Lipid hydroperoxides in plants

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Abstract

Hydroperoxides are the primary oxygenated products of polyunsaturated fatty acids and were determined spectrophotometrically based on their reaction with an excess of Fe³⁺ at low pH in the presence of the dye Xylenol Orange. Triphenylphosphine-mediated hydroxylation formation was used to authenticate the signal generated by the hydroperoxides. The method readily detected lipid peroxidation in a range of plant tissues including Phaseolus hypocotyls (26 ± 5 nmol·g of fresh weight⁻¹; mean ± S.D.), Alstroemeria floral tissues (sepal, 66 ± 13 nmol·g of fresh weight⁻¹; petals, 49 ± 6 nmol·g of fresh weight⁻¹), potato leaves (334 ± 75 nmol·g of fresh weight⁻¹), broccoli florets (568 ± 68 nmol·g of fresh weight⁻¹) and Chlamydomonas cells (602 ± 40 nmol·g of wet weight⁻¹). Relative to the total fatty acid content of the tissues, the percentage hydroperoxide content was within the range of 0.6–1.7 % for all tissue types (photosynthetic and non-photosynthetic) and represents the basal oxidation level of membrane fatty acids in plant cells. Leaves of transgenic potato with the fatty acid hydroperoxide lyase enzyme expressed in the antisense orientation were elevated by 38 %, indicating a role for this enzyme in the maintenance of cellular levels of lipid hydroperoxides.

Introduction

Fatty acid hydroperoxides are key intermediates in the octadecanoid signalling pathway in plants and stand at the branch point of a number of competing metabolic pathways [1,2]. Fatty acid hydroperoxides can be converted into a family of cyclopentenone compounds collectively referred to as jasmonates [3,4], can undergo chain cleavage by hydroperoxide lyase [5,6], generating oxoacids and volatile aldehydes, or can serve as substrates for peroxigenases and hydroperoxide isomerase (epoxy alcohol synthase), generating products which may have roles in cutin monomer formation [7]. Little is known about the physiological levels of lipid hydroperoxides (LHPOs) in plants and how these are regulated. Here we report a simple and rapid method for their estimation based upon the hydroperoxide-mediated oxidation of ferrous to ferric ions under acidic conditions [8,9] following rapid extraction of total lipids [10].

Materials and methods

The ferrous oxidation of Xylenol Orange (version 2) reagent (FOX2) was prepared as described in [8] and total lipids were extracted as given elsewhere [9]. Lipids were quantified by GC of their fatty acid methyl esters using heptadecanoic acid as an internal standard and the chlorophyll content determined using the equations in [11].

Results and discussion

Fatty acid profiles, LHPO and chlorophyll content of various tissues

We anticipated that photosynthetic tissues may contain higher levels of LHPO than non-photosynthetic tissues, resulting from reactive oxygen species damage during the extraction process. In order to determine whether this was so, we chose a range of plant tissues, namely non-photosynthetic dark-grown hypocotyls, floral tissues, leaf tissue and the photosynthetic unicellular alga, Chlamydomonas, to investigate this possibility. The fatty acid profile of total lipids extracted from the various tissues is given in Table 1. Palmitate (16:0), stearate (18:0) oleate (18:1 Δ⁹), linoleate (18:2 Δ⁹,Δ₁₂) and α-linolenic acid (18:3 Δ⁹,Δ₁₂,Δ₁₅) were ubiquitously present. The series of C₁₄ unsaturated fatty acids up to the level of hexadecatrienoic acid (16:3 Δ⁹,Δ₁₂,Δ₁₅) was also present in all tissues except Phaseolus hypocotyls. In Chlamydomonas, γ-linolenic acid (γ-18:3 Δ⁹,Δ₁₂,Δ₁₅) and stearidonic acid (18:4 Δ⁹,Δ₁₂,Δ₁₅) were also present. In all tissues, the predominant polyunsaturated fatty acids were 18:2 and α-linolenic acid (18:3 Δ⁹,Δ₁₂,Δ₁₅).
Table 1

Fatty acid compositions of the tissues used for LHPO determination

Values are means±S.D. (n = 4), except for potato leaves, where n = 14.

<table>
<thead>
<tr>
<th>Fatty acid composition (mol%)</th>
<th>16:0</th>
<th>16:1</th>
<th>16:2</th>
<th>16:3</th>
<th>18:0</th>
<th>18:1</th>
<th>18:2</th>
<th>γ-18:3</th>
<th>α-18:3</th>
<th>18:4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlamydomonas cells</td>
<td>21.6±0.4</td>
<td>13±0.4</td>
<td>2.5±0.4</td>
<td>2.8±0.6</td>
<td>1.4±0.5</td>
<td>24.7±0.6</td>
<td>12.2±0.8</td>
<td>7.3±0.8</td>
<td>25.6±0.5</td>
<td>0.9±0.2</td>
</tr>
<tr>
<td>Broccoli florets</td>
<td>16.4±0.4</td>
<td>0.1±0.1</td>
<td>1.7±0.3</td>
<td>2.7±0.4</td>
<td>2.0±0.4</td>
<td>5.3±0.9</td>
<td>17.1±1.9</td>
<td>—</td>
<td>54.4±2.4</td>
<td>—</td>
</tr>
<tr>
<td>Potato leaves</td>
<td>12.1±0.6</td>
<td>0.2±0.1</td>
<td>1.4±0.5</td>
<td>13.2±1.0</td>
<td>0.3±0.1</td>
<td>2.3±1.1</td>
<td>17.8±2.8</td>
<td>—</td>
<td>52.8±3.7</td>
<td>—</td>
</tr>
<tr>
<td>Alstroemeria</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sepals</td>
<td>19.5±0.2</td>
<td>—</td>
<td>0.3±0.2</td>
<td>2.7±0.3</td>
<td>1.2±0.8</td>
<td>1.6±0.8</td>
<td>46.6±1.0</td>
<td>—</td>
<td>28.2±0.9</td>
<td>—</td>
</tr>
<tr>
<td>Petals</td>
<td>19.7±1.5</td>
<td>—</td>
<td>0.1±0.0</td>
<td>1.1±0.6</td>
<td>1.2±0.5</td>
<td>2.6±1.1</td>
<td>52.0±1.4</td>
<td>—</td>
<td>23.3±2.2</td>
<td>—</td>
</tr>
<tr>
<td>Phaseolus hypocotyls</td>
<td>31.5±1.5</td>
<td>—</td>
<td>—</td>
<td>2.4±0.3</td>
<td>6.5±0.7</td>
<td>34.5±2.1</td>
<td>25.0±1.9</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

Table 2

LHPO, fatty acid and chlorophyll contents of various plant tissues

Values presented are the means±S.D. (n = 6), except for potato leaves, where n = 14. g of fresh weight; nd, not detected.

<table>
<thead>
<tr>
<th>Tissue type</th>
<th>Chlorophyll (μg·gfw·⁻¹)</th>
<th>Fatty acid (nmol·gfw·⁻¹)</th>
<th>LHPO (nmol·gfw·⁻¹)</th>
<th>Total fatty acids oxidized (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlamydomonas cells</td>
<td>794±1291</td>
<td>7374±44068</td>
<td>602±40</td>
<td>0.8±0.1</td>
</tr>
<tr>
<td>Broccoli florets</td>
<td>608±145</td>
<td>3392±5988</td>
<td>568±68</td>
<td>1.7±0.2</td>
</tr>
<tr>
<td>Potato leaves</td>
<td>1307±256</td>
<td>2245±3216</td>
<td>334±75</td>
<td>1.5±0.3</td>
</tr>
<tr>
<td>Alstroemeria</td>
<td>142±2</td>
<td>629±332</td>
<td>66±13</td>
<td>1.0±0.2</td>
</tr>
<tr>
<td>Sepals</td>
<td>193±17</td>
<td>806±160</td>
<td>49±6</td>
<td>0.6±0.1</td>
</tr>
<tr>
<td>Petals</td>
<td>nd</td>
<td>2602±247</td>
<td>26±5</td>
<td>1.0±0.2</td>
</tr>
</tbody>
</table>

The results expressed as fatty acid content/g of fresh weight show that tissues vary significantly in their lipid contents (Table 2). The highest lipid content was in Chlamydomonas cells, which have over 2-, 3- and 28-fold higher levels than broccoli, potato leaf and dark-grown Phaseolus hypocotyls, respectively. The fatty acid content in Alstroemeria floral tissues is about one-third of that in potato leaf. The content of LHPOs expressed per g of fresh weight also varies widely from tissue to tissue (26–602 nmol·g of fresh weight⁻¹). The highest levels are observed in those tissues that also have the highest lipid contents (e.g. Chlamydomonas), while those with the lowest lipid contents (Phaseolus hypocotyls) have the lowest LHPOs. The relationship between the fatty acid content and the LHPO content is not stoichiometric, but generally the higher the tissue's lipid content the higher the LHPO content, and by expressing the data as the percentage of total fatty acids oxidized it is evident that the levels are in the range of 0.6–1.7% for all tissue types examined. In addition, the levels of LHPOs are not related to the chlorophyll content of the tissues. Thus the relative LHPO levels are similar in photosynthetic and non-photosynthetic tissues, indicating little reactive oxygen species damage during the lipid-extraction process of chlorophyllous tissues.

Determination of LHPO content in the leaves of transgenic potatoes with altered LHPO metabolism

The LHPO content of potato leaves with the fatty acid hydroperoxide lyase gene (HPL; which degrades hydroperoxides) expressed in the antisense orientation (anti-HPL) was compared with that of wild type. The results indicated that anti-HPL plants accumulated hydroperoxides to a level almost 40% higher than the wild type (407±21 compared with 566±16 nmol of LHPO·g of fresh weight⁻¹).
weight \(^1\); means ± S.D.), demonstrating a role for this enzyme in the maintenance of cellular LHPO levels.

**Conclusion**

The FOX2 method readily detected the LHPO content of plant tissues. The levels of LHPO in plant tissues are maintained at a basal level of between 0.6 and 1.7 \(^\text{\%}\) of total fatty acids in a wide range of tissues examined. The method of LHPO determination reported here is a rapid and simple technique and may be useful for the determination of LHPO in tissues where the levels of these compounds are expected to vary, such as in plants grown under conditions of abiotic and biotic stress or during the course of senescence in plant organs.

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**Lipoxygenase-catalysed degradation of carotenoids from tomato in the presence of antioxidant vitamins**

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**Abstract**

Carotenoid extract from ripe tomato fruit was subjected to a lipoxygenase-catalysed co-oxidation in the presence of vitamin C and vitamin E at different concentrations. Relative retention (\(^\%\)) of major carotenoids by the experimental mixture was used as an index of their degradation and interaction with the antioxidants. Oxidation-prevention activity of each antioxidant against pigment co-oxidation as impacted by their molar concentration was studied. \(^\alpha\)-Carotene was found to be the most sensitive pigment, followed by lycopene and lutein. Ascorbic acid when added in the range of 0–1.8 mM interacted with the different carotenoids by different modes. Evidence was given on regeneration, by ascorbic acid, of lycopene during the course of co-oxidation. The concentration required for \(\alpha\)-tocopherol acetate to exhibit antioxidative effect was 10 times higher than that of ascorbic acid. \(\beta\)-Carotene was prevented, by \(\alpha\)-tocopherol acetate, faster than lutein and lycopene. The latter carotenoids differed substantially in their interaction with the lipophilic antioxidant at only the lowest concentration (0.3 mM). It was established that under the given conditions there is no synergism between vitamin C and vitamin E that improves their oxidation prevention against co-oxidation of carotenoids. Moreover, the combined use of antioxidants caused more oxidative degradation of \(\beta\)-carotene.

**Introduction**

The colour of ripe tomato fruit is attributed to carotenoid-type pigments that have technological, nutritional and biological importance. Most of the carotenoids from tomatoes have been found in different tissues of human and animal bodies \([1–3]\). Owing to their potential vitamin A activity some carotenoids are of nutritional importance. It is essential to be able to distinguish among different carotenoids because, due to structural differences, the biological activity of all carotenoids is not the same \([3,4]\). This becomes particularly important in products such as tomatoes where the major carotenoid, lycopene, has no vitamin A activity.

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**References**


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\(^2\)Abbreviation used: LOX, lipoxygenase.