Temperature Induced Spectral Changes of Chlorophyll in Micelles and Solution

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Effects of temperature on the spectral properties of chlorophyll in solution and in micelles are reported. After correcting for the volume expansion coefficient of the solvent, it is observed that temperature has no detectable effects on the spectral properties of chlorophyll (between 14 °C to 35 °C). Solutions examined include acetone, chloroform and ethyl alcohol. It is concluded that the temperature induced change in refractive index, of the solvent, has no significant affect on the chlorophyll spectrum.

In micelles containing chlorophyll there are significant temperature induced spectral changes. Heating and cooling results in an irreversible redistribution of monomeric and oligomeric forms of chlorophyll.

A. Introduction

Temperature induced changes in spectra were reported *in vivo* and for pigments in liposomes [1–3]. Changes were also reported in the fluorescence intensity of chlorophyll *in vivo* and in liposomes as a function of temperature [4, 5]. The spectral changes might originate from several sources. The present experiments are designed to determine the contribution of two of these sources. Specifically, the possible effects on the absorption spectrum of the temperature induced change in index of refraction, of the solvent, and the state of pigment oligomers in micelles.

B. Materials and Methods

The techniques used to measure the temperature induced changes in absorption spectrum were described previously [1].

Chlorophyll was prepared chromatographically and purified by recrystallization as described by Aghion *et al.* [6]. Soy bean lecithin was obtained from Sigma Chem. Co. (St. Louis, Mo.), and used without further purification.

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Micelles were prepared by injecting 200 µl, of an acetone solution of chlorophyll, into 5 ml of buffered water (5 mM phosphate, pH 7.8).

C. Results and Discussion

Chlorophyll in solution. The absorbance of chlorophyll *a* in acetone solution was examined as a function of temperature over the range 14 to 35 °C. The temperature induced spectral changes are measured relative to a chlorophyll sample maintained at 24 °C. The absorbance of the red and blue maximum increases as the temperature is lowered, and decreases as temperature is raised. Solutions of chlorophyll in chloroform or ethyl alcohol undergo similar spectral changes.

The temperature induced change in absorbance is completely accounted for by the relatively large thermal expansion coefficient of these solvents (the volume expansion coefficient for water is much smaller). The change in volume, V_t, with temperature t is given by $V_t = V_0\,(1 + at + bt^2 + ct^3)$, where a, b, c are empirically determined coefficients and V_0 is the volume at 0 °C [7].

Since the temperature induced change in the absorption spectrum of chlorophyll in solution is completely accounted for by the expansion coefficient of the solvent, the absorption coefficients and wavelengths of the absorption maxima are not significantly altered by the small change in index of refraction of acetone, chloroform or ethanol (between 10 and 36 °C). The refractive index of water changes even less over this temperature interval. Thus the temperature induced spectral changes reported *in vivo* probably do not arise from changes in index of refraction [1, 2]. Furthermore, there are no temperature induced spectral changes associated with pure chlorophyll in true solution.

Micelles. The absorption spectrum of the chlorophyll micelles has absorption maxima at 673 and 438 nm plus a shoulders at about 705 and 420 nm (Fig. 1).

As temperature is lowered there appears to be a red shift of both the red and blue absorption bands. Associated with the red band there are small increases in absorbance at 710 and 685 nm plus a decrease in absorbance at 656 nm. For the blue band there is a small increase in absorbance at 450 nm and a decrease at 430 nm. These spectral changes show an increase in absorbance on the red

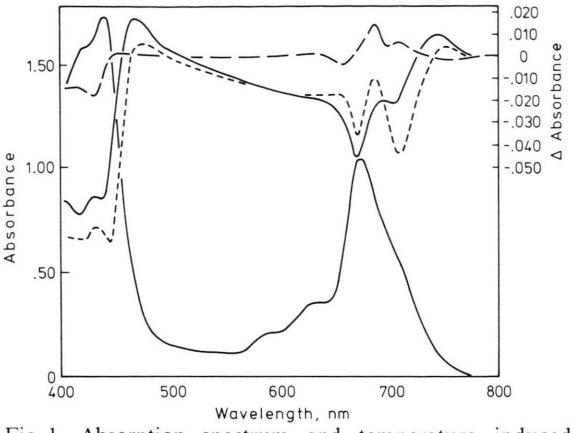

Fig. 1. Absorption spectrum and temperature induced difference spectra of chlorophyll *a* micelles. Scale for absorption spectrum is given on left. Difference spectra are measured relative to a sample held constantly at 24 °C. The difference spectrum on cooling a sample to 13 °C is shown by long dashes (——). On heating to 31 °C the difference spectrum is shown by short dashes (----). After heating a sample to 31 °C and subsequently cooling to 26 °C the difference spectrum is shown by a solid line.

side of the absorption bands and a decrease on the blue side of the bands. This is what one expects for a red spectral shift.

The volume expansion coefficient of water is quite small. While it could account for the magnitude of the increase in absorbance upon cooling, it could certainly not account for the decrease in absorbance nor the wavelengths of the spectral changes. The small temperature dependent expansion of water can not account for are relatively large decreases in absorbance observed upon heating.

As temperature is raised there is a general decrease of the red and blue absorption bands. There are decreases in absorbance at 708, 669, 440 and 417 nm. The decreases in absorbance on the long wavelength side of the absorption bands suggest a blue spectral shift.

If micelles are first heated to 33 °C and subsequently cooled to room temperature (25 °C), it appears that there is a redistribution of the relative population of chlorophyll monomers to oligomers. The increase in absorbance at 742 nm is interpreted as arising from chlorophyll oligomers; the decrease at 670 nm is associated with chlorophyll monomers. The ratio of the minima at 708 to 669 nm, does not remain constant as the temperature is changed. The direction of the change in ratio indicates that the annealing process, decreases the population of chlorophyll monomers and increases that of the chlorophyll oligomers.

In short, in micelle systems temperature can cause a redistribution of monomeric and oligomeric forms of chlorophyll.

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