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Sugar Regulation of Plastid Interconversions in Epicarp of Citrus Fruit[1]

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ABSTRACT

Seasonal transformations between chloroplasts and chromoplasts, as measured by changes in chlorophyll content, in the epicarp of degreening and regreening *Citrus sinensis* (L.) Osbeck cv Valencia fruit closely parallelled the accumulation and later loss of soluble sugars. At any stage of development, reversing the relative soluble sugar content in the epicarp by culturing pericarp segments on agar media with low (15 millimolar) or high (150 millimolar) sucrose concentrations reversed the direction of change in chlorophyll content. Fruit of *C. madurensis* Lour., which mature year around and do not regreen, also accumulated soluble sugars in the pericarp as degreening was initiated.

The epicarp of *C. sinensis* fruit accumulated nitrogen, but total nitrogen concentrations and amino acid concentrations changed little, during degreening and regreening of *C. sinensis* fruit. Cessation of nitrogen fertilization reduced the tendency of pericarp segments to regreen *in vitro* during subsequent years, but regreening tendency was restored by inclusion of KNO_3 in the media.

It is concluded that chloroplasts become chromoplasts and citrus fruit degreen partially in response to the accumulation of sugars in the epicarp and that the reverse transformation accompanying regreening of certain citrus species occurs when accumulated sugars disappear. Change in nitrogen flux to the fruit is probably not a factor in regulating seasonal transformations, but an abundance of nitrogen in the epicarp diminishes the effects of high sugar concentrations in inducing transformation of chloroplasts to chromoplasts, thereby retarding degreening and promoting regreening.

Certain citrus fruit degreen in the winter and regreen during the ensuing spring and summer. This degreening and regreening reflects reversible transformations between chloroplasts and chromoplasts (19) and is, therefore, useful for studying factors controlling plastid metamorphosis. Degreening of most citrus fruit is induced by cool, winter air and soil temperatures (22) and regreening of late season fruit, most notably Valencia oranges (*Citrus sinensis* [L.] Osbeck), occurs in response to warm spring and summer temperatures (2). However, fruit of species such as *C. madurensis* Lour., which bloom often and have fruit maturing several times each year, do not require cool temperatures to induce degreening. Since plastid form is probably more a function of cell and tissue type than environment (12) and since cool temperatures are not obligatory for inducing degreening in all citrus, it is likely that environmental factors such as temperature act indirectly by influencing cellular metabolism.

Cool temperatures have been reported to reduce markedly nitrogen uptake (3, 20) and translocation (20) by citrus trees, and the pericarp of *C. paradisi* Macf. (grapefruit) cease to accumulate nitrogen in the winter (11). The pericarp of *C. paradisi* Macf. fruit also accumulate reducing sugars in response to cool temperatures, and those sugars largely disappear in response to warm spring temperatures (17). Nitrogen metabolism is further implicated by observations that degreening is delayed and regreening promoted by excessive nitrogen fertilization (6, 10, 11).

In a previous paper (9) it was demonstrated that transformation between chloroplasts and chromoplasts in citrus epicarp could be controlled *in vitro* by manipulating sugar and nitrogen supplies in agar media upon which pericarp segments were cultured. High concentrations of sucrose in media usually promoted degreening and inhibited regreening, whereas nitrogen as nitrate and certain amino acids acted in opposition to sucrose by inhibiting degreening and promoting regreening. In the presence of increasing nitrogen concentrations, the sucrose effect was much reduced and, when nitrogen supplies were sufficiently high, high concentrations of sucrose sometimes promoted regreening.

In addition to promoting loss of Chl, high sugar concentrations in media increased epicarp sugar concentrations, and in media lacking nitrogen high concentrations of sugars caused a marked reduction in epicarp amino acid concentrations. In those *in vitro* experiments there was a significant negative correlation between Chl and the molar ratio of sugars to amino acids in the epicarp.

Based on these observations, it is hypothesized that most citrus fruit degreen in response to a reduced flux in nitrogen to the fruit accompanied by increased concentrations of sugars in the epicarp, both usually induced by cool temperatures. Regreening of late season citrus fruit in the spring and summer could be attributed to renewed nitrogen flux and a reduction in sugar concentrations. Degreening of species such as *C. madurensis* Lour., could also be attributed to elevated sugar concentrations and reduced nitrogen content, but cool temperatures would not be the causative factor.

To further examine the relationship between plastid transformations and sugar and nitrogen status, Chl, sucrose, reducing sugars, total nitrogen, and amino acids were monitored in the epicarp of *C. sinensis* (L.), Osbeck cv Valencia and pericarp of *C. madurensis* Lour. fruit degreening *in situ*, and in the epicarp of regreening *C. sinensis* fruit. Also, pericarp sections from *C. sinensis* fruit at different stages of development were tested for their response to sugars and nitrate *in vitro*. The results, reported here, suggest that accumulation of sugars in the epicarp is indeed a major factor regulating plastid metamorphosis in citrus fruit, and that while the abundance of nitrogen is an influential factor, nitrogen flux is not a major factor in seasonal changes in plastid form.

MATERIALS AND METHODS

One fruit from each of twenty trees of *Citrus sinensis* (L.) Osbeck, cv Valencia, growing at the University of Arizona Experimental Citrus Farm at Tempe, were periodically collected

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and prepared for analysis on the following day. The 20 fruit were divided into 4 replicas of 5 fruit, and each fruit divided into a proximal quarter (stem end), middle half, and distal quarter (styler end). A 1-cm diameter segment was removed from the pericarp in the equatorial region of the middle section of each fruit and Chl determinations made *in situ* on 20 segments using a reflectance spectrophotometer as previously described (9). Since the fruit were still enlarging, especially in October, there was a decrease in absorption by dilution due to expansion of the epicarp rather than actual loss of Chl. Absorptions were, therefore, normalized to a constant surface area so that changes in absorption represented actual changes in the amount of Chl in the fruit epicarp. Pericarp segments, which included the colored epicarp and some of the white mesocarp, were then placed upon 1.5% (w/v) agar media under continuous fluorescent light to degreen or regreen *in vitro* (9).

The epicarp of segments initially weighed 0.136 ± 0.007 g. After 14 d on media the epicarp weighed 0.197 ± 0.004 g. The basal medium (B) was as previously described (9) and contained macro and micro elements, vitamins, the fungicide benomyl (methyl 1-[butylcarbamoyl]-2-benzimidazole-carbamate) and 150 mM Mes (pH 5.5), but neither nitrogen nor sugars. The remaining epicarp from each section of the five fruit in each replica was collected and combined to give for further analysis four replicate samples of each section.

Samples of 5 g each were homogenized in 80% (v/v) ethanol with a Brinkmann Polytron for analysis of free sugars and amino acids, and the remainder of each sample dried for analysis of total nitrogen. Alcoholic extracts were centrifuged 20 min at 10,000*g* and 0°C. The residues were rinsed three times with additional 80% ethanol and the combined supernatants made aqueous by evaporation to a small volume and diluting to 100 ml with deionized H_2O. Reducing sugars were estimated by Cu (II) reduction (13) and nonreducing sugars estimated as the increase in reducing sugars after treating a 1-ml aliquot with 0.86 IU of invertase for 2 h at room temperature. Amino acids were determined by the method of Yemm and Cocking (21). Total nitrogen in dried samples were determined by micro-Kjeldahl analysis.

C. madurensis fruit growing on trees in Tucson were tagged and 10 fruit periodically collected, the pericarp removed, rinsed thoroughly with deionized H_2O and finely chopped with a razor blade. Two-g samples were extracted with 80% acetone in dim light, and Chl determined according to the method of Arnon (1). Samples of 1 g each were extracted with 80% (v/v) ethanol and aliquots analyzed for sugars and amino acids as described above.

RESULTS

Chl and Sugars in Epicarp of Degreening and Regreening *C. sinensis* Fruit. The equatorial region of *C. sinensis* fruit accumulated Chl until the first week of November, at which time there commenced a gradual loss of this pigment so that the epicarp were nearly devoid of Chl from February through April (Fig. 1A). By May, the fruit were again accumulating Chl and had regained about 30% of their maximum values by July. Loss of Chl was somewhat irregular over the fruit surface with a slightly greater retention of Chl in the proximal quarter. In contrast, regreening was strongly directed with the proximal end accumulating the greatest amounts of Chl.

Concentrations of total soluble sugars in the middle half epicarp tended to be low when equatorial Chl was high (Fig. 1A) and highest when Chl was lowest. Linear regression of Chl on total sugars in the middle half epicarp was negative and significant ($r = -0.92$, $P < 0.01$). During the last stages of Chl accumulation prior to degreening, the epicarps were also accumulating sugars (Fig. 1A). On October 1 there was a strong gradient in total sugars with highest concentrations in the distal

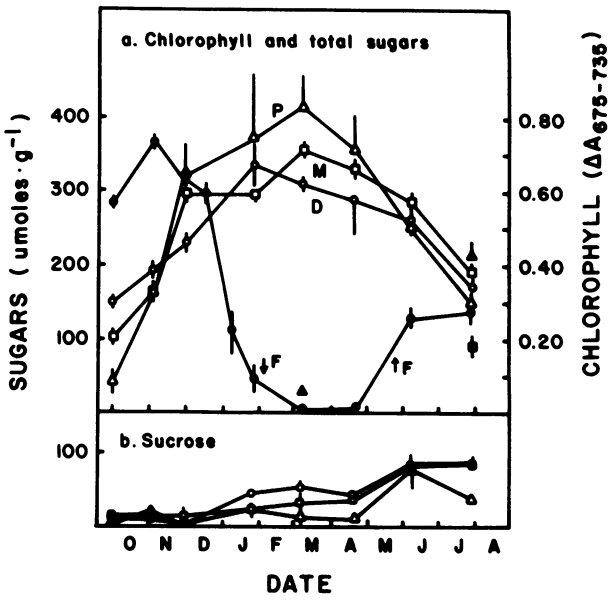

FIG. 1. Chl and sugars in epicarp of *C. sinensis* fruit. Chl was measured *in situ* in segments removed from the equator (●), and occasionally the proximal quarter (▲) and distal quarter (■) of 20 fruit. Sugar determinations were made on each of four pooled epicarp samples from the proximal quarter (△, P), middle half (□, M), and distal quarter (○, D) of 5 fruit. Each data point represents the mean ± SE of the four samples. Trees were fertilized in February and June (arrows, F) with 0.55 kg ammonium in irrigation water.

quarter of the epicarp and lowest concentrations in the proximal quarter. Subsequently, sugars increased prior to degreening and until most Chl had been lost and began to reaccumulate. The rate of sugar accumulation was highest in the proximal epicarp and lowest in the distal epicarp so that by December 19 there was a reversal in the sugar concentration gradient, with the proximal epicarp having the highest concentrations from December through April. Sugars in the proximal epicarp increased 10-fold from 40 μmol/g fresh weight to 400 μmol/g fresh weight. When the epicarp began to reaccumulate Chl and regreen, they also began to lose sugars most rapidly from the proximal quarter, that portion of the fruit most intensely regreening.

The greatest fluctuation in sugars during degreening and regreening of *C. sinensis* fruit was in reducing sugars. At the beginning of the study period, sucrose concentrations ranged from 0 to 13 μmol/g fresh weight (Fig. 1B). There was then a somewhat steady accumulation of sucrose so that by July 27 sucrose concentrations ranged from 39 to 87 μmol/g fresh weight and made their greatest contribution to the total sugars, about 25 to 50%. Reducing sugars in the epicarp increased by 116 to 364 μmol/g fresh weight during degreening and decreased by 173 to 294 μmol/g fresh weight during regreening. A maximum of only 38% of the loss in reducing sugars (distal quarter) can be accounted for by condensation to sucrose.

Regreening and Degreening of Pericarp Segments on Agar Media Lacking Nitrogen. Although there was a close parallel between loss or gain in Chl and sugar concentrations, the importance of sugars in affecting epicarp Chl levels is more apparent by considering the response of pericarp segments removed from the fruit and cultured on media lacking nitrogen (B medium) and with low (15 mM) and high (150 and 300 mM) concentrations of sucrose. Results are presented in Figure 2 of experiments in which pericarp segments were removed from the equatorial portion of fruit at various stages of degreening and regreening and kept 14 d under continuous fluorescent light on medium B with only 15 mM sucrose, conditions usually resulting in a decline in endogenous sugar concentrations (Tables I and II).

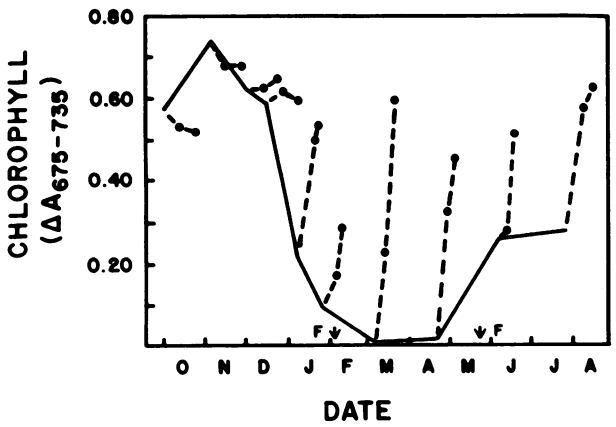

FIG. 2. Chl changes in epicarp of cultured pericarp segments of *C. sinensis* fruit sampled at different dates. Pericarp segments, removed from the equators of fruit and used to estimate Chl in epicarp of fruit on the tree (●——;● and Fig. 1), were placed on B media with 15 mM sucrose and no nitrogen, and Chl determined in 10-segment subsets at two subsequent dates (- - -). Each data point represents the mean of 10 segments. Trees were fertilized in February and June (↓) with 0.55 kg ammonium in irrigation water.

Segments taken from the equator of fruit still accumulating Chl tended to lose a small amount of Chl in culture (Fig. 2). After degreening had commenced, the epicarp of equatorial segments placed on low sugar media without nitrogen promptly regreened although only to a slight degree when Chl loss had only just begun. Segments from fruit harvested at any time after they began the most intense period of degreening, and during regreening, regained much or all the lost Chl when placed on B media with only 15 mM sucrose.

In contrast to their behavior on low sugar media, epicarp of segments from fruit kept 14 d on medium B with 150 mM sucrose and 300 mM sucrose gained sugars and either degreened (Table I) or regreened only slightly (Table II).

When pericarp segments were kept on agar media without a source of nitrogen, the amino acid content of the epicarp decreased (Tables I and II). In addition to increasing endogenous sugar concentrations, the loss of amino acids was increased by higher sucrose concentrations in the media. Inclusion of potassium nitrate in the media reduced the endogenous sugar content of the epicarp and countered the sucrose effects on both Chl and amino acids (Table II). In similar experiments using various nitrogen sources (9), Chl correlated well with the ratio of endogenous sugars to amino acids. Similarly, Chl during the *in vitro* degreening experiments reported here, while negatively correlating with sugars ($r = 0.78$, $P < 0.05$), correlates best with the molar ratio of endogenous sugars to amino acids ($r = 0.92$, $P < 0.01$).

Total Nitrogen and Amino Acids in Epicarp of Degreening and Regreening *C. sinensis* Fruit. While sugar concentrations increased and decreased inversely with Chl in epicarp of *C. sinensis* fruit, amino acid concentrations remained relatively constant at about 30 μmol/g fresh weight (Fig. 3). Total nitrogen concentration dropped significantly during October and November, especially in the proximal quarter, but deviated little from about 320 μmol/g fresh weight after that time. Much of the initial decrease in total nitrogen concentration was due to epicarp enlargement and when total nitrogen concentration was converted to total nitrogen per fruit epicarp, it was seen that the epicarp continued to accumulate nitrogen until March (Fig. 3).

When nitrogen was withheld from the trees beginning with a last application of 0.55 kg anhydrous ammonia in June 1981, pericarp segments began to lose their tendency to regreen on media lacking nitrogen (Fig. 4). The tendency to regreen was restored by inclusion of 60 mM KNO_3 in the media. Endogenous nitrogen components were not determined in all experiments contributing to results presented in Figure 4; however, typical epicarp concentrations in 1981 were 300 ± 10 μmol total N/g fresh weight and 33 ± 2 μmol amino acid/g fresh weight, and in 1983, 169 ± 8 μmol total N/g fresh weight and 9.6 ± 0.7 μmol amino acid/g fresh weight.

Comparison of Degreening Pericarp Segments in Light and Dark. On media with 15 mM sucrose and lacking nitrogen, pericarp segments lost Chl in the dark at a mean daily rate of −18 ± 3 units (Table III). This rate of loss was markedly reduced to almost zero by light. Nitrogen, supplied in this experiment as proline, the most abundant amino acid in maturing *C. sinensis* fruit (4), virtually prevented the loss of Chl in the dark, and resulted in a significant net gain in Chl in the light. The use of proline avoided the possibility of light effects on nitrogen reduction confounding results; however, similar results were obtained when nitrogen was supplied with KNO_3. Increasing the sucrose concentration to 300 mM nearly doubled the rate of Chl loss in

Table I. *In Vitro Degreening of C. sinensis Epicarp and Changes in Chl, Sugars, and Amino Acids*

Segments of pericarp from *C. sinensis* fruit were kept 14 d under continuous fluorescent light on agar media (B) with 15, 150, and 300 mM sucrose. Each Chl value represents the mean ± SE of 10 segments. Each sugar and amino acid value represents the mean ± SE of three epicarp samples from three or four segments each. Fruit were collected November 17. Similar results were obtained in repeated experiments.

Sucrose in Media	Chl	Reducing Sugars	Sucrose	Amino Acids	Total Sugars/ Amino Acids
mM	$A_{675-735}$	*μmol/g fresh wt*	*μmol/g fresh wt*	*μmol/g fresh wt*	*mol/mol*
Initial	0.61 ± 0.01	319 ± 7	0 ± 7	22 ± 1	15 ± 1
After 14 Days on Media					
15	0.55 ± 0.02	269 ± 18	0 ± 18	15 ± 2	18 ± 3
150	0.49 ± 0.03	357 ± 30	71 ± 23	8.4 ± 0.4	51 ± 5
300	0.12 ± 0.05	320 ± 19	63 ± 19	6.2 ± 0.1	61 ± 2
After 28 Days on Media					
15	0.56 ± 0.01	170 ± 11	0 ± 11	11.6 ± 0.3	15 ± 1
150	0.27 ± 0.04	390 ± 23	130 ± 31	7.7 ± 0.6	68 ± 6
300 (21 d)	0.05 ± 0.01	365 ± 36	150 ± 31	6.1 ± 0.3	84 ± 6

Table II. *In Vitro Regreening of C. sinensis Epicarp and Changes in Chl, Sugars, and Amino Acids*

Segments of pericarp from *C. sinensis* fruit were kept 14 d under continuous fluorescent light on agar media (B) with 15 and 150 mM sucrose. Each Chl value represents the mean ± SE of 10 segments. Each sugar and amino acid value represents the mean ± SE of three epicarp samples from three or four segments each. Fruit were collected February 22. Similar results were obtained in repeated experiments.

Sucrose in Media	Chl	Reducing Sugars	Sucrose	Amino Acids	Total Sugars/ Amino Acids
mM	$A_{675-735}$	*μmol/g fresh wt*	*μmol/g fresh wt*	*μmol/g fresh wt*	*mol/mol*
Initial	0.018 ± 0.003	324 ± 4	63 ± 4	21.1 ± 1.6	18 ± 1
After 14 Days on Media					
15	0.22 ± 0.04	20 ± 4	21 ± 2	6.1 ± 0.2	7 ± 1
150	0.032 ± 0.004	76 ± 6	130 ± 4	5.1 ± 0.3	42 ± 2
150 + 6 mM KNO_3	0.23 ± 0.02	69 ± 4	103 ± 9	13.7 ± 0.2	12.4 ± 0.4
150 + 60 mM KNO_3	0.65 ± 0.01	46 ± 3	53 ± 3	45.2 ± 5	2.2 ± 0 1

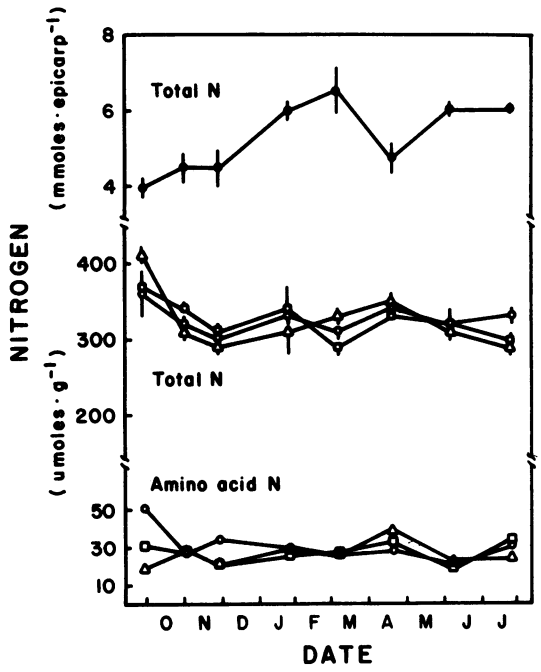

FIG. 3. Nitrogen and amino acid content of *C. sinensis* epicarp. Epicarp from the proximal quarter (△) middle half (□) and distal quarter (○) of 20 fruit were collected into four samples of 5 fruit each. A subset of each sample was analyzed for amino acids and the remainder dried for total nitrogen analysis. Total nitrogen per epicarp (●) was calculated as one-fifth the product of total nitrogen per g and total weight of epicarp from 5 fruit. Each value represents the mean ± SE of four samples.

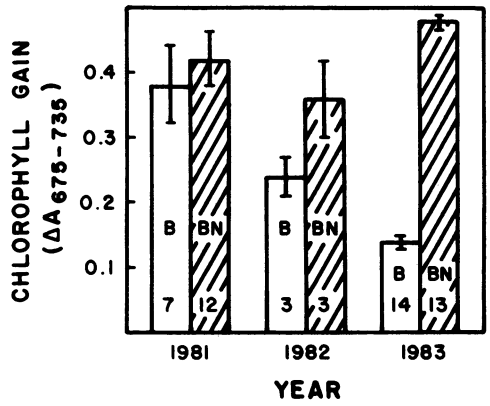

FIG. 4. *In vitro* regreening of *C. sinensis* epicarp following cessation of nitrogen fertilization. Trees were fertilized each year in February and June with 0.55 kg ammonium in irrigation water until June 1981. Pericarp segments from degreened fruit were cultured on B media with 15 mM sucrose (no nitrogen) and B media with 15 mM sucrose and 60 mM KNO_3 (BN), and the increase in Chl after 14 d under continuous fluorescent light measured. Data from several experiments during each season were combined. The number of experiments is indicated in each bar and the SE indicated by the line at top of each bar. Typical concentrations of total N and amino acids were 300 ± 10 μmol/g fresh weight and 33 ± 2 μmol/g fresh weight respectively in 1981 and 169 ± 8 μmol/g fresh weight and 9.6 ± 0.7 μmol/g fresh weight, respectively in 1983.

the dark, and light did not significantly effect this high rate of Chl loss. Thus, on media with 15 mM sucrose there was a net increase in Chl in the light compared with dark that was not significantly affected by nitrogen, but was completely eliminated by increasing the sucrose concentration in the media.

Accumulation of Sugars during Degreening of *C. madurensis* Fruit. Small, immature fruit of *C. madurensis* had pericarp with 307 ± 17 μmol Chl/g fresh weight, relatively high sugar concentrations of 177 μmol/g fresh weight, and amino acid concentrations of 15.6 μmol/g fresh weight for a molar sugar to amino acid ratio of 11 ± 2 (Table IV). As the fruit enlarged, they continued to accumulate Chl while sugar and amino acid concentrations dropped slightly. However, the molar ratio of sugars to amino acids stayed about the same at 9 ± 1. As the fruit began to lose Chl, there was a marked increase in sugar concentration while amino acid concentrations did not change significantly.

Table III. *Rate of Change in Chl in C. sinensis Pericarp Segments Kept in Light and Darkness*

Segments of pericarp from fruit harvested December 6 were cultured 14 d in darkness or under continuous fluorescent light, and on B media with 15 and 300 mM sucrose, and 15 mM sucrose with 30 mM proline. Chl was determined periodically in subsets and the rate of change calculated as the slope of the regression line. Values are reported ± SE.

Addendum to Medium B	Rate of Chl Change		
	Dark	Light	Light-Dark
	$(\Delta A_{675-735} d^{-1}) \times 10^3$		
15 mM sucrose	−18 ± 3	−1 ± 4	16 ± 5
15 mM sucrose, 30 mM proline	−3 ± 3	20 ± 2	22 ± 3
300 mM sucrose	−30 ± 4	−35 ± 3	−4 ± 5

Table IV. *Sugars, Amino Acid, and Chl Concentrations in Pericarp of C. madurensis Lour. Fruit before and during Degreening*

Each value represents the mean ± SE of three samples from the combined pericarp of 10 fruit.

Time	Chl	Reducing Sugars	Sucrose	Amino Acids	Total Sugars/ Amino Acids
d	*μmol/g fresh wt*	*μmol/g fresh wt*	*μmol/g fresh wt*	*μmol/g fresh wt*	*mol/mol*
—[a]	307 ± 17	177 ± 31	0 ± 16	15.6 ± 0.3	11 ± 2
0	340 ± 1	78 ± 5	26 ± 7	11.3 ± 0.7	9 ± 1
20	213 ± 3	179 ± 6	42 ± 3	11.5 ± 0.4	19 ± 1
28	117 ± 3	154 ± 1	30 ± 1	9.4 ± 0.4	20 ± 1
35	99 ± 2	178 ± 2	53 ± 1	11.4 ± 0.5	20 ± 1
49	19 ± 2	212 ± 2	41 ± 2	12.9 ± 0.9	20 ± 1
50	15 ± 1	185 ± 2	31 ± 2	10.0 ± 0.2	22 ± 1
55	0.4 ± 0.1	338 ± 14	0 ± 7	10.0 ± 0.5	34 ± 2

[a] Immature fruit, much smaller than in subsequent samples of mature fruit.

The ratio of sugars to amino acids doubled. Niether sugars nor amino acids exhibited much further change and their molar ratio remained at about 20 until 96% of the Chl had been lost. At that time, sugar concentrations again increased so that the sugar to amino acid ratio increased to 34 ± 2 when less than 0.1% of the Chl remained.

DISCUSSION

Ultrastructural evidence (19) indicates that regreening of *Citrus sinensis* fruit epicarp involves reversion of chromoplasts to chloroplasts so that degreening and regreening of *C. sinensis* fruit represents a reversible interconversion between these two plastid forms.

Epicarp of mature *C. sinensis* fruit responded to removal from a source of sugars by failing to degreen or, if already degreened, by regreening. Replacement of the sugar supply by culturing pericarp segments on media with 150 mM sucrose promoted degreening and inhibited regreening. Previous studies using mannitol demonstrated that the effects of sucrose on degreening and regreening were not due to low osmotic potentials (9). These results taken with the observed parallel between Chl loss and sugar accumulation in the epicarp of both *C. sinensis* fruit and *C. madurensis* fruit, and between the reaccumulation of Chl and sugar loss in the epicarp of *C. sinsensis* fruit indicates that some components of carbohydrate metabolism participate in the regulation of the interconversions between chloroplasts and chromoplasts in citrus fruit.

Since Chl synthesis does not occur in the dark, differences in the rate of Chl loss in the dark are ascribed to differences in the rate of degradation. Assuming that the rate of Chl degradation is either unaffected by light or increased somewhat through photooxidation, differences in the rates of change in Chl in the light and dark are an estimate of the minimum rate of Chl synthesis. Using these assumptions, the results given in Table III indicate that nitrogen inhibited Chl degradation, but had little effect on Chl synthesis, whereas sucrose both inhibited Chl synthesis and promoted Chl degradation.

Sucrose then seems to have general effects promoting chloroplast to chromoplast transformations while nitrogen appears to have a more protective role stabilizing chloroplast forms, but not necessarily promoting chloroplast formation from chromoplasts.

The specific components of carbohydrate metabolism that might regulate these interconversions have not been identified, but it seems unlikely that glucose, fructose, or sucrose would be directly involved as these chemicals do not communicate directly across the plastid outer membranes (12). Triose phosphates and 3-P-glycerate, which do communicate easily between the plastid and cytoplasm, are possible regulators of plastid morphology, but Horrum and Schwartzbach (8) suggest glyoxylate as the actual repressor in the analogous carbon repression of *Euglena* chloroplast development. Whatever the specific factors that regulate these plastid transformations might be, they are clearly related to the abundance of soluble sugars.

Accumulation of sugars in the epicarp of citrus fruit when mean weekly temperatures dropped to 10°C or less has been previously noted in *C. paradisi* Macf. (17). In *C. sinensis* epicarp the proximal quarters exhibited the greatest change in sugars both during accumulation and subsequent loss, whereas the distal quarters exhibited the least change, suggesting that translocation from and to the tree was responsible for the observed changes. At temperatures as low as 5°C, photosynthesis in *C. sinensis* leaves can be as much as 50% their maximum photosynthetic rate (16), yet growth of *C. sinensis* trees nearly ceases at temperatures below 13°C (7, 15). Thus, during the months of October through January there is virtually no growth of *C. sinensis* trees while the fruit do continue to enlarge (5). The fruit then would be a principle sink for photosynthate still being produced.

In February and March new shoots appear (5) and midbloom at Tempe, Arizona usually occurs during the first 2 weeks of April (18). Thus, from February onward, renewed vegetative and reproductive growth strongly competes with the mature fruit for available photosynthate. Whether sugars are actually translocated out of the epicarp of mature fruit at this time, as suggested by the more rapid decline in sugars from the regions closest to the tree, is not known, and I am unaware of any data demonstrating that translocation of carbohydrate from citrus fruit to the tree ever takes place. The decline in sugars possibly results from a decrease in translocation to the fruit and continued metabolism of the sugars already present in the epicarp.

C. madurensis fruit mature at several times during each year so that the development of chloroplasts into chromoplasts in the pericarp of these fruit is not associated with cool temperatures as it is in *C. sinensis* fruit. Nevertheless, the degreening of *C. madurensis* fruit is closely associated with the accumulation of sugars in the pericarp. However, for *C. madurensis* this increase in sugars appears to be simply a gradual accumulation with time, not necessarily related to temperature.

In addition to components of sugar metabolism, components of nitrogen metabolism also affect interconversions between the two plastid forms. This is evident by the ability to overcome the influence of high sugar concentrations by inclusion of various nitrogenous substances in media, and by the gradual decline in regreening tendency with decreasing tree fertility that can be restored by inclusion of nitrate in the media.

As with sugars, the specific component of nitrogen metabolism responsible for affecting plastid interconversion is not identified. *In vitro* experiments indicated a strong correlation between Chl content and the molar ratio of sugars to amino acids. On the other hand, Chl was more closely correlated with just sugars in the *in situ* observations. However, since the amino acid concentrations, on a fresh weight basis, were nearly constant for *C. sinensis* and *C. madurensis*, there was still a high degree of correlation between the ratio of sugar to amino acids. In fact, dividing the data presented here into those samples losing Chl and those samples not losing Chl, and analyzing the populations by the maximally selected Chi square method of Miller and Siegmund (14), suggests that a sugar to amino acid ratio of 11 or 12 is a critical value ($\chi^2_{max} = 12.2$; $P < 0.05$) above which the epicarp chloroplasts develop into chromoplasts and below which the chromoplasts will revert to chloroplasts.

However, it is probable that the relationship between plastid transformations and total amino acids is coincidental and that the total amino acid concentrations simply reflect the general

state of nitrogen metabolism as well as the abundance of specific nitrogenous effecters of plastid development such as specific amino acids, polyamines or even Chl-binding proteins (12).

While the nitrogen status of the fruit epicarp clearly influences the tendency of the fruit to regreen, changes in the nitrogen flux to the fruit is not a part of the seasonal pattern of degreening and regreening of *C. sinensis* fruit because that flux does not cease in the winter and resume in the spring, but rather continues unabated through the winter when the fruit degreen and slows or ceases in the spring when the fruit are regreening.

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