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CONTENT OF RNA AND PROTEIN OF THE RIPENING BANANA

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Abstract—A possible net movement of nitrogen between peel and pulp tissues of ripening bananas was evaluated. No evidence of a movement of nitrogen from the peel to the pulp was found, nor with either tissue did the proportion of the total nitrogen extracted by 5%(w/v) trichloracetic acid change significantly during the climacteric period. Neither the content of RNA in the pulp tissue, nor the base composition of the RNA changed during the climacteric. It is concluded that no substantial increase in the amount of ribosomal material accompanies the increase in the rate of protein synthesis early in the climacteric.

INTRODUCTION

IN APPLE fruit, the rate at which proteins are formed,[1] and the amount of protein in the fruit tissue[2] increases during the period of the respiration climacteric. During this time, there is evidence of a substantial increase in the RNA content,[3] suggesting that the content of ribosomes increases during the period when enhanced protein synthesis is measured. In pear fruits, there is evidence that the enhanced rate of protein synthesis as ripening commences is directed to the synthesis of enzymic proteins whose activities increase during ripening.[4] There is evidence, too, of an increased rate of synthesis of ribosomal RNA during the early climacteric in pears.[5]

In the pulp tissue of banana fruits, the portion of the total nitrogen present as protein[6] and protein content per unit fresh weight[7] does not change significantly during the climacteric. These results suggest that there is no increase in the protein content of the pulp cells during ripening, but there is the possibility that they are influenced by movements of nitrogen, dry matter or water from the peel to pulp tissues. For this reason we have examined the distribution of nitrogen between peel and pulp as well as between protein and non-protein components in ripening banana fruits. We have also measured the content and base distribution of RNA in the pulp of ripening bananas to determine whether there are changes in RNA as the rate of protein synthesis changes during ripening.[6]

RESULTS

Protein

When individual banana fruits are ripened in a stream of moist air, the nitrogen content of the system must remain constant unless there is gain or loss from or to the atmosphere.

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Discounting gaseous exchange, the possibility of net movements of nitrogen between the peel and pulp was investigated by measuring the total nitrogen content of both peel and pulp at three stages of ripening. The distribution of nitrogen between the tissues was similar in pre-climacteric (day 0), peak climacteric (day 3) and ripe (day 6) fruits (Table 1).

TABLE 1. DISTRIBUTION OF NITROGEN AND DRY MATTER BETWEEN PEEL AND PULP

Time of ethylene application (days)	% of nitrogen in		% of dry matter in	
	Peel	Pulp	Peel	Pulp
0	28·5	71·6	22·4	77·6
3	29·0	71·0	22·4	77·6
6	27·9	72·2	21·3	78·7

L.S.D. 5%: Nitrogen 1·3%; dry matter 0·3%.
Figures are the mean of three harvests, at each of which four fruits were analysed at each stage of ripeness.

Since there is no net change in the nitrogen content of peel and pulp tissues during ripening, changes in the protein content may be measured in terms of the distribution of nitrogen between protein and non-protein fractions in each tissue. Such a measure will be independent of the changes in fresh weight and dry weight (Table 1) which occur during ripening.

Despite the almost complete loss of chlorophyll by the peel in 7 days of exposure to ethylene, there is no significant loss of protein (Table 2). Nor is there any significant decrease in the proportion of nitrogen extracted by 5% trichloracetic acid (TCA) from pulp tissue as the climacteric developed (Table 3).

TABLE 2. DISTRIBUTION OF NITROGEN WITHIN PEEL TISSUE

Time of ethylene application(days)	Chlorophyll (mg/g fresh wt.)	Nitrogen (mg/g fresh wt.)		TCA-soluble N
		TCA-soluble	Total	Total N
0	0·116 ± 0·002	0·46 ± 0·03	1·63 ± 0·10	0·28 ± 0·02
7	0·007 ± 0·000	0·51 ± 0·05	1·58 ± 0·08	0·32 ± 0·05

At each time, five fruits all from the same hand were analysed. Figures are means with standard errors.

TABLE 3. DISTRIBUTION OF NITROGEN WITHIN PULP TISSUE

Time of ethylene application (days)	Whole fruit respiration (mg CO_2 kg^{-1} hr^{-1})	Nitrogen (mg/g fresh wt.)		TCA-soluble N
		TCA-soluble	Total	Total N
0	44·29 ± 2·26	1·33 ± 0·08	2·68 ± 0·09	0·50 ± 0·05
2	135·72 ± 6·52	1·22 ± 0·11	2·68 ± 0·22	0·45 ± 0·08
3	151·38 ± 8·30	1·21 ± 0·04	2·65 ± 0·07	0·45 ± 0·03

Pulp from four fruits was analysed on each day. The results are means with standard errors.

RNA

When ^{3}H-uracil (0·3 μc) was added to the homogenate of pulp tissue in methanol reagent, 95·5 $\pm$ 2·3% of the radioactivity was recovered in the combined methanol, TCA and ethanol extracts, and there was no radioactivity in the 0·3 M KOH fraction. *E. coli* ^{3}H-RNA added to the initial homogenate contributed no radioactivity to the methanol, TCA or ethanol extracts, and 101·6 $\pm$ 1·0% of the radioactivity was recovered in the 0·3 M KOH soluble fraction. Four acidic UV absorbing zones were resolved by ion exchange chromatography of the acid-soluble fraction of alkali extracts of pulp tissue. By comparison with published elution curves, calibration of the column with nucleotide standards, and co-chromatography with nucleotide standards, these peaks were identified as racemic mixtures of cytidylic, adenylic, guanylic and uridylic acids. TLC and UV spectroscopy confirmed the identifications. When 0·5 μmole of each of the nucleotide standards was added to the tissue suspensions prior to alkaline hydrolysis, recoveries were in the range 97·3–104·8%.

In banana fruits induced to ripen by exposure to ethylene, the RNA content of the pulp was found to be 0·29 $\pm$ 0·02 mg/g fresh wt. and remained constant during the 3 days over which the climacteric developed (Table 4). The base composition of the RNA in these samples did not change significantly during the climacteric (Table 5).

TABLE 4. RNA CONTENT OF PULP TISSUE OF BANANAS SAMPLED THROUGH THE CLIMACTERIC

Time of ethylene application (hr)	Whole fruit respiration (mg CO_2 kg^{-1} hr^{-1})	RNA (mg/g fresh wt.)
0	19·2 $\pm$ 1·4	0·29 $\pm$ 0·02
16	28·6 $\pm$ 0·9	0·31 $\pm$ 0·02
24	55·1 $\pm$ 1·2	0·30 $\pm$ 0·02
48	125·9 $\pm$ 2·4	0·29 $\pm$ 0·00
72	131·4 $\pm$ 2·1	0·27 $\pm$ 0·03

Figures are means with standard errors. A sample of three fruits was analysed at each time.

TABLE 5. BASE COMPOSITION OF RNA FROM THE PULP OF BANANAS

Time of ethylene application (hr)	Base composition (molar %)			
	Cytosine	Adenine	Guanine	Uracil
0	18·8 $\pm$ 1·9	21·2 $\pm$ 0·8	42·0 $\pm$ 3·1	18·1 $\pm$ 2·0
16	19·6 $\pm$ 1·3	21·1 $\pm$ 0·5	40·2 $\pm$ 0·8	19·2 $\pm$ 1·7
24	19·9 $\pm$ 0·7	22·2 $\pm$ 0·9	38·3 $\pm$ 1·1	19·5 $\pm$ 2·1
48	19·0 $\pm$ 2·8	21·1 $\pm$ 1·4	41·8 $\pm$ 0·9	18·2 $\pm$ 1·1
72	18·5 $\pm$ 1·0	23·9 $\pm$ 0·2	40·8 $\pm$ 0·4	16·8 $\pm$ 0·4

Figures are means with standard errors. A sample of three fruits was analysed at each time.

DISCUSSION

The conclusion that the protein content of the pulp of the banana remains constant over the ripening period has rested upon comparisons made on a fresh weight, dry weight or

nitrogen basis. An appreciable net movement of water from peel to pulp occurs during ripening,[8] while dry matter changes due to fixation, respiration and translocation can be anticipated. A movement of newly-fixed carbon from peel to pulp can be demonstrated,[9] and a changed distribution of dry matter between peel and pulp occurs in the latter stages of ripening (Table 1). Neither fresh weight nor dry weight then are constant bases on which to make comparisons.

The nitrogen content of individual fruits remains constant after harvest, and net changes in the distribution between peel and pulp are not greater than about 1% of the nitrogen in the system (Table 1). Comparisons on a nitrogen basis are valid within this limit, and confirm the conclusion[6,7,10] that there is no change in the protein content of banana fruit pulp tissue during ripening.

On a fresh weight basis, the RNA content of the pulp tissue did not change during the respiratory rise (Table 4). There was a tendency for the RNA content to fall at the end of the climacteric, though this may reflect water movement rather than loss of RNA. Analysis of the base composition provided no evidence that RNA components were changed markedly during the climacteric.

While a decided increase in the content of protein and RNA occurs during the ripening of apples,[3] and the protein content of tomatoes increases during ripening,[11] such changes are not a general feature of ripening fruits.[12] An increase in the rate of synthesis of RNA and protein during the early stages of ripening may proceed without a net increase in the content of these macromolecules, or may even accompany a rapid net decrease of RNA and protein as has been reported in barley leaves.[13] An increase in the RNA content will probably only occur if the ribosomal system is expanded to support the protein synthesis associated with ripening. No such increase in ribosomal components occurs during ripening of the pulp of banana fruits. This may mean that any initiation of protein synthesis when ripening is induced involves a small portion of the protein complement of the tissue.

EXPERIMENTAL

Fruit. The source of fruit, and the methods of applying ethylene and measuring respiration have been published.[6]

Nitrogen distribution. Bananas from one hand were used in each experiment. Green bananas were sampled at the time ripening was induced by exposure to 10 ppm ethylene. Bananas were separated into peel and pulp, and the whole of both peel and pulp samples was homogenized in 5% (w/v) Na_2SO_3. Samples were taken from the homogenates for the estimation of total and non-protein nitrogen.[6] Non-protein nitrogen was measured as that extracted by 5% (w/v) TCA, and the efficiency of TCA extraction in the separation of non-protein and protein nitrogen was confirmed by molecular sieve chromatography of both extract and residue in phenol–HOAc–H_2O.[14]

Estimation of RNA in pulp tissue. The method of Smillie and Krotkov[15] was modified for use with banana pulp. Extractions were with ice-cold reagents and centrifuging was at 39,000 *g* for 20 min. The initial extraction of finely sliced tissue was by grinding in a mortar 1 part of tissue with 2·8 parts of MeOH containing 0·19% (v/v) HCOOH, 0·008% (w/v) Na 2-mercaptobenzothiazole and 0·1% (w/v) ascorbic acid. The residue was extracted with 10% (w/v) TCA containing 0·1% (w/v) ascorbic acid (3×), with 95% (v/v) EtOH saturated with NaOAc (1×) and with 95% (v/v) EtOH (2×). The residue was suspended in 0·3 M KOH and incubated at 37 ± 1° for 16 hr. The alkali-soluble fraction was cooled and adjusted to pH 1–2 with

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cold 72% (w/v) $HClO_4$. After 30 min, insoluble material was removed by centrifuging and washed twice with 1% (w/v) $HClO_4$. The bulked supernatant fractions gave a negative biuret reaction for protein and negative Dische reaction for DNA.[16]

This acid soluble fraction was adjusted to pH 7 with KOH and concentrated *in vacuo*. The volume was adjusted to 5·0 ml with 0·10 M formate buffer, pH 3·0, and the solution was filtered. A 2·0-ml sample of the filtrate was applied to a 10 × 0·6-cm column of anion exchange resin (Dowex 1 X-8, 100–200 mesh analytical grade, from Bio-Rad Laboratories, Richmond, California, U.S.A.). The column was successively eluted with 0·10 M formate buffer, pH 3·0, 1·00 M, and 3·26 M HCOOH to yield 45 × 10 ml fractions. The UV absorbance of each fraction was measured. Cytidylic acid was estimated from the absorbance at 280 nm using the molar absorptivity cited by Markham.[16] Adenylic, guanylic and uridylic acids were estimated from the absorbance at 260 nm, using the molar absorptivities given by Beaven *et al.*[17]

The purity of nucleotide standards, from Calbiochem, Los Angeles, California, U.S.A., was ascertained by UV spectroscopy, ion exchange chromatography and chromatography on thin layers of cellulose in *n*-BuOH–acetone–HOAc–5% NH_4OH–H_2O (4·5 : 1·5 : 1 : 1 : 2, by vol.) and in saturated $(NH_4)_2SO_4$–1 M NaOAc–*iso*PrOH (80 : 18 : 2, by vol.),[18] 5-^{3}H-Uracil was obtained from the Radiochemical Centre, Amersham, U.K. *E. coli* ^{3}H-RNA was a gift from Dr. N. S. Scott, and was freed of acid-soluble radioactivity by precipitation and washing on a membrane filter with cold 5% (w/v) TCA. Radioactivity was measured by liquid scintillation spectrometry in the "Mix I" scintillation fluid described by Patterson and Greene.[19]

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Key Word Index—Musa sp.; banana; ribonucleic acid; protein; climacteric changes.