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# Inhibition of fatty acid desaturation in sycamore cells deprived of iron

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**Abstract** The growth of isolated sycamore cells in a medium devoid of iron induced a marked reduction of the unsaturation level of fatty acids: the proportion of linolenic acid (C18:3) in polar lipids (phospholipids and galactolipids) decreased whereas a parallel increase in the proportions of oleic (C18:1) and linoleic (C18:2) acid was observed. In our experimental conditions, no direct effect of iron deprivation on fatty acid and glycerolipid biosynthesis could be observed. When sycamore cells were incubated in the presence of [$^{14}$C]acetate, the level of unsaturation in fatty acids was very strongly reduced: no polyunsaturated fatty acids were synthesized in iron-deprived cells: only [$^{14}$C]palmitic and [$^{14}$C]oleic acids accumulated in glycerolipids. In contrast, sycamore cells grown in an iron-containing medium in the presence of [$^{14}$C]acetate were able to synthesize glycerolipids containing $^{14}$C-labelled C18:2 and C18:3. We concluded that, in sycamore cells, iron is essential for C18:1→C18:2→C18:3 desaturations. In contrast, C18:0→C18:1 desaturation is much less sensitive to iron deprivation under our experimental conditions.

*Key words:* Iron deficiency; Fatty acid saturation; Acetate incorporation; Galactolipid; Phospholipid; Sycamore cell

## 1. Introduction

Fatty acid desaturation in the different plant cell compartments, including plastid (stroma and envelope membranes) and endoplasmic reticulum, involves iron-containing proteins (iron-sulfur proteins) and various cytochromes [1]. In addition, stearoyl-acyl carrier $\Delta_9$-desaturase, a soluble enzyme, was shown to be a diiron-oxo protein [2]. Therefore, one can expect that under iron deficiency, fatty acid biosynthesis and desaturation could be inhibited. Indeed, Abadia et al. [3] found that in pea plants grown in absence of iron the level of linolenic acid in thylakoids was lower than in normal conditions. However, in such experiments using whole plants, all leaves are at different developmental stage and are not equally affected by iron deprivation. In addition, galactolipids are the major membrane lipid constituents of thylakoids and it was not clear whether typical extraplastidial lipids were also affected by iron deprivation. Because plant cell suspension cultures rapidly generate large amounts of cell material that offers a remarkable tool to study the impact of deficiencies on plant cell metabolism [4], we undertook a series of experiments using isolated sycamore cells to analyze the effect of iron deficiency on fatty acid biosynthesis and desaturation. In this article, we describe the impact of iron deficiency on glycerolipid and fatty acid compositions of sycamore cells deprived of iron. We also analyzed the effect of iron deficiency on in vivo desaturation of fatty acids by following [$^{14}$C]acetate incorporation into glycerolipids and fatty acids.

## 2. Materials and methods

### 2.1. Biological material

Sycamore (*Acer pseudoplatanus* L.) cells were grown as a suspension either in a medium containing 10 $\mu$M $FeSO_4$ supplied with an equimolar amount of $Na_2EDTA$ (control conditions) or in a medium devoid of iron. Cell growth conditions, determination of cell number at all stages of growth are described by Pascal and Douce [5]. All culture flasks were soaked overnight in 5 M HCl to remove contaminating iron, as described by Pascal and Douce [5].

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### 2.2. [$^{14}$C]Acetate incorporation into sycamore cells

Sycamore cells were first grown in 200 ml of normal and iron-free culture medium as described above. At the beginning of the exponential phase of growth (i.e. after 3 days of culture), 10 mM [$^{14}$C]acetate (3.7 kBq) were added to the medium. The cells were harvested 2 days after the addition of [$^{14}$C]acetate for lipid analyses.

### 2.3. Glycerolipid and fatty acid analyses

Lipids were extracted from sycamore cells (10 g wet weight) and analyzed according to Douce et al. [6]. Polar lipids were resolved by two-dimensional chromatography [6]. The identity of methyl esters of the constituent fatty acids of glycerolipids was analyzed on 5% $AgNO_3$ silica gel as described by Hawke and Stumpf [7].

### 2.4. Iron determination

Iron (in sycamore cells and in culture media) was determined with a Perkin-Elmer (model 2380) atomic absorption spectrophotometer as described by Pascal and Douce [5]. After 7 days of iron deficiency, when growth had ceased, the total iron content in sycamore cells averaged 55 ± 5 ng/$10^6$ cells, compared with 140 ± 15 ng/$10^6$ cells in control cells [5]. Iron could not be detected in the iron-deprived culture medium. The limit of detection was 0.1 mg Fe/ml.

### 2.5. Protein determination

Cell protein content was determined by the method of Lowry et al. [8] using bovine serum albumin as standard.

## 3. Results and discussion

### 3.1. Effect of iron deficiency on the polar lipid composition of sycamore cells

The major polar lipids found in sycamore cells are phospholipids. Galactolipids represent only 5% of the total polar lipid content because of the small development of amyloplast membranes within the cells. During the lag phase (up to 3 days), the amount of polar lipids (phospholipids as well as galactolipids) increased by 40% (on average) in control as well as in iron-deprived cells (Table 1), corresponding to a development of membranes within the cells, shown by the increase of sycamore cell size during the first 2–3 days [5]. No difference between control cells and iron-deprived cells were noted at this stage. The most significant increase was observed for cardiolipin (70%), a marker for the inner membrane of mitochondria, and galactolipids (130%). The increase of the galactolipid content


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Table 1
Polar lipid composition of control sycamore cells (+ Fe) and cells deprived of iron (−Fe).

| Culture time (days) in presence (+ Fe) or absence (-Fe) of iron | Polar lipid content ($\mu g/10^6$ cells) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 0 | | 3 | | 5 | | 7 | |
| | + Fe | − Fe | + Fe | − Fe | + Fe | − Fe | + Fe | − Fe |
| Galactolipids | 3.5 | 3.5 | 7.8 | 8 | 5.3 | 7.1 | 3.5 | 6.3 |
| PC | 30 | 30 | 36 | 37 | 33.4 | 40 | 30 | 46 |
| PE | 18 | 18 | 26 | 26 | 22 | 28 | 18 | 30 |
| PG | 2.5 | 2.5 | 3.5 | 4.0 | 3.2 | 4.2 | 2.5 | 4.0 |
| PI | 4.5 | 4.5 | 7.3 | 7.5 | 6.1 | 8.5 | 4.5 | 8.7 |
| DPG | 1.5 | 1.5 | 2.4 | 2.5 | 2.0 | 2.2 | 1.5 | 2.0 |
| Total | 60 | 60 | 83 | 85 | 72 | 90 | 60 | 97 |

Lipids were extracted from the same number of protoplasts ($167 \cdot 10^6$) derived from cells grown for 0 to 7 days on culture medium containing (+Fe) or devoid of (−Fe) iron, as described in section 2. Abbreviations used: PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; DPG, diphosphatidylglycerol or cardiolipin. Galactolipids: MGDG (monogalactosyldiacylglycerol) + DGDG (digalactosyldiacylglycerol).

can be correlated with a 3-fold increase in starch accumulation, corresponding to an increase of the size of amyloplasts [5].

The main effect of iron deficiency was observed during the exponential phase of growth (i.e. between 3 and 7 days of culture. Indeed, when cells began dividing, the glycerolipid content (per $10^6$ cells) of control cells decreased markedly, whereas it remained roughly stable or even increased slightly in cells deprived of iron (Table 1). Again, the evolution of the polar lipid content of cell was parallel to that of cell size: normal cells became much smaller (up to 7 days) whereas the size of iron-deprived cells (which divide much more slowly) increased [5]. During the stationary phase of growth, the size of both type of cells increased, as well as their polar lipid content (not shown). From all these observations, we can conclude that, in sycamore cells, iron deprivation has no direct effect of fatty acid and glycerolipid biosynthesis. The glycerolipid content of iron deprived cells is tightly related to the development of cell membranes. For instance, the galactolipid content of sycamore cells reflects the expansion of amyloplast membranes. Interestingly, Nishio et al. [9] found that the amount of galactolipids in iron-deprived sugar-beet leaves increased linearly with time during iron resupply, together with the increase of thylakoid surface during chloroplast development.

### 3.2. *Effect of iron deficiency on the fatty acid composition of sycamore cells*

To characterize further the effect of iron on sycamore lipids from cells grown for 6 days in a medium devoid of iron, we analyzed their fatty acids. Little evolution of the percentage of palmitic acid (C16:0) in sycamore polar lipids was observed (Table 2). In contrast, we observed major changes in C18-fatty acids composition: the proportion of linolenic acid (C18:3) in sycamore cells decreased whereas a parallel increase in the proportions of oleic (C18:1) and linoleic (C18:2) acids was observed (Table 2). This evolution occured within all sycamore polar lipids, but since galactolipids contain much higher proportions of C18:3 than phospholipids, the changes were even more obvious in these polar lipids: the proportion of C18:3 in galactolipids decreased from about 75% to 35% whereas that of C18:2 increased from 10% to more than 40% during the 6-day period of growth in absence of iron (Table 2). Our results confirm previous observations on thylakoid polar lipids from iron-deprived pea plants [3], and suggest that iron deprivation induces a marked inhibition of C18:3 formation in membrane glycerolipids. This was true for typical plastid glycerolipids (galactolipids) as well as for extraplastidial constituents (phosphatidylcholine and phosphatidylethanolamine). Time-course evolution of the fatty acid content of polar lipids provided further convincing evidence for this conclusion (not shown). However, the amount of glycerolipids present in sycamore cells at the begining of the growth in a medium devoid of iron, and containing significant amounts of 18:1 and 18:2, was rather high. Therefore, sycamore cells grown in culture medium with or without the presence of iron were incubated in presence of [$^{14}$C]acetate to directly investigate the effect of iron deficiency on in vivo fatty acid desaturation. The fatty acid composition of newly synthesized glycerolipid molecules was analyzed.

### 3.3. *[$^{14}$C]Acetate incorporation into glycerolipids from sycamore cells deprived of iron*

After a 2 day-incubation in the presence of [$^{14}$C]acetate, radioactive fatty acids were synthesized and incorporated in glycerolipids from sycamore cells grown in a culture medium containing or not iron. No direct effect of iron deprivation on the level of acetate incorporation into cell lipids was observed (not shown). In a total lipid extract from control cells, about 25 and 50% of the radioactivity was found in C16:0 and C18:2, respectively (Fig. 1). Since sycamore cells mostly contain phospholipids, these values reflect the distribution of radioactivity among fatty acids from the major glycerolipids: indeed,

Table 2
Fatty acid composition of glycerolipids from control sycamore cells (+Fe) and cells deprived of iron (−Fe).

| | | Fatty acids (%) | |
|---|---|---|---|
| | | + Fe | − Fe |
| Galactolipids | | | |
| | C16:0 | 12.6 | 13.1 |
| | C18:1 | 3 | 9.5 |
| | C18:2 | 10.3 | 42.1 |
| | C18:3 | 74.1 | 35.3 |
| Phospholipids | | | |
| | C16:0 | 25.3 | 27.3 |
| | C18:1 | 3.9 | 9.8 |
| | C18:2 | 42.5 | 52.5 |
| | C18:3 | 28.3 | 10.4 |
| Total Lipids | | | |
| | C16:0 | 24.5 | 26.4 |
| | C18:1 | 3.8 | 9.8 |
| | C18:2 | 40.7 | 51.9 |
| | C18:3 | 31 | 11.9 |

Lipids were extracted from the same number of protoplasts ($167 \cdot 10^6$) derived from cells grown for 7 days on culture medium containing (+Fe) or devoid of (−Fe) iron, as described in section 2. Galactolipids represent the total MGDG + DGDG. Since sycamore is a C18 plant, only negligible amounts of C16:0 are present in MGDG, the presence of this fatty acid in galactolipids is mostly due to DGDG. Results are expressed as a percentage of total fatty acids.

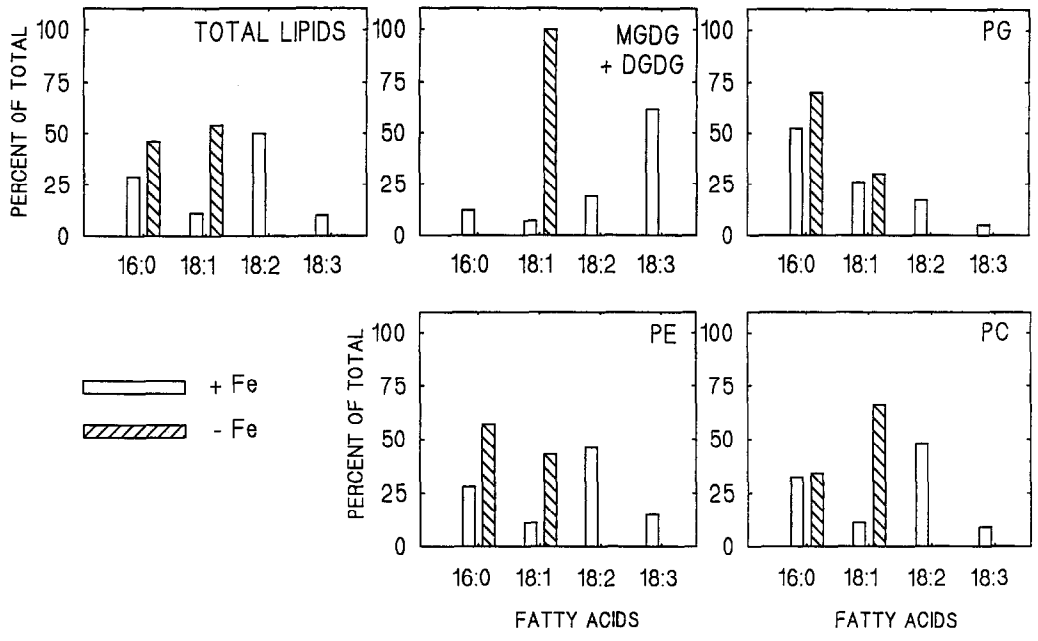


Fig. 1. Distribution of radioactive fatty acids in glycerolipids from control sycamore cells (+Fe) and cells deprived of iron (−Fe). Conditions for growth and labelling of sycamore cells, lipid extraction and analyses are described in section 2. Fatty acid radioactivity in each fatty acid is expressed as a percentage of the total radioactivity in the total lipid fraction and in the different glycerolipids (PG, PE, PC, MGDG + DGDG). Abbreviations used: see legend for Table 1. White bars: +Fe; dashed bars: −Fe.

similar proportions were found in phosphatidylcholine and phosphatidylethanolamine (Fig. 1). However, C16:0 and C18:3 represented more than 50% of the radioactivity found in fatty acids from phosphatidylglycerol and galactolipids, respectively (Fig. 1). The patterns of radioactive fatty acids changed completely in iron-deprived cells: no $^{14}$C-labelled C18:2 or C18:3 were detected in phospholipids or in galactolipids (Fig. 1). For instance, in a total lipid extract from iron-deprived cells, most of the radioactivity was found in C16:0 and C18:1 (Fig. 1), reflecting the fatty acid pattern of phosphatidylcholine and phosphatidylethanolamine. The radioactivity in galactolipids (in fact mostly in MGDG because the radioactivity in DGDG was very low) was almost exclusively in C18:1 (Fig. 1). Such an observation is in agreement with previous data [10] demonstrating that sycamore is a C18 plant and does not contain C16:0 fatty acid at the *sn*-2 position of the diacylglycerol backbone of MGDG. Together, our results demonstrate that iron deprivation induces an almost complete inhibition of C18:1 desaturation to C18:3. In contrast, C18:0 to C18:1 desaturation seems to be much less sensitive to iron deprivation, iron is both directly (the desaturase contains iron, [2]) and indirectly (through ferredoxin) involved in this desaturation. Interestingly, similar selectivity for fatty acid desaturation was obtained by Rebeillé et al. [11] with sycamore cells grown under low oxygen concentration. However, in the case of oxygen deprivation, the total incorporation of acetate in sycamore cells was very low compared to the control culture conditions [11], whereas in our experiments, the level of acetate incorporation into sycamore cells was almost identical in the presence or absence of iron in the culture medium. Concerning the fatty acid desaturation, our results may support several hypotheses. Indeed C18:0 to C18:1 desaturation is catalyzed by a soluble, stromal enzyme from plastids, whereas C18:1 to C18:2 and C18:3 desaturations are catalyzed by membrane-bound enzymes, localized on the plastid envelope membranes and on the endoplasmic reticulum [1]. Thus the electron transfer chains involved in these two desaturation pathways are necessarily different and their components probably could not have the same sensitivity to iron deficiency. Moreover, it is possible that the different cell compartments are not affected to the same extent by iron deprivation. So in these cells, iron would be mobilized to sustain mainly biosynthesis of the soluble desaturase at the expense of the membrane-bound forms. Finally, in cells, the soluble desaturase could be in a large excess in comparison to the membrane- bound desaturases. Therefore, iron deprivation during cell growth which affect mainly desaturase biosynthesis would be more effective on linoleic and linolenic acids synthesis. Such a selectivity of the impact of iron deprivation was also observed at the level of the components of the respiratory chain in mitochondria: Pascal and Douce [5] have demonstrated that although the iron content of sycamore cells, together with the level of cytochromes, decreased to about half of the value in control cells, only citrate and succinate oxidations were affected by iron deprivation.

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