Temperature and oxygen regulation of microsomal oleate desaturase (FAD2) from sunflower
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Abstract
The effect of temperature and oxygen on the in vivo oleate desaturation and microsomal oleate desaturase (FAD2) activity was studied in peeled developing sunflower seeds. Using an oxygen concentration that was saturating for FAD2 enzyme, the amount of linoleic acid increased for all studied temperatures, being maximal at 20°C. Under these conditions, FAD2 activity increased at the beginning of the incubation, remaining constant for the rest of the time, but reaching a lower level at 30°C. Anoxia brought about a decrease in the FAD2 activity for all studied temperatures, becoming faster as the temperature increased. All these data suggest that temperature and oxygen control the level of FAD2 activity by separate mechanisms.

Introduction
Previous studies on developing sunflower seeds have shown that microsomal oleate desaturase (FAD2) activity is regulated by temperature. When the whole plant or the capitulum was transferred to high temperature, FAD2 activity was decreased, whereas low temperatures brought about an increase in the activity level [1]. In addition, the level of FAD2 activity in developing sunflower seeds seems to be regulated by oxygen availability [2]. Whether these changes in the FAD2 activity level are due to the temperature itself or to an indirect effect is still under debate. Recent results from our group (M. T. García-Díaz and M. Mancha, unpublished work) indicate that the presence of a hull, which acts as a barrier for oxygen, could explain the observation that oleate desaturation was completely inhibited when the achenes were transferred to high temperature, whereas it was little affected by temperature changes in peeled seeds.

In this work we have used peeled developing sunflower seeds to study temperature and oxygen regulation separately, in order to elucidate if both effects are independent.

Experimental
Sunflower (Helianthus annuus L.) plants were cultivated in a growth chamber with a photoperiod
of 16 h light/8 h dark, at 25/15 °C. Seeds were harvested at 18–19 days after flowering. Total lipids from the seeds were converted into the corresponding methyl esters and their fatty acid compositions determined by GLC. FAD2 activity was measured as previously described [3] using the microsomal fractions of the seeds.

Results and discussion
The effect of different incubation temperatures on the in vivo oleate desaturation and FAD2 activity was studied using peeled developing sunflower seeds blown with air. In these conditions the oxygen concentration is saturating for FAD2 enzyme; therefore, there is no influence of oxygen on the level of activity.

The amount of linoleic acid increased at the three studied temperatures over time, being maximal at 20 °C (Figure 1). This temperature coincides with the optimal temperature of the sunflower FAD2 assayed in vitro [3], showing that the thermal stability of the enzyme could play an important role in its regulation by temperature.

On the other hand, the level of FAD2 activity increased at the beginning of the incubation at the three temperatures, remaining constant the rest of the time (Figure 1). This rapid increase of the activity could be explained by the adaptation to a new physiological situation, mainly due to the absence of the hull, which facilitates the availability of oxygen for desaturation. A differential temperature effect is also observed. The levels of FAD2 activity reached at 10 and 20 °C were similar, and both were higher than the level obtained at 30 °C. This result confirms that high temperatures decrease FAD2 activity and suggests that there is a temperature regulation independent of oxygen.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>10 °C</th>
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<tr>
<td>30</td>
<td>77</td>
<td>38</td>
<td>41</td>
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Figure 1
Time course of in vivo oleate desaturation (% 18:2) and FAD2 activity (nmol of 18:2·g of fresh weight⁻¹·h⁻¹) in peeled developing sunflower seeds incubated at different temperatures and blown with air.
We have also studied the effect of anoxia on FAD2 activity using peeled developing sunflower seeds blown with nitrogen and incubated at different temperatures. The level of FAD2 activity decreased for all temperatures (Table 1). However, this decrease was faster as temperature increased. This inactivation process due to the absence of oxygen was independent of temperature and indicates, for the first time in a plant FAD2, that oxygen is not only a necessary co-substrate for the enzyme but also plays a role in the regulation of FAD2 activity, as has been previously reported for other organisms [4].

References

Received 26 June 2000

The tolerance of cyanobacterium Cylindrospermopsis raciborskii to low-temperature photo-inhibition affected by the induction of polyunsaturated fatty acid synthesis

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Abstract

Acyl-lipid desaturation introduces double bonds (unsaturated bonds) at specifically defined positions of fatty acids that are esterified to the glycerol backbone of membrane glycerolipids. Desaturation patterns of the glycerolipids of Cylindrospermopsis raciborskii, a filamentous cyanobacterium, were determined in cells grown at 35 °C and 25 °C. The lowering of the growth temperature from 35 °C to 25 °C resulted in a considerable accumulation of polyunsaturated octadecanoic fatty acids in all lipid classes. The tolerance to low-temperature photo-inhibition of the C. raciborskii cells grown at 25 °C and 35 °C was also compared. The lower growth temperature increased the tolerance of C. raciborskii cells. These results strengthen the importance of polyunsaturated glycerolipids in the tolerance to environmental stresses and may give a physiological explanation for the determinative role of C. raciborskii in algal blooming in Lake Balaton (Hungary).

Key words: environmental stress, fatty acid desaturation, lipids.

Introduction

The physical and biochemical properties of glycerolipids depend on the degree of unsaturation of the fatty acids [1]. In membranes, the level of unsaturation is modulated by a specific enzyme system, fatty acid desaturases. In cyanobacterial strains acyl-lipid desaturases are the most efficient regulators of the extent of lipid unsaturation in response to changes in ambient temperature [2].

The photosynthetic apparatus of photosynthetic organisms is embedded in thylakoid membrane and surrounded by a lipid matrix. The main constituents of the lipid matrix are glycerolipids, which provide the necessary structural background for the functioning of protein complexes [3]. In cellular physiology, the unsaturation of membrane glycerolipids plays a key role in the tolerance of living organisms to low-temperature stress [4,5]. In our earlier studies we demonstrated the importance of polyunsaturated glycerolipids in the tolerance of photosynthetic organisms to low-temperature photo-inhibition by using transgenic cyanobacterial strains [6].

Cylindrospermopsis raciborskii is a filamentous, toxic blue–green alga inhabiting subtropical/tropical water bodies, and has an optimal growth temperature above 25 °C. It appeared in