Research Article

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Ultraweak photon emission in strawberry fruit during ripening and aging is related to energy level

https://doi.org/10.1515/biol-2017-0046
Received June 23, 2017; accepted September 5, 2017

Abstract: *Background:* Ultra-weak photon emission (UPE), or biophoton emission, is a phenomenon observed in various living organisms, including plants. In this study, we analyzed the UPE from ripening strawberry fruits, to elucidate its source and association with cellular energy. *Methods:* Freshly harvested and stored strawberry fruits were measured for levels of UPE and energy molecules adenosine triphosphate (ATP), adenosine monophosphate (AMP) and adenosine diphosphate (ADP). The associations between them were calculated. *Results:* In ripening fruit, a decrease in UPE positively correlated with declining levels of ATP, AMP, and energy charge. In harvested fruits, levels of UPE, ATP, and energy charge declined, but ADP and AMP increased. *Conclusion:* Changes in UPE levels synchronized with changes in ATP and energy charge, which reflect cellular energy levels. Thus, cellular energy may be related to UPE, and may be an energy source for UPE.

Keywords: aging; energy; delayed luminescence; fruit development; ultra-weak luminescence

1 Introduction

Spontaneous ultra-weak photon emission (UPE; also known as biophoton emission at wavelength of 180-800 nm), in the order of 10-16 W/m^2, has been detected in various living systems, including microbial, plant, and animal species [1-3]. UPE is putatively associated with oxidative metabolism, intra- and inter-cellular communication, photosynthesis, growth, cell division and apoptosis, and death [4-6]. The development of highly sensitive detectors has enabled new investigation of UPE in the life and medical sciences, and in agriculture [3,7]. For example, two-dimensional images of UPE can be captured using a highly sensitive charge-coupled device camera. This is an excellent non-invasive method of investigation for the fields of microbiological, plant, and medical research [3]. In medical research, UPE is being studied as a diagnostic tool [8,9]. In the plant sciences, UPE has been used to investigate the responses of plants to lunisolar tides and wounding [2]. In agriculture, UPE is being used to identify new types of agrochemicals that potentiate plants' defenses, or to differentiate herbicide-resistant from herbicide-susceptible weeds [10].

Despite the above studies, relatively little is known about the molecular mechanisms underlying UPE. An earlier study showed that UPE in bull spermatozoa is associated with adenosine triphosphate (ATP) levels. The increase in photon emission due to lipid peroxidation highly correlated with increases in cell ATP levels induced by thermal stress [11].

Most studies of UPE have focused on its link to plant growth and development [12,13]. The biological role of UPE in plants is not well understood, although it may be generated from the relaxation of electronically excited species formed during oxidative metabolic processes [14-16]. UPE has been shown to be closely related to photosynthesis, lipid peroxidation, catabolism, free radical reactions, radiation effects, detoxification, carcinogenic effects, aging, and the death process [17].

Strawberry (*Fragaria x ananassa* Duch.) fruit is soft and juicy when ripe, with high nutritional value. It is a typical non-climacteric fruit in that it ripens without ethylene or dramatic changes in cellular respiration. Although there have been many studies of the ripening, softening, and decay of the strawberry fruit, there is little known of how these processes are related to cellular energy levels. Recent studies showed that post-harvest softening and decay of the fruit are regulated by energy level [18,19].

To the best of our knowledge, there has been no report on UPE conducted in strawberry fruit. This study

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was conducted to further our understanding of UPE in plants and to determine whether there is an association between UPE and cellular energy levels during ripening of the strawberry fruit. The data from this study should provide new insights into the association between UPE and fruit development and senescence, the source of UPE production in plants, and a better understanding of the role of UPE in plants.

2 Materials and Methods

2.1 Fresh fruits

The strawberry fruits (cv. 'Hongyan') used in this study were collected from a farmers' cooperative greenhouse at 5 ripening stages, I-V: I, green; II, light red; III, half-red; IV, red; and V, ripe. Within 30 min of harvest, the fruits were measured for UPE and cut into pieces for frozen storage in liquid nitrogen at –196°C for subsequent analysis.

2.2 Stored fruits

For the post-harvest studies, fruits were harvested at stage IV (red), washed, air-dried, packed into zip bags, and stored at 25 ± 1°C. After storage of 1-5 days, the fruits were sampled for UPE and biochemical analysis in the same manner as the fresh fruits.

2.3 UPE measurements

UPE measurements were performed as described [20,21], with some modifications, using the UPE detection system BPCL-SH15-TGC (Rio Tinto Technology, Beijing, China), in accordance with the manufacturer's instructions. Ten randomly-selected fruits were measured at each timepoint, and each fruit was measured 10 times. A 1 cm (diameter) × 1.5 cm (height) tissue sample was punched from each fruit and placed into a cup in a dark chamber of the instrument for UPE measurement. The machine was pre-warmed for 30 min before use. UPE at each timepoint was calculated based on 10 fruits, each measured 10 times.

2.4 Biochemical analysis

To determine ATP, adenosine diphosphate (ADP), and adenosine monophosphate (AMP) content of the fruits, one gram of each frozen fruit was analyzed using high performance liquid chromatography (ELITE Lachrom Pump L-2130 equipped with Hitachi UV-VIS detector L-2420, Hitachi, Japan). All samples were measured in 3 replications. A Hitachi liquid chromatography column (LaChrom-C18; 4.6 mm × 250 mm, 5 μm) was eluted with 35 mmol/L phosphate (NaH_2PO_4) buffer (pH 6.8) at a flow rate of 1.0 mL/min and column temperature of 30 °C.

Standards of the highest quality for ATP, ADP, and AMP (Sigma, USA) were used to draw the concentration curves. The energy charge was calculated as (ATP + 0.5 ADP) / (ATP + ADP + AMP) [22].

2.5 Statistical analysis

Data were statistically analyzed using SPSS software and expressed as mean ± standard error.

Ethical approval: The conducted research is not related to either human or animals use.

3 Results

3.1 Changes in energy levels during fruit ripening

The levels of ATP, ADP, and AMP in the ripening strawberry fruits were measured (Figure 1). The data showed that ATP dropped quickly from stage I (0.89 mg/g) to the lowest at stage IV, and increased slightly afterward to 0.43 mg/g. ADP content was relatively low (about 0.22 mg/g) and stable before stage IV, and more than doubled at stage V. AMP was barely detectable and declined in the fruits

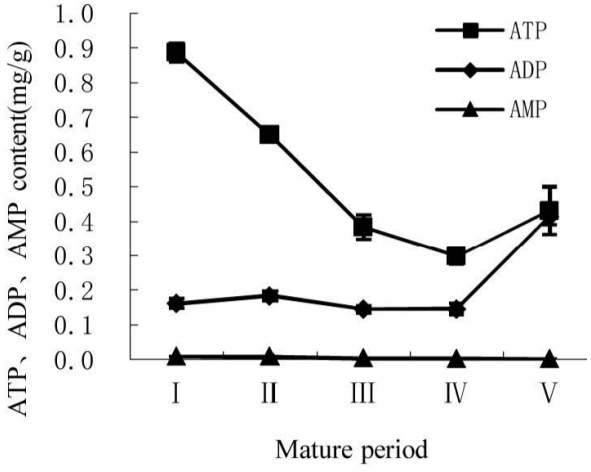

Figure 1. Content of ATP, ADP, and AMP in the ripening strawberry fruits.

over the entire ripening period. The energy charge from the three molecules (ATP, ADP, and AMP) varied between 0.75-0.92, and decreased as the fruit ripened (Figure 2).

3.2 Changes in UPE during fruit maturation

UPE during fruit ripening decreased almost linearly from stage I (63 count/s) to stage V (32 counts/s; Figure 3). The reduction was most remarkable between stages III and V.

3.3 Association between UPE intensity and energy in ripening fruits

Statistical analyses showed that there were linear correlations between the intensity of UPE (y) and energy charge (x), represented as AMP, ADP, or ATP levels (Table 1). Among them, ADP levels negatively correlated with UPE, while the others positively correlated.

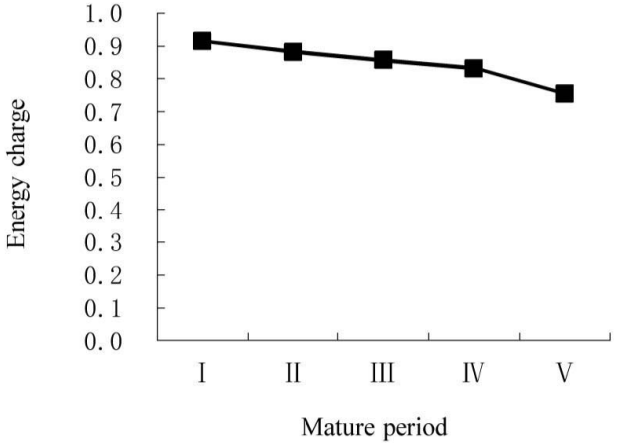

Figure 2. Energy charge of ripening strawberry fruits

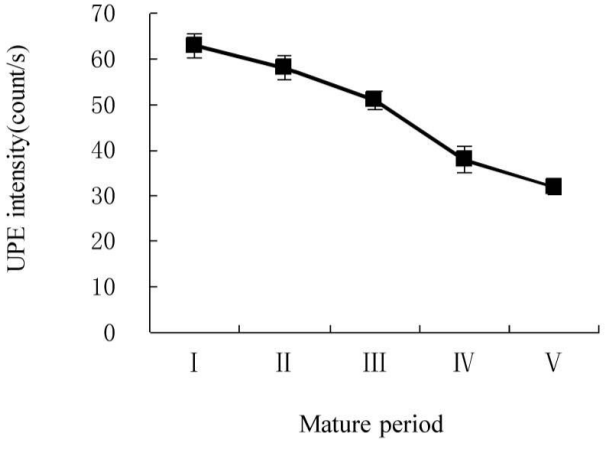

Figure 3. The intensity of UPE in ripening strawberry fruit

Table 1. The correlation analysis of UPE intensity (y) and energy level (x) in ripening strawberry fruits

	UPE regression equation	r *
AMP	$y = 3889.961\,x_1 + 30.1172$	0.9539
Energy charge	$y = 204.886\,x_2 - 125.343$	0.9500
ATP	$y = 44.109\,x_3 + 25.0295$	0.8004
ADP	$y = -71.692\,x_4 + 63.4568$	−0.6302

* Correlation coefficient

3.4 Change in energy levels in post-harvest fruit

We then analyzed the energy changes after storage at room temperature in fruits harvested at stage V. The data showed that ATP content declined over the storage period, particularly 3 days after harvest, from 0.62 mg/g on day 1 to 0.13 mg/g on day 5. For ADP, the reduction was slow from 0.30 mg/g (day 1) to 0.22 mg/g (day 3), and it slightly increased after day 3 to 0.35 mg/g on day 5. AMP was barely detectable and the content increased from 0.01 mg/g on day 1 to 0.06 mg/g on day 5 (Figure 4). The energy charge from the 3 molecules declined from 0.85 (on day 1) to 0.57 (on day 5) during the period (Figure 5).

3.5 Change in UPE during post-harvest storage period

During the storage period, fruits continued to ripen and soften. Measurements showed that UPE increased slightly between days 1 and 2, and declined constantly afterward from 46 count/s (day 1) to 29 count/s (day 5; Figure 6).

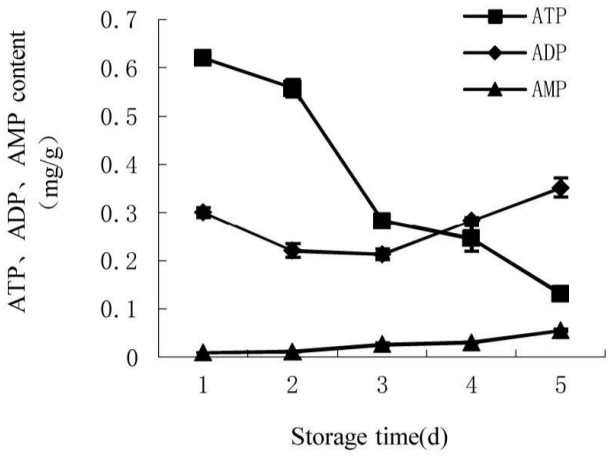

Figure 4. Content of ATP, ADP, and AMP in ripe strawberry fruits during post-harvest storage.

3.6 Association between UPE intensity and energy during the post-harvest period

Regression analyses showed that there were linear correlations between the intensity of UPE (y) and energy charge (x) represented as AMP, ADP, or ATP levels (Table 2). Among them, ADP and AMP levels negatively correlated with UPE, while ATP and energy charge positively correlated.

Table 2. The correlation analysis of UPE intensity (y) and energy level (x) in ripe strawberry fruits during post-harvest storage period

	UPE regression equation	r *
Energy source	$y = 72.327\,x_1 - 13.6758$	0.9807
ATP	$y = 36.827\,x_2 + 26.0633$	0.9403
AMP	$y = -417.633\,x_3 + 50.6589$	−0.9457
ADP	$y = -91.535\,x_4 + 64.6953$	−0.6433

* Correlation coefficient

4 Discussion

Strawberry is a typical non-climacteric fruit. Many studies of the physiology of this important fruit have shown that during ripening numerous changes occur at the gene and molecular levels. However, to our knowledge, UPE has not investigated in the fruit. Using a highly sensitive detector, we were able to detect UPE in the fruit during ripening and the post-harvest storage period. Our data showed that UPE declines during the ripening stages, and we further found that this decline is concurrent with decline in cellular energy levels.

The association between UPE and plant development is largely unclear, and how UPE is generated in the plant is still an open question. Tang et al. [23] speculated that UPE resulted from energy released as photons from ATP. In their study with Chinese cabbage, Li et al. [24] proposed that UPE is derived from the electron transfer chain during photosynthesis. In an *in vitro* study of UPE with mitochondria, it was shown that the intensity of UPE in mitochondrial extract was positively related to the mitochondrial concentration [25]. Mitochondria and chloroplasts are the main organelles in which oxidation and energy conversion take place. These results suggest that UPE may originate from subcellular organelles such as mitochondria and chloroplasts.

Furthermore, a study showed that, during flowering, UPE from the apricot flower was concurrent with changes in ATP levels [26], suggesting that ATP and energy levels are likely associated with UPE. In the present experiment, we found that UPE intensity decreased gradually during

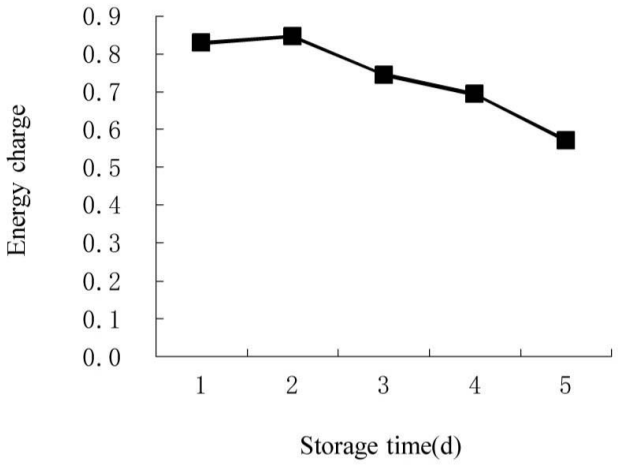

Figure 5. Energy charge of ripe strawberry fruits during post-harvest storage.

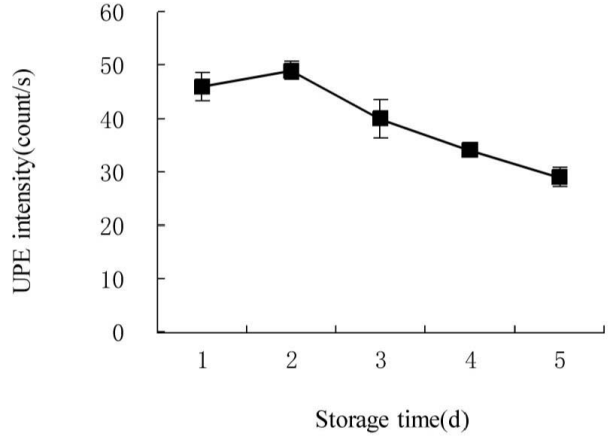

Figure 6. The intensity of UPE in ripe strawberry fruits during post-harvest storage.

the maturation of strawberry fruit, and was significantly and positively related to cellular ATP and energy charge levels. Thus, it appears likely that ATP is the energy source for UPE. This is consistent with earlier observations [11,18].

The associations between UPE and the other 2 energy molecules, ADP and AMP, were not consistent during the ripening and post-harvest storage period (Table 1 and 2). This might be because the metabolic processes of ATP, which is the precursor of ADP and AMP, during the two periods are different. Furthermore, measurements showed that the AMP content was very low (Figure 1 and 4), and may therefore contribute very little to UPE. The negative association between ADP and UPE levels suggests that conversion of ATP to ADP reduces the cellular energy charge effective for UPE. Therefore, the levels of ATP and energy charge may be the main determinant of UPE level. Although the energy charge is based on the ATP, ADP, and AMP contents, our study showed that during the fruit

ripening and post-harvest period, most of the contribution to energy charge is from ATP. This is consistent with previous work [27].

Earlier studies showed that the generation of active oxygen species can accelerate decay of the fruit [28], and active oxygen production is related to NAD(H) and NADP(H) [29]. A more recent study showed that storage of fruit in pure oxygen increased respiration and the generation of ATP, which helps to maintain the integrity of the cell membrane and delay fruit decay [30]. Further study is needed to determine how other energy carriers and molecules are involved in UPE and whether UPE can be used for profiling the quality or other agronomic traits of the fruit.

5 Conclusions

UPE from ripening strawberry fruits declined during ripening and the post-harvest period, and the decline is concurrent with the decline in cellular ATP and energy charge levels. The significant and positive correlations between the two parameters suggest that ATP is the main source of energy for UPE in the fruit.

Conflict of interest: Authors state no conflict of interest.

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