USE OF POLARIZED FLUORESCENCE SPECTROSCOPY TO MEASURE FLUIDITY OF MITOCHONDRIAL MEMBRANES FROM COLD-TREATED TULIP BULBS

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Abstract

Polarized-fluorescence spectroscopy using diphenyl hexatriene as a probe can be used as a method to assess the overall fluidity changes of cell membranes. To demonstrate the use of this method, changes in membrane fluidity of cold-treated tulip bulbs were studied.

Cold treatment of tulip bulbs is common practice. For the production of good quality flowers a cooling period is necessary: for the cv. Apeldoorn a 12-week period at 5°C is optimal. The adaptation of the cell membranes to this cold treatment was studied with membrane preparations from isolated mitochondria. A rapid increase of the membrane fluidity was observed after transfer of the bulbs from 17 to 5°C, although clear phase transitions were not observed during measurement of the fluidity at temperatures between 4 and 30°C. When after cooling bulbs were transferred back to 17°C the membrane fluidity remained at the 5°C level for at least one week. The changes in membrane fluidity were not reflected in a changed fatty-acid composition of the mitochondrial membrane. Apparently, polarized-fluorescence spectroscopy is a rapid method to detect overall changes in membrane fluidity. These changes in fluidity relate to the storage temperature but not to the length of this storage period.

1. Introduction

Many plants are able to withstand the large fluctuations in temperatures that accompany the seasonal changes normally occurring in temperate climate zones. Many species are even dependent on such seasonal temperature changes for the realization of their developmental program. They will only germinate or produce flowers

after they have undergone a cold period for several weeks or months. This capability to withstand long periods of low temperatures requires adaptations of the plant cell membranes. To prevent a decline of the correct functioning of membrane-bound enzyme systems the fluidity of the membranes has to be changed: at lower ambient temperatures the cells need membranes with an increased fluidity. Such fluidity changes can be realized by increasing the percentage of unsaturated fatty acids but also by changes in the lipid type or in the lipid:protein ratio (Alberdi & Corcuera, 1991).

In order to produce well-developed flower stalks, tulip bulbs (*Tulipa gesneriana*) need a period of low temperature. For the cultivar Apeldoorn the optimum period has been established at 12 weeks 5°C (Moe and Wickstrøm, 1973). In earlier experiments differences in respiratory characteristics between 5°C- and 17°C-stored bulbs were observed (Kanneworff & Van der Plas, 1990). The respiratory capacity of mitochondria from 5°C-stored bulbs was higher than that from 17°C bulbs. In bulbs cooled for a short period, mitochondrial respiration remained at a high level when the bulbs were transferred back to 17°C. In bulbs cooled for a longer period a serious decline in the respiratory capacity was observed after such a transfer. Other authors reported changes in Arrhenius plots of mitochondrial respiration after cooling (Hobson, 1979 and Davies & Hobson, 1980). The noted differences could be caused by changes in membrane fluidity. To investigate these possible correlations, the fluidity was measured with polarized-fluorescence spectroscopy. Parallel to these experiments the lipid:protein ratio and the saturation level of the fatty acids in these mitochondrial membranes were determined.

2. Materials and Methods

Tulipa gesneriana bulbs cv Apeldoorn, circumference 12-12.5 cm, were obtained from a commercial grower via the Bulb Research Centre, Lisse and stored at 20°C until stage G (Beijer, 1942) was reached. Over a period of 20 weeks every 4 weeks a new 5°C-cooling series was initiated, beginning on 13 August, 1990. Bulbs that did not receive a low temperature treatment were stored at 17°C.

Mitochondria were isolated from bulb scales of cooled and 17°C-stored bulbs as described by Marissen et al. (1986) and Kanneworff & Van der Plas (1990). However, the isolation was carried out without the addition of bovine serum albumin (BSA), to prevent disturbance in the fluidity measurements. The mitochondrial suspensions were frozen in liquid nitrogen and stored at -85°C. After protein determination the mitochondria were thawed and diluted to a concentration of 1.25 mg protein ml^{-1} with isolation medium. For the actual measurements the mitochondrial suspension was diluted to 18.75 µg protein ml^{-1} in a solution of 10 mM tricine, 5 mM $MgCl_2$ and

10 mM KCl, pH 7.4. To this suspension 5 mM diphenyl hexatriene (DPH) in dimethyl sulphoxide (DMSO) was added to a final concentration of 1 μM. To accelerate the incorporation of DPH in the membranes the suspension was sonicated at low power for 15 seconds with a MSE soniprep. The polarized steady-state fluorescence measurements were performed on a SLM Aminco 500c fluorescence spectrophotometer fitted with a polarization accessory. Excitation took place at 354 nm and emission was measured at 450 nm (slit width both 5 nm). Anisotropy was determined between 4 and 30°C. Each value was the mean of 20 separate determinations. Ln-transformed anisotropy values were plotted against the reciprocal of the absolute temperature to create Arrhenius plots.

Determination of protein was performed according to Bradford (1976) with BSA as standard. Lipids were extracted by the addition of 10 ml chloroform/methanol (1:2) mixture, vigorously mixing and the addition of 3 ml 0.1 M KCl. After phase separation the lipids were dried under nitrogen. After saponification and methylation with methanol/5 % sulphuric acid (2 h at 70°C) of the lipids, the fatty acids were extracted with hexane. Finally the relative abundance of the different fatty acids was determined on a Perkin-Elmer SIGMA 300 gas chromatograph equipped with a glass capillary column, FID and an integrator according to Ykema et al. (1989).

3. Results and Discussion

DPH readily diffused into the mitochondrial fragments after gentle sonication. The excitation and emission spectra obtained are shown in figure 1. The form of the spectra resembled those reported by other authors (Shinitzky & Barenholz, 1974).

Arrhenius plots of 5°C and 17°C mitochondria revealed differences in membrane fluidity (figure 2). The fluidity of mitochondria isolated from 5°C bulbs was greater than the fluidity of mitochondria from 17°C bulbs. Within 4 weeks of cooling at 5°C this adaptation was accomplished. The fluidity of mitochondria from 5°C-cooled bulbs was comparable with the fluidity of mitochondria from 17°C bulbs measured at a 5°C higher temperature. The altered fluidity might enable the mitochondria to consume oxygen more adequately at lower temperatures. The Arrhenius plots were more or less straight lines and did not reveal the clear phase transitions described by Hobson (1979) for mitochondrial respiration. The adaptation of the membrane fluidity to 5°C was already completed after these 4 weeks. After a longer cooling period no further changes were observed (figure 3). Thus there was no direct relation with the fulfilment of the optimum cooling requirement of 12 weeks.

Transfer of cooled bulbs back to 17°C caused no changes in membrane fluidity (figure 4). After one week at 17°C membrane

fluidity was still at the 5°C level. This lack of reaction was observed in all series, independent of the length of the previous cooling period at 5°C. The results of earlier experiments, in which bulbs cooled for a 11- to 16-week period showed a dramatic decline in the capacity of their mitochondrial respiration as a reaction on transfer to 17°C (Kanneworff & Van der Plas, 1990), cannot be explained by these changes in membrane fluidity. Therefore, changes in respiratory proteins may have caused the observed decrease in respiration.

When the changes in mitochondrial-membrane fluidity were compared with data for fatty-acid composition and the lipid:protein ratios, no clear relations could be demonstrated. In the mitochondrial membranes the fatty-acid composition did not alter significantly after cooling (figure 5). The relative amounts of palmitic acid (16:0), oleic acid (18:1), linolic acid (18:2) and linoleic acid (18:3) for both 5°C bulbs and 17°C bulbs were equal. Of these fatty acids linolic acid was the most abundant followed by palmitic acid as, is generally observed for mitochondria (Harwood, 1985). Linoleic acid and oleic acid contributed for only minor percentages. Also the lipid:protein ratio did not change after cooling (table 1). Mitochondria isolated from 5°C and 17°C bulbs showed the same ratio. Possibly other membrane-determining qualities caused the differences between 5°C- and 17°C-stored bulbs.

When mitochondrial membranes were incubated with DPH without sonication, a striking difference was observed (figure 6). The diffusion of DPH into the membranes from 5°C bulbs occurred faster than into membranes from 17°C bulbs. The presence of phospholipids, sterols and maybe certain proteins in the membranes of mitochondria may have contributed to these differences. This taken into consideration, the changes in membrane fluidity as observed with DPH may be caused by changes in these membrane constituents and not by the saturation level of the fatty acids nor by the lipid:protein ratio.
Polarized-fluorescence spectroscopy with DPH is a powerful tool to determine membrane-fluidity changes in plant membranes and, as a consequence, is very suitable to detect the effect of ambient temperature on membrane characteristics. The properties of tulip mitochondrial membranes appeared to change with the storage temperature, but not with the length of the storage period. The polarized-fluorescence spectroscopy technique thus is not suitable to diagnose the length of the cooling period applied to tulip bulbs.

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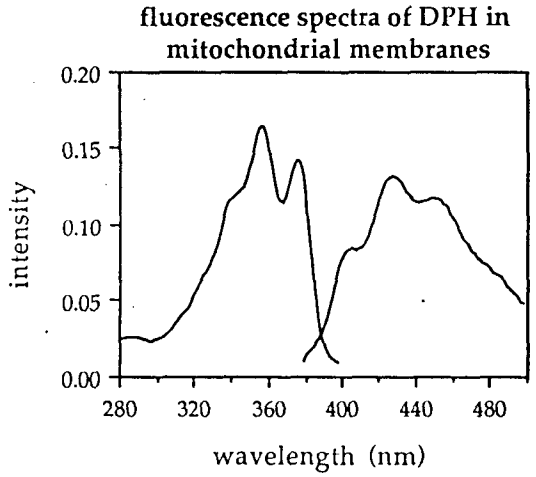

Figure 1: Excitation (left) and emission (right) spectra of 1 μM DPH in mitochondrial membranes. Excitation at 354 nm, emission at 430 nm.

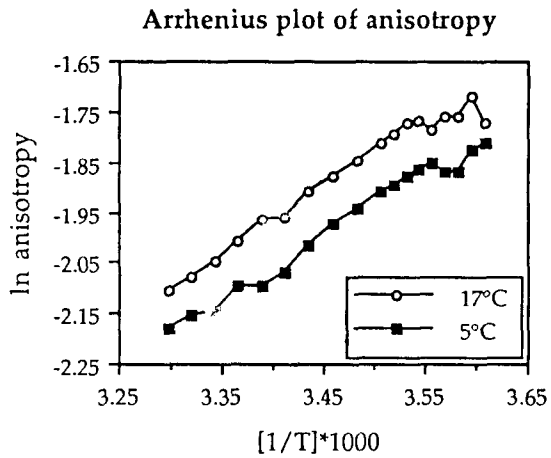

Figure 2: Arrhenius plot of fluorescence anisotropy of mitochondrial membranes from 17°C-stored bulbs (open circles) and 5°C-stored bulbs (solid squares) over the interval 4-30°C.

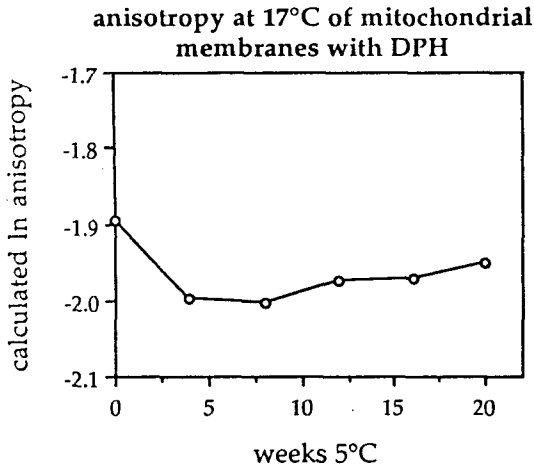

Figure 3: Calculated anisotropy of isolated mitochondrial membranes with 1 μM DPH at 17°C. Values calculated by linear regression of Arrhenius plots over the 12-30°C interval.

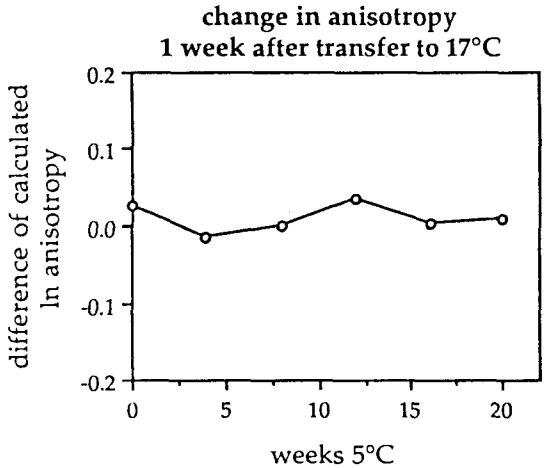

Figure 4: Change in ln anisotropy at 17°C, 7 days after transfer of 5°C-stored bulbs to 17°C when compared with values derived from 5°C-stored bulbs. Values calculated by linear regression of Arrhenius plots over the 12-30°C interval.

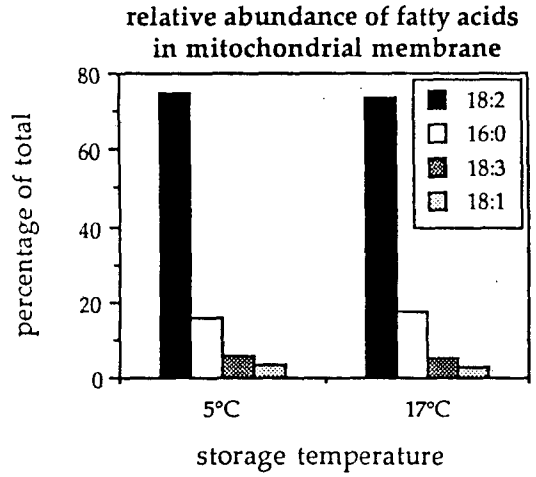

Figure 5: Relative presence of the major fatty acids in mitochondrial membranes isolated from 5°C-stored and 17°C-stored bulbs.

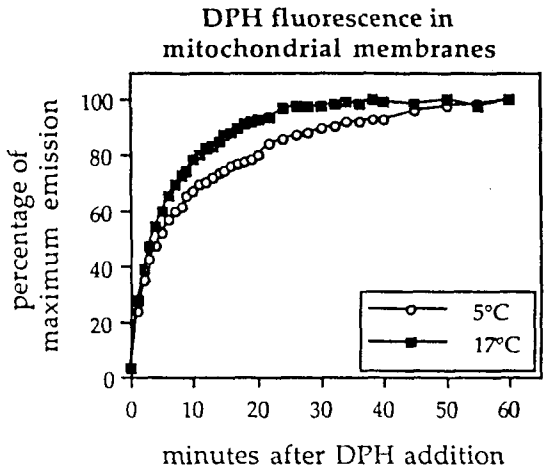

Figure 6: Incorporation of DPH in the mitochondrial membranes isolated from 5°C-stored bulbs (open circles) and 17°C-stored bulbs (solid squares) as percentage of the value after 60 minutes (maximum emission, measured at 450 nm).

Table 1: Yield of mitochondrial proteins and lipids in mg [g FW]$^{-1}$ (BSA used as standard).

	proteins	lipids	ratio
bulbs stored at 5°C	0.37	0.60	1.6
bulbs stored at 17°C	0.35	0.56	1.6