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SPECTRAL PROPERTIES OF CHLOROPLAST MEMBRANES AS A FUNCTION OF PHYSIOLOGICAL TEMPERATURES

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SUMMARY

The absorption spectrum of chloroplasts changes as a function of temperature. As chloroplasts are cooled from room temperature to 10^0C there are increases in absorption at 675, 500 and 436 nm, plus a small decrease at 685 nm. As the chloroplasts are heated to 34^0C there are decreases in absorption at 675, 500 and 436 nm plus increases in absorption at 690 and 400 nm. It is concluded that the temperature dependent change in phase of the membrane lipids (solid to liquid crystal state) modifies the state of chlorophyll aggregation.

INTRODUCTION

Chlorophyll has been used as a fluorescence probe to detect phase changes in the lipid phase of vesicles (1) and in intact photosynthetic systems (2). In the latter, the transition temperature depended on the culture conditions of the organisms.

In general the transition temperature of lipids is lower the lower the degree of bond saturation (3). Biomembranes have a high concentration of polyunsaturated fatty acids so that the lipids are primarily in the liquid crystal phase at room temperature (4). There can be a temperature range where regions of the membrane, separated laterally, are in the solid and liquid crystal states (5).

As the state of the lipid changes from solid (low temperature) to the liquid crystal (high temperature) state there is a decrease in the fluorescence yield of chlorophyll. This has been interpreted, in liposomes, as resulting from the formation of chlorophyll aggregates in the liquid crystal state (4). As the temperature increases the solubility of chlorophyll in the lipid changes and more aggregates are formed.

The absorption spectra of photosynthetic pigments in the chloroplast membrane, as a function of temperature, are described in the present work. Spectral changes

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are determined by measuring the difference in absorption between two identical samples at different temperatures.

MATERIALS AND METHODS

Chloroplasts were isolated from mature barley in 0.4M sucrose, 10mM phosphate buffer pH 7.8. The leaves were chopped in a Waring blender for 20 seconds, then squeezed through cheese cloth and centrifuged at 500xg for 5 minutes. Chloroplasts were collected by centrifuging at 1000g for 10 minutes. The resuspended chloroplasts were sonicated for about 15 seconds. This was done to reduce the rate of settling of chloroplasts (fragements) in the cuvette during absorption measurements.

A Shimadzu (MPS-5000) was used to measure absorption spectra. Difference spectra were recorded using two identical samples. One sample was heated or cooled by circulating water from a thermostated bath through the cuvette holder. The rate of heating of the chloroplast suspension was 0.5^0C/min, the rate of cooling was 0.4^0C/min. Temperature was measured with a calibrated copper-constantan thermocouple immersed in the solution.

The difference spectrum between the two identical samples yields a baseline that is complex in shape. The initial difference spectrum can be used as a 'baseline' from which to quantitatively measure changes in spectra. There is a slow drift in the 'baseline' spectrum arising from settling of the chloroplast fragments in the cuvettes. However, the relative peak heights remain unchanged. To compensate for the small drift the OD of all spectra were set equal at 730 nm where there is essentially no absorption.

The optical density of the red absorption band in each sample was between 1 and 2. Difference spectra were measured on either an OD scale of $\pm$ 0.1 or $\pm$ 0.05.

The spectral changes do not result from artifacts associated with scattering properties or settling of the chloroplast fragments. Vigorous shaking one of the sample cuvettes to thoroughly mix the chloroplast fragment suspension does not result in any spectral changes; there is only a small shift of the baseline.

RESULTS AND DISCUSSION

The details of the spectral changes depend on whether the chloroplasts are subjected first to cooling then heating of first to heating then cooling. The final spectral changes are similar but there is a pronounced hysteresis effect. The data reported here are for a cool-heat cycle.

Upon cooling the chloroplast suspension from room temperature to 10^0C there are increases in absorbance at 675, 500 and 436 nm. There is a small decrease in absorbance at 680 nm. In Figure 1 (upper curve) is shown the change in absorption spectrum upon cooling, ΔOD = OD (10^0C) - OD (25^0C).

Upon heating from 10^0C to 34^0C there is a progressive decrease in absorbance at 675 nm. At the same time there is an accompanying increase in absorbance at 690 nm. The increase in absorption at 690 nm is almost certainly associated with an increase in concentration of aggregated forms of chlorophyll, formed upon

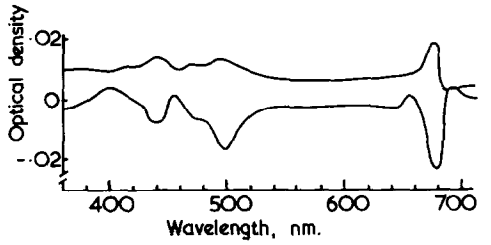

Figure 1 Difference in the absorption spectra of two identical samples of barley chloroplasts but at different temperatures. The upper spectrum is the difference in absorption between a sample at 10°C and another at 25°C; there is a general increase in absorption on cooling. The lower spectrum is the difference between a sample at 35°C and another at 25°C; there is a general decrease in absorption.

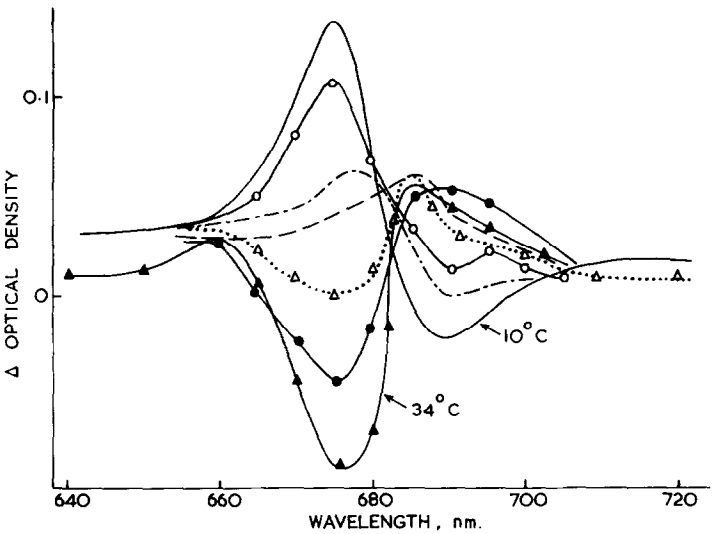

Figure 2 Change in absorption spectrum at various temperatures between 10°C and 34°C; 10°C solid line, 20°C open circles, 22°C dot-dash line, 25°C dash line, 27°C open triangles, 30°C solid circles, 34°C solid triangles.

warming. This observation would agree with the report of Lee (4) using chlorophyll containing liposome systems.

There may be an isobestic point at 682 nm (Figure 2). The net change in OD at 675 nm, of about 0.02, amounts to a total change in OD of about 1%.

During the heating cycle there are accompanying decreases in absorbance at 500, 480 and 436 nm plus an increase in absorbance at 400 nm. In Figure 1 (lower

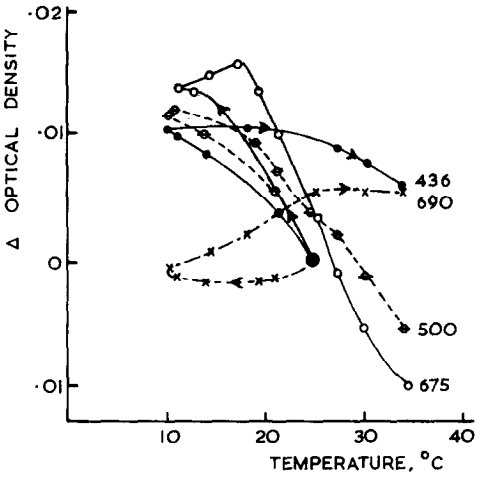

Figure 3 Change in optical density at 690, 675, 500 and 436 nm as a function of temperature. Barley chloroplasts were first cooled from 25°C to 10°C then heated to 35°C. A hysteresis effect is observed in the spectral changes at 436 and 690 nm.

curve) is shown the change in absorption spectrum upon heating, OD = OD (35°C) - OD (25°C). A notable exception to the general decrease in OD in the blue part of the spectrum, upon heating, is an increase in OD at 400 nm (Figure 1, lower curve).

The spectral changes at 690, 675, 500 and 436 nm as a function of temperature are shown in Figure 3. There is almost complete spectral reversibility 675 and 500 nm when the chloroplasts are subjected to a temperature cycle of 25°C to 10°C to 25°C. At other wavelengths the hysteresis effect is quite apparent.

Where heating is continued to 55°C the spectral changes reported above continue to grow larger. Cooling a chloroplast suspension after a heating cycle does not restore the original spectrum. There are large hysteresis effects at all wavelengths.

Hysteresis effects upon cooling and heating plant material was also observed in studies of the fluorescence of chlorophyll in situ as a function of temperature (2). The hysteresis in the spectral changes could arise from several sources. Heating in air for an extended period might cause irreversible changes in the pigments. One possible product is chlorophyll pheophytin. There is no spectral evidence, however, of the appearance of any of the bands characteristic of pheophytin.

The effects of temperature on the absorption (and fluorescence) properties of pigments in the thylakoid might, in addition, be a function of hydrogen bonding or water bonding or other weak forces. As temperature is varied, weak bonds or interactions might be altered resulting in a modification of certain types of aggregated species of chlorophyll. It is quite possible that the spectral changes are associated with irreversible changes in pigment aggregation. The lipids in the chloroplast membrane undergo a phase change, on heating, from the solid to the liquid crystal state. This phase change may directly or indirectly effect the state of chlorophyll in or associated with the membrane. Perhaps on heating, more of the chlorophyll can go into the liquid phase of the lipid, thereby increasing the chlorophyll concentration which in turn increases aggregation. These long wavelength absorbing forms of chlorophyll could act as an energy sink. The decrease in chlorophyll fluorescence from plant materials upon warming reported by Murata and Fork (2), could result from energy transfer to such a non-fluorescent energy sink.

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