Patterns of pigment changes in apple fruits during adaptation to high sunlight and sunscald development

Mark N. Merzlyak *, Alexei E. Solovchenko, Olga B. Chivkunova

Department of Cell Physiology & Immunology, Faculty of Biology, Moscow State University, 119899 GSP Moscow W-234, Russia

Received 18 November 2001; accepted 31 January 2002

Abstract

Reflectance spectra of four apple (Malus domestica Borkh.) cultivars were studied and chlorophyll, carotenoid, anthocyanin and flavonoid content in sunlit and shaded peel was determined. In all cases sunlit peel accumulated high amounts of phenolics (flavonoid glycosides). Adaptation to strong sunlight of an apple cultivar with limited potential for anthocyanin biosynthesis (Antonovka) was accompanied by a decrease in chlorophyll and a significant increase in total carotenoid content. The increase in carotenoids also took place in sunlit sides of the Zhigulevskoye fruits, accumulating high amounts of anthocyanins, but chlorophyll content in sunlit peel was higher than that in shaded peel. Significant increases in carotenoids and anthocyanins were detected during fruit ripening when chlorophyll content fell below 1.5–1.8 nmol cm⁻². Chlorophyll in sunlit fruit surfaces of both cultivars was considerably more resistant to photobleaching than in shaded (especially of Zhigulevskoye) sides. Induced by sun irradiation, the photoadaptive responses were cultivar-dependent and expressed at different stages of fruit ripening even after storage in darkness. The development of sunscald symptoms in susceptible apple cultivars (Granny Smith and Renet Simirenko) led to a dramatic loss of chlorophylls and carotenoids, which was similar to that observed during artificial photobleaching. The results suggest that apple fruits exhibit a genetically determined strategy of adaptation of their photoprotective pigments to cope with mediated by reactive oxygen species photodynamic activity of chlorophyll under strong solar irradiation. This includes induction of synthesis and accumulation of flavonoids, anthocyanins and carotenoids that could be expressed, if necessary, at different stages of fruit development © 2002 Éditions scientifiques et médicales Elsevier SAS. All rights reserved.

Keywords: Adaptation; Apples; Anthocyanins; Carotenoids; Chlorophylls; Flavonoids; Photodamage

1. Introduction

Under certain conditions solar radiation induces mediated by production of Chl excited states and reactive oxygen species photodamages to plants [2,5,7,11,12,19], particularly sunscald that considerably reduces fruit quality and life in storage [1,13]. Plant responses to strong light include remarkable changes in pigment content and composition suggesting that these events are involved in their adaptive and/or protective mechanisms.

Apples represent an interesting natural system for studies of light-induced effects on pigments and elucidation of the mechanisms of photoadaptation, photoprotection in, as well as photodamage to, plants since only one side of a fruit is mainly exposed to strong sun radiation which allows paired comparison. It has been reported that sunlit surfaces of apple fruit contain much higher quantities of Flv and other phenolics than those in the dark [3,14]. UV–B is known to induce build up of Flv and Anth (mainly, cyanidin 3-galactoside) [3,4,10,14,15]. In many apple cultivars strong radiation in the visible range also induces the synthesis of the latter pigments responsible for the red coloration of fruit [15]. Recently, using reflectance spectroscopy we examined sunlit and shaded sides of freshly collected fruits of two apple cultivars, commonly found in production orchards in Russia, and found a great difference in the sensitivity of their Chl to photo-induced bleaching that has been attributed to a massive accumulation of Anth in Zhigulevskoye apple. Fruit peel pigment content estimated from the spectral measurements indicated that a response to strong sunlight of Antonovka apples (which do not accumulate Anth), involved an increase in Car and a decrease in Chl content [10].
In the progress of these studies it was essential to establish the extent of Flv, Chl, Car and Anth changes quantitatively and simultaneously and to reveal the patterns of pigment adaptation at different stages of fruit development. This has been done by comparing optical properties of pigment content in sunlit and shaded surfaces of fruits and by using Chl as a marker of fruit ripeness in both apple cultivars. In addition, two apple cultivars (Granny Smith and Renet Simirenko) susceptible to and in the progress of sunscald development have been included in the investigation. The quantitative pigment and in situ spectral analyses demonstrate a strong sunlight effect on apple peel pigment content and composition, and revealed common responses and also different adaptation strategies in individual apple cultivars. Pigment changes characteristic of the injury caused by excessive solar irradiation in apple (sunscald) have been also established.

2. Results

2.1. Reflectance spectra

In Fig. 1 average reflectance spectra of shaded and sunlit sides of apple fruits as well as the difference ‘sunlit–shaded’ \( f(R) \) spectra are presented. The common effect observed in all cases on sunlit-fruit surface compared with shaded-fruit surfaces was a strong decrease of reflectance \( (R \) as low as 5\%) and, an apparent maximum in the difference \( f(R) \) spectrum near 400–420 nm. The spectra of Antonovka and Zhigulevskoe fruits (Fig. 1A, B) suggest that sun radiation induces significant cultivar-dependent changes in Chl, Car and Anth content throughout all stages of their ripeness.

Sunlit surfaces of Zhigulevskoe apples possessed lower reflectance in the orange–red range and a positive peak in \( f(R) \) difference spectrum near 670–680 nm indicating an accumulation of Chl on sunlit compared with shaded surface. Unlike Zhigulevskoe, sunlit sides of Antonovka possessed higher reflectance and a negative peak in the difference \( f(R) \) spectrum in the red suggesting lower Chl content on the sunlit-fruit side. Much more pronounced an increase of reflectance in 650–700 nm range occurred in sunscald-affected surfaces of Granny Smith and Renet Simirenko fruits. The changes observed in this case reflect a considerable Chl decrease occurring on sun-exposed fruit sides.

Low reflectance in the green region of the spectrum and the band near 540–550 nm in the difference \( f(R) \) spectrum was due to Anth and characteristic of sunlit (but not shaded) surfaces Zhigulevskoey apples (see also [10]). Several marked changes were found in the blue part of the spectrum. Despite an increased reflectance in the red, sunlit sides of Antonovka fruit possessed lower reflectance in the blue region relative to shaded sides. Peaks near 450 and 480 nm in the difference \( f(R) \) spectrum corresponded to Car absorption maxima thus suggesting their accumulation in sunlit peel. The spectra of Zhigulevskoye apples possessed (on the background of Anth absorption) a spectral feature near 480 nm, also attributable to Car (Fig. 1). Reflectance of sunscald-affected Granny Smith and Renet Simirenko fruits was higher than that of undamaged fruit and the difference \( f(R) \) spectrum was consistent with a massive degradation of Chl.

2.2. Pigment content

2.2.1. Flavonoids

Comparison of absorbance spectra of chloroform and water–methanol phases of apple peel extracts indicated that Flv rutin together with Chl and Car contribute to fruit reflectance near 400–420 nm and at shorter wavelengths (not shown). A strong decrease of reflectance in this range occurring in peel of sunlit sides of fruits (Fig. 1) is in agreement with the extensive accumulation of Flv observed in fruits at all stages of their ripening (Fig. 2). Although an increase in Flv content in some Antonovka and Zhigu-levskoye apples was recorded after storage for 4–5 months in the dark, nevertheless, their content in sun-exposed sides of the fruits was much higher (five-fold increase, on an average). A similar extent of Flv accumulation was also found in the majority of Granny Smith and Renet Simirenko apples in the progress of sunscald development.
2.2.2. Anthocyanins

The analytical data showed that only Zhigulevskoye possessed high Anth content: 34 ± 0.7 nmol cm$^{-2}$ (mean ± standard error) in sunlit peel, whereas other cultivars possessed low (< 3 nmol cm$^{-2}$, Antonovka) or did not accumulate Anth pigments. As a rule, in Zhigulevskoye apples a significant difference in Anth content between sunlit and shaded surfaces was found (Fig. 3). The accumulation of Anth in the range of 10–20 nmol cm$^{-2}$ was observed in sunlit surfaces of freshly collected Zhigulevskoye apples with Chl content ranging from 2.5 to 4.5 nmol cm$^{-2}$. Much higher Anth quantities (35–50 nmol cm$^{-2}$) were recorded in stored fruits when Chl content dropped below 1.5–1.6 nmol cm$^{-2}$; at this stage some shaded fruits also displayed an increase in Anth content.

2.2.3. Chlorophyll and carotenoids

A significant difference in total and relative Chl and Car content and between sunlit and shaded surfaces was found in the apple fruit cultivars studied (Fig. 4). Average Chl content in Antonovka sunlit peel was lower compared with shaded peel (1.59 ± 0.15 vs. 2.03 ± 0.18 nmol cm$^{-2}$); Zhigulevskoye fruit contained more Chl on sunlit than on shaded sides (2.21 ± 0.21 vs. 1.62 ± 0.16 nmol cm$^{-2}$). Unlike these two cultivars, a dramatic decrease of Chl content was observed in sunscald-affected peel of Granny Smith and Renet Simirenko (up to 3.85 ± 0.76 and 1.96 ± 0.54 nmol cm$^{-2}$, respectively) in comparison with undamaged fruit zones where Chl content was as high as 8.49 ± 0.55 and 6.98 ± 0.19 nmol cm$^{-2}$, respectively (Fig. 4). On an average, sunlit peel of Antonovka and Zhigulevskoye peel possessed higher Car content in comparison with shaded peel: 1.42 ± 0.07 vs. 1.16 ± 0.07 and 2.07 ± 0.10 vs. 1.42 ± 0.07 nmol cm$^{-2}$, respectively. However, no Chl decline was detected in Zhigulevskoye sunlit peel. Zhigulevskoye apple contained more Car in shaded peel than Antonovka (Fig. 4A, B).
Sunlit peel of Antonovka fruits possessed lower Chl content and a higher Car content (see trends in Fig. 4A, B) and relative Car/Chl ratio compared with shaded sides (Fig. 4D). In sunlit peel of Zhigulevskoye apples an increase in both Chl and Car content took place (Fig. 4). Due to a decrease in Chl content, sunlit peel of Antonovka fruits possessed higher Car/Chl ratios than shaded peel (0.75 ± 0.03 vs. 0.47 ± 0.01). Despite an increase in Chl content, a Car/Chl ratio in sunlit peel of Zhigulevskoye was also higher compared with shaded peel (1.18 ± 0.09 vs. 1.07 ± 0.09), but a difference was considerably lower than in the case of Antonovka (10% vs. 60%, Fig. 4D).

In shaded surfaces of both Antonovka and Zhigulevskoye fruits, Car content followed a decrease in Chl; relative rates of both pigments’ disappearance were close and the Car/Chl ratio was not changed significantly until Chl content reached ca. 1.5–1.8 nmol cm⁻². At lower Chl content in Antonovka fruits a sharp increase in Car content suggesting induction of Car synthesis in the progress of fruit ripening was observed. This increase in Car over Chl was not observed much in Zhigulevskoye apples. Additional amounts of Car accumulated in sunlit peel of both cultivars were nearly constant during fruit development (see the trends in Fig. 4) even after prolonged storage in darkness (Fig. 4A, B).

In synchrony with Chl, a sharp decline in Car content was observed in sunscald-affected sides of Granny Smith and Renet Simirenko fruits. In undamaged zones of fruits the pigment content was high and the relationships between peel Car and Chl were similar to those of shaded sides of Antonovka and Zhigulevskoye (Fig. 4C, D).

### 2.3. Light-induced chlorophyll photobleaching

To estimate the sensitivity of fruits to strong light as related to the changes in pigment content and composition the bleaching experiments were carried out and the rates of Chl destruction were determined in situ with reflectance measurements (see also [10,11]) in Antonovka and Zhigulevskoye apples. Fig. 5 demonstrates that Chl in sunlit surfaces of both fruit cultivars exhibited higher resistance to light-induced bleaching as compared to shaded surfaces. In Antonovka apples half time of Chl destruction was increased by 60–70%. In accordance with our previous observations [10] Chl in sunlit surfaces of Zhigulevskoye apples accumulating Flv significantly under light-induced bleaching as compared to shaded surfaces. In Antonovka apples half time of Chl destruction was increased by 60–70%. In accordance with our previous observations [10] Chl in sunlit surfaces of Zhigulevskoye apples accumulating Flv significantly under light-induced bleaching as compared to shaded surfaces.

### 3. Discussion

The analysis of optical properties and pigment content in this and our previous [10] works show several distinct effects of strong sunlight on apple fruit and individual fruit cultivars, related to the changes pigment content and composition.
ment in susceptible Granny Smith and Renet Simirenko apples.

Both spectral analysis (features near 450 and 480 nm in Fig. 1A, B) and pigment quantitative data (Fig. 4) indicated that together with Flv, lower reflectance of sunlit Antonovka and Zhigulevskoye apple surfaces in the blue is related to an increase in Car content. As could be seen from Fig. 4, in both cultivars, the changes in peel Chl and Car content on sunlit and shaded fruit surfaces proceeded synchronously, thus Car/Chl ratios were not significantly changed during fruit ripening until Chl content reached 1.5–1.8 nmol cm$^{-2}$. Additional amounts of Car, accumulated in sunlit peel of Antonovka and Zhigulevskoye in response to high sunlight, were nearly constant at different stages of fruit developments (Fig. 4A, B) and remained so in Antonovka apple even after prolonged storage in darkness when induction of Car synthesis was observed.

Accumulating Cars could play an important role in protecting Chl from photooxidation due to their abilities to quench excited states and singlet oxygen, intercept oxygen and organic free radicals and dissipate energy of excessive light quanta via violaxanthin cycle [5,19]. Accordingly, Chl in sunlit surfaces of Antonovka fruit was found to be less sensitive to light-induced bleaching (Fig. 5). In addition, it could be noted that in yellow Antonovka apple extremely small light-induced Car degradation was observed (Solovchenko and Merzlyak, unpublished) that suggests high light stability of Car in the absence or in the presence of minute quantities of Chl.

The build up of Anth in sunlit peel of Zhigulevskoye apple (Fig. 3) resulted in a considerable increase of absorptions (Fig. 4A, B) and pigment quantitative data (Fig. 4) indicated strongly that together with increases in Flv and Car content) provides an efficient photoprotection of photosynthetic apparatus in Zhigulevskoye apples. It could also be mentioned that higher Chl content in sunlit compared with shaded peel is to maintain sufficient level of photosynthesis, when lower portions of photosynthetically active radiation reach chloroplasts as a result of a strong Anth screening effect.

Significantly, the data show that as a response to strong sun light the synthesis of all other main plant pigments (i.e. Flv, Anth and Car) able to exert photoprotective (screening) effects at particular wavebands of solar spectrum is induced to reduce and/or to prevent photodynamic activity of Chl. Furthermore, Fig. 4 demonstrates the accumulation of Car in sunlit peel in Antonovka and Car and Anth and Zhigulevskoye apples resembles the progress of fruit ripening but it occurs in fruits with much higher Chl content. The data also argue that a significant increase in both Car and Anth takes place when Chl content decreases below 1.5–1.8 nmol cm$^{-2}$. In Antonovka apples, at terminating stages of ripening, Car content was close to that in green fruits (Fig. 4). Much higher quantities of Anth have been recorded in sunlit peel of Zhigulevskoye apples during storage than in fruits with higher Chl level (Fig. 3). This indirectly suggests the existence of (light-)sensitive stages during Chl breakdown and dismounting of photosynthetic apparatus in the course of fruit ripening/leaf senescence [11,12]. It seems that the cultivar-dependent synthesis of photoprotective Car and Anth in apples is genetically programmed and it could be expressed, if necessary, under certain environmental conditions.

4. Methods

4.1. Plant material

The fruits of four apple (Malus domestica Borkh.) cultivars, Antonovka obykovenennaya, Zhigulevskoye, Granny Smith, and Renet Simirenko were used. Both freshly collected and stored at +4 °C up to 4–5 months Antonovka and Zhigulevskoye fruits were from the Botanical Garden of Moscow State University and All-Russian Institute of Horticulture (Michurinsk, Tambov region, Russia). Granny Smith and Renet Simirenko were delivered from Krasnodar region of Russia and studied immediately or within 3–4 weeks after harvesting. Healthy (Antonovka and Zhigulevskoye) and affected by sunscald, but without visual symptoms of browning (Granny Smith and Renet Simirenko), fruits were selected. Among fruit cultivars involved in the study Zhigulevskoye and Antonovka (the latter, to a significantly lesser degree) apples exhibited red coloration on their sunlit surfaces.

4.2. Pigment analysis

Pigment analysis allowing to quantify Chl, Car, Flv and Anth in an extract was carried simultaneously as follows (for more details, see [16]). Peel disks (16 mm in diameter and ca. 1 mm thickness) were homogenized in chloroform–methanol (2/1, v/v) in the presence of MgO in amounts sufficient to prevent Chl pheophytinization and to avoid Ant absorption. After completion of extraction homogenates were filtered through a paper filter and distilled water (1/5 of total extract volume) was added to them. Then the extracts were centrifuged at 3000 × g for 10 min until phase separation. Chl and Cars concentrations were quantified spectrophotometrically in lower (chloroform) phase using coefficients of Wellburn [18]. The upper (water–methanol) phase was used for assay of Flv and Anth. In consistence with the data in the literature [6,14], spectrophotometric analysis, acid hydrolysis and TLC chromatography on 5565 Merck cellulose plates with n-butanol/acetatic acid/water (4/1/5, v/v/v.) as a developing system [9] showed that chlorogenic acid and rutin (or other quercetin glycosides) are dominant phenolics both in sunlit and shaded apple and that quercetin glycosides mainly contribute to absorption of
water–methanol phase of extracts between 300 and 420 nm. Therefore, Flv content was expressed in equivalent quantities of rutin using the absorption coefficient of 25.4 mM$^{-1}$cm$^{-1}$ at 358 nm determined for authentic rutin (Chemapol, CSSR) in methanol or 5% water–methanol. Anth were quantified in water–methanol fraction of an extract after addition of concentrated HCl (final concentration of 0.1%); absorption coefficient of 30 mM$^{-1}$cm$^{-1}$ at 530 nm [17] was accepted. Pigment contents were expressed on fruit surface area basis.

4.3. Spectral measurements

Whole fruits reflectance spectra in 400–800 nm range were recorded with a 150–20 Hitachi spectrophotometer equipped with an integrating sphere against barium sulfate as a standard. Spectral data were interfaced to IBM-compatible personal computer and processed using spreadsheet software. Reflectance were converted to \( (1 - R)^2 \) values formally analogous to Kubelka-Munk remission function [10,11].

4.4. Bleaching experiments

The fruits were irradiated through a 0.4 cm UV-cut off BS-8 filter and a 5 cm layer of water as a heat filter from a slide projector with a KGM 150W/24 tungsten-halogen lamp (NPO Svetotekhnika, Saransk, Russia) as a light source at an irradiance of 2500 W m$^{-2}$; during illumination the samples were blown off with a stream of air to prevent their heating [10,11]. The rate of Chl photodestruction was estimated as time required for the decrease of \( f(R) \) at 678 nm by 50%.

References