative profiles of the polyribosomes extracted from pulp tissues at different stages during cold storage followed by normal ripening, and storage at 15°C immediately after the harvest with or without ethylene or ethephon treatment. The surface areas under the different portions of the profiles were measured, and the amount of estimated mRNA was followed (3). These values, related to the 1979 harvest, were determined from the average of three different polysome extractions, and identical results have been obtained for three previous harvests.

Figure 1 shows the evolution of estimated amount of available mRNA of peel and pulp tissues from fruits ripened under different conditions.

Cold Storage Followed by Normal Ripening. During cold storage, identical changes occur in peel and pulp tissues (Fig. 1A). After 12 weeks of cold storage (preripening delay is usually needed before a normal ripening), ribosome and mRNA synthesis occurred. After 16 weeks of cold storage which resulted in a lower ripening ability, a smaller amount of ribosomal material and mRNA was found. Therefore, in peel (4) and pulp tissues, an optimum ripening ability seems to be closely linked to a larger amount of ribosomes and mRNA.

During normal ripening and senescence, which followed 12 weeks of cold storage, two different phases can be distinguished (Fig. 1A). Just after the transfer at 15°C, an increase in mRNA and ribosome production is found in pulp tissues while in peel tissues a constant level of mRNA production occurs concomitant with an increase in large mRNA species. This has been related to the ripening process (4). After the climacteric peak, changes in

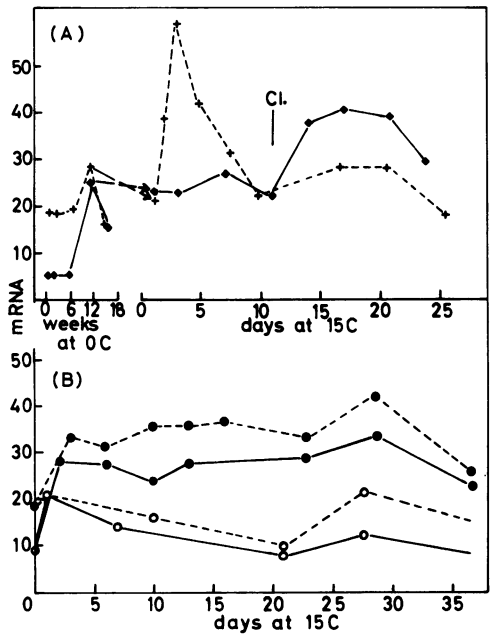

FIG. 1. Evolution of estimated amount of available mRNA of peel and pulp tissues during ripening and senescence. A, Cold storage followed by normal ripening; (◆), peel tissues; (+), pulp tissues. B, Abnormal ripening; (●), with an ethylene treatment; (○), without any ethylene treatment. Peel tissues (——); pulp tissues (----); Cl, climacteric peak; mRNA, arbitrary units.

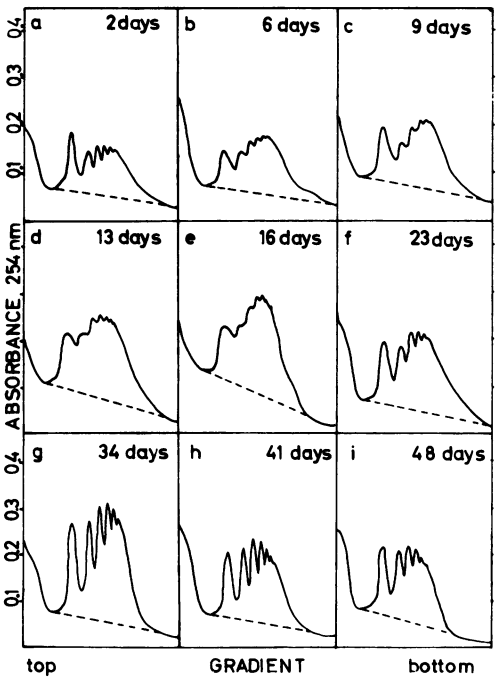

FIG. 2. Ripening after an ethephon treatment (2-chloroethylphosphonic acid at 0.2%) 3 d before the harvest. Sucrose density-gradient profiles of ribosomal populations extracted from pulp tissues of pear fruit held in air at 15°C for different number of days immediately after the harvest: *i.e.* a, 2 d; b, 6 d; c, 9 d; d, 13 d; e, 16 d; f, 23 d; g, 34 d; h, 41 d; i, 48 d.

pulp and peel tissues are similar. First, ribosome and mRNA synthesis is followed by a constant level of mRNA production concomitant with an increase in large mRNA species. This has been related to the overripening process (4). Then, a decrease in mRNA and ribosome production takes place.

This increase in ribosomes noted at the beginning of ripening is in good accordance with the increase in ribosomal synthesis found in avocado (16), figs (12), and pear fruit (9, 17) at the beginning of the climacteric. Yet, the increase in polyribosomes noted with progressive ripening of our pear fruit is contrary to these studies. These differences in results may come from varietal differences since we have used similar methods except for the extraction from lyophilized tissues.

Without Cold Storage. Pear fruit stored in air at 15°C immediately after harvest did not exhibit the usual climacteric respiratory pattern and were unable to ripen. Changes in pulp and peel tissues (Fig. 1B) are identical and characterized by the loss of the distinctive changes that occur in peel and pulp tissues during the ripening process, *i.e.* the increase in large mRNA species which occurred in peel tissues and the increase in mRNA and ribosomal material found in pulp tissues.

An ethylene treatment restored the normal ripening process and a respiratory climacteric occurred after 22 d at 15°C. Pulp and peel tissues exhibit identical changes, *i.e.* two maxima for protein synthetic capacity that are related to the ripening process and the senescence process (Fig. 1B).

The application of an ethylene-releasing substance, 2-chloroethylphosphonic acid, 3 d before harvest or immediately afterwards, had identical effects on fruits ripening. Fruit ripened normally and exhibited the respiratory climacteric after 30 d. Figure 2 shows representative profiles of the polyribosomes extracted from pulp tissues at different stages of ripening following an ethephon treatment 3 d before harvest. Identical results have been obtained with fruits treated with ethephon immediately after harvest. The amount of available mRNA estimated from these profiles is identical to the one obtained in Figure 1A when fruits are transferred to 15°C after 12 weeks of cold storage.

DISCUSSION

Once picked, pear fruits are unable to ripen at 15°C and to synthesize ethylene (10). An ethylene treatment restores the ripening process, but it does not cause an increase in mRNA, ribosomal material, or ethylene synthesis (10); similar results were obtained with banana (18). Moreover, an ethephon treatment restores the ripening process, the ethylene synthesis (10), and an increase in mRNA and ribosomal material (Fig. 2). Consequently, ethylene synthesis may be closely linked with this increase in mRNA and ribosomal material. Similar conclusions have also been elaborated for the ripening of apples (5) and the aging of disks of apple peel (6). We found it more convenient to use ethephon to release small amounts of ethylene at the cellular level, although low concentrations of gaseous ethylene would probably have had the same effect. We assumed that a single application of ethephon (0.2%) should not cause artifacts.

Leblond (10) has shown that the ethylene synthesis is initiated very quickly at 0°C but remains at a low level until transferred to 15°C. Ethephon is well known for its ability to release ethylene by degradation in plant tissues (19, 20). These results suggest that ethylene at a low level is required at the beginning of storage at 15°C to obtain a high level of auto-stimulated synthesis of ethylene; an increase in the level of endogenous ethylene is considered the immediate trigger of ripening in climacteric fruit (14). McMurchie *et al.* (12) proposed two systems for the regulation of ethylene biogenesis; in line with this model, we propose that, for our fruits, the sequence of events is as follows (Fig. 3).

During cold storage, ethylene biogenesis should be initiated by some factor (system 1) that is inactive at 15°C but active at 0°C and is involved in regulation of aging. At 0°C, ethylene remains at a low level (10) and should trigger ripening after 12 weeks of cold storage (ribosomes and mRNA synthesis) (4). When transferred to 15°C, fruits are able to ripen normally, and the ethylene

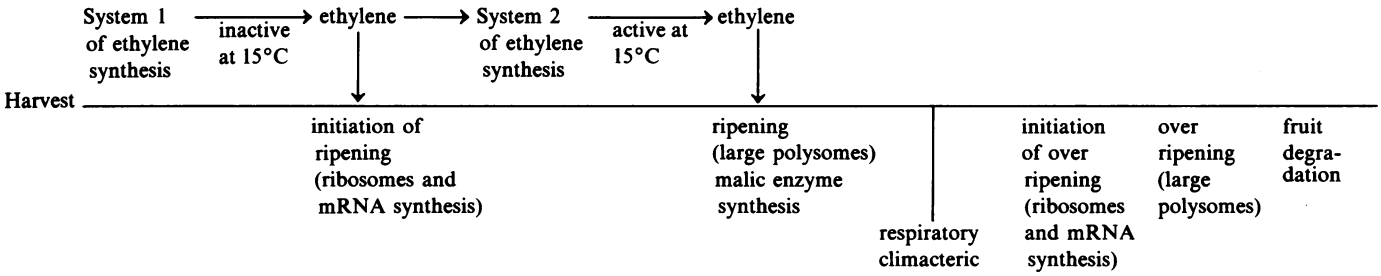

FIG. 3. Sequence of events which occur during the senescence (ripening) of the pear Passe-Crassane.

present in the fruit should trigger system 2 for ethylene synthesis at 15°C; apparently, an excess of ethylene is required for the full integration of the various changes associated with natural ripening (15). Natural ripening, associated previously with the presence of large polysomes (4) and malic enzyme synthesis (7, 8), evolves and leads to the respiratory climacteric.

Once picked, fruits are unable to ripen at 15°C because system 1 can not produce ethylene. Ethylene, added to pear fruits, should circumvent system 1 and trigger system 2 and ripening; however, system 2 is suppressed by the continued presence of a high concentration of ethylene or propylene (13). Ethylene released by an ethephon treatment in fruit tissues (18, 19) should circumvent system 1 and initiate system 2 and ripening. These results suggest that, for our fruits, the chain of ethylene biosynthesis reactions starts only at 0°C. Once the presence of ethylene is established (in endogenous or exogenous form) at a sufficient level, ripening is initiated. For normal ripening of climacteric fruits, they need a period of time that varies according to temperature (10) and an increased ethylene production (system 2) that is triggered by ethylene. On the other hand, it has been suggested (13) that the critical difference between climacteric and nonclimacteric fruits rests in their relative ability to produce ethylene in response to low concentrations of ethylene. Consequently, because nonclimacteric fruits lack system 2 entirely, the synthesis by system 2 of ribosomes and mRNA linked to ethylene production must be absent in the ripening of nonclimacteric fruits.

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