208 © 1983 The Chemical Society of Japan Bull. Chem. Soc. Jpn., 56, 208—211 (1983) [Vol. 56, No. 1

Temperature Dependence of Absorption Spectra of Chlorophyll a in Hydrocarbons over the Temperature Range 300—77 K. Enthalpy and Entropy Changes for the Formation of Dimeric Chlorophyll a

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(Received May 17, 1982)

The absorption spectra of chlorophyll a in isopentane, 3-methylpentane, and methylcyclohexane solutions were studied over the temperature range 300—77 K. In those hydrocarbon solutions which were prepared using methanol treatment, chlorophyll a forms hydrated dimer at low temperatures. The dimer has an absorption peak at 695 nm at 160 K, which gradually shifts to 706 nm with decreasing temperature from 160 K to 77 K. The enthalpy change ($-\Delta H$) for the formation of the dimer was obtained to be 41.0 ± 0.8 kJ mol^{-1}, independent of the solvent used. The entropy changes ($-\Delta S$) were 113 ± 8 J K^{-1} mol^{-1} for isopentane, 118 ± 8 J K^{-1} mol^{-1} for 3-methylpentane and 128 ± 8 J K^{-1} mol^{-1} for methylcyclohexane solutions. Comparative studies carried out of the solutions prepared without the methanol treatment suggest the existence of two kinds of hydrated monomeric chlorophyll a.

Chlorophyll (Chl) a is the best understood of Chl's[1)] and has been subject to a variety of studies in connection with the photochemical conversion of solar energy. The optical absorption spectrum of Chl a is known to vary markedly with nature of solvent, temperature, and degree of hydration and aggregation.[2,3)]

The absorption spectra of Chl a reported so far are in good agreement with one another as far as polar solvents are used. In nonpolar solvents, on the other hand, the absorption spectrum varies substantially depending on the procedures of preparing the solutions, in particular at low temperatures. Cotton *et al.*[4)] reported that the amount of water causes substantial changes in the absorption spectrum of Chl a in hydrocarbon solvents. For instance, the absorption spectrum of Chl a in dry methylcyclohexane+isopentane shows only a slight shift of the peak wavelength from 672 nm at room temperature to 678 nm at 77 K, which is in sharp contrast to the absorption spectral change observed in a toluene solution containing 2 mol H_2O per mol Chl a. For the latter solution, a new absorption band centered at 708 nm was observed to appear with a slight shift of the 667-nm peak to 672 nm at 77 K.

Fong and Koester[5)] reported the temperature dependence of the absorption spectrum of Chl a in a 1:1 methylcyclohexane+pentane mixture, in which the absorption peak in the red band region is located at 667 nm at room temperature. The spectrum observed at 121 K indicates that the major Chl a shows the absorption peak at 702.5 nm. This is the first observation that a greater part of Chl a in the hydrocarbon mixture is transformed into a $\approx$700 nm absorbing species at low temperatures.

The formation of 706-nm absorbing species of Chl a (S706) and the optical absorption and ESR studies of its cation radical produced by γ-irradiation, both in glassy 3-methylpentane solutions at 77 K, were reported by Hoshino *et al.*[6)] The comparative study on the ESR spectra of the cation radicals of the S706 and monomeric Chl a demonstrates that the S706 is a dimeric Chl a. This result is consistent with the suggestion made by Brody and Broyde[3)] that the absorption band centered at around 700 nm appearing at low temperatures is attributed to dimeric Chl a.

The present paper reports the temperature dependence of the absorption spectra of Chl a in isopentane, 3-methylpentane, and methylcyclohexane solutions over the temperature range 300—77 K, and the thermodynamic data for the formation of the dimers.

Experimental

Chl a extracted from spinach leaves with acetone was chromatographed on powdered sugar columns according to standard procedures.[7)] Solid Chl a obtained from a Chl a zone of a last column was chromatographed with petroleum ether containing 0.5% 2-propanol as developer. The purified Chl a was dissolved in dry diethyl ether and stored in a freezer at −20 °C. The absorption spectrum of the stock solution had absorption peaks at 428.6 and 660 nm with a ratio of the Soret to red absorption maxima of 1.29 at room temperature.

Isopentane, 3-methylpentane, and methylcyclohexane were distilled and stored on Na–K alloy *in vacuo* in order to remove traces of water.

Absorption spectra were recorded on a Cary 14R spectrophotometer. The temperature was controlled by using an Oxford CF204 cryostat. Optical pathlength of cells were 0.5—1.5 mm.

A suitable volume of the Chl a stock solution was transferred into a cell, and diethyl ether was removed on a vacuum line. After pumped at 10^{-6} Torr (1 Torr=133.322 Pa) at room temperature for 10—20 min, the cell was detached from the vacuum line and freshly distilled methanol was added into the cell. The solid Chl a was completely dissolved in methanol by shaking the cell for a few min. The sample cell was again attached to the vacuum line to remove methanol completely under pumping to 10^{-6} Torr for 10—20 min. This procedure using methanol will be referred to as methanol treatment. The solution was cooled to 77 K and sealed off under pumping.

Comparative studies were also carried out on the Chl a solutions prepared by omission of the methanol treatment described above; unless otherwise specified, the Chl a solutions were prepared using the methanol treatment.

The concentration of Chl a was *ca.* 10^{-4} mol/dm^3 for all the solutions used in the present study.

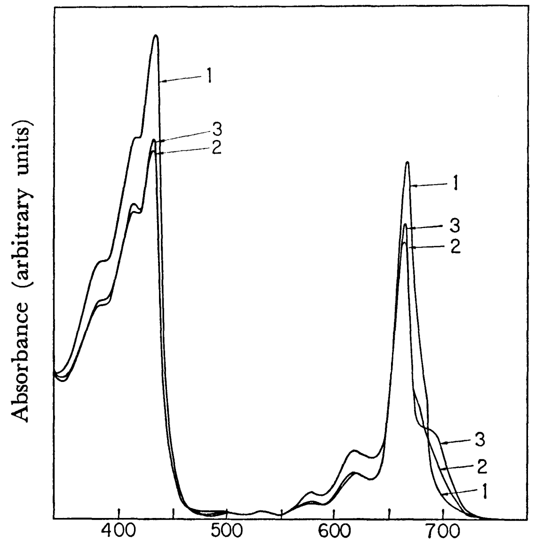

Fig. 1. Temperature-dependent absorption spectra of 3-methylpentane solution of Chl a in the temperature range 300—240 K.
1): 300 K, 2): 270 K, 3): 240 K.

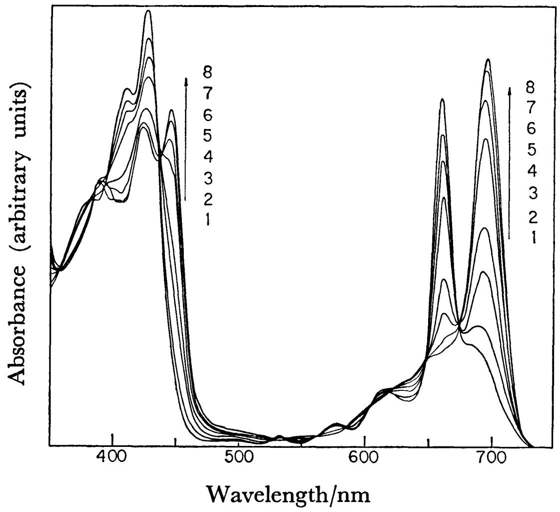

Fig. 2. Temperature-dependent absorption spectra of 3-methylpentane solution of Chl a (the same solution as that in Fig. 1) in the temperature range 240—160 K.
1): 240 K, 2): 219 K, 3): 210 K, 4): 200 K, 5): 190 K, 6): 179 K, 7): 170 K, 8): 161 K.

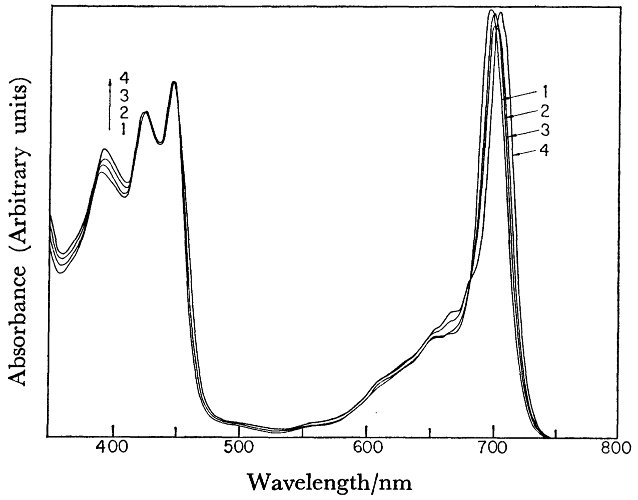

Fig. 3. Temperature-dependent absorption spectra of 3-methylpentane solution of Chl a (the same solution as that in Fig. 1) in the temperature range 150—95 K.
1): 150 K, 2): 130 K, 3): 110 K, 4): 95 K.

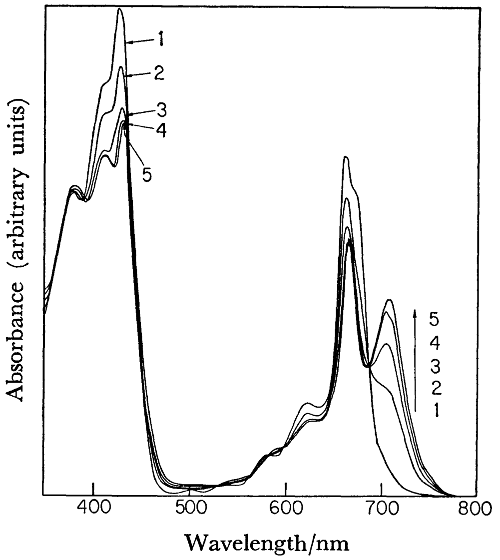

Fig. 4. Temperature-dependent absorption spectra of 3-methylpentane solution of Chl a prepared without methanol treatment (see text) in the temperature range 260—180 K.
1): 260 K, 2): 240 K, 3): 220 K, 4): 200 K, 5): 180 K.

Results

Figure 1 shows the absorption spectra of Chl a in 3-methylpentane in the temperature range 300—240 K. The absorption spectrum at 300 K has an absorption peak at 662 nm with a shoulder at 680 nm in the red-band region. No isosbestic points were observed for the spectral change of the Chl a solution in this temperature range. The complicated features observed for the temperature-dependent absorption spectra are essentially the same as those reported.[5]

Figure 2 shows the temperature-dependent absorption spectra of Chl a in 3-methylpentane in the temperature range 240—160 K. A series of absorption spectra measured in this temperature range evidently shows several isosbestic points, indicating a simple equilibrium between the species absorbing at 663 and 695 nm. The absorbance at 695 nm increases with decreasing intensity of the peak at 663 nm, which ultimately vanishes at 160 K.

The spectral change below 160 K is shown in Fig. 3. The 695 nm peak at 160 K shows only a slight shift toward long wavelengths without showing isosbestic points with decreasing temperature; no equilibrium seems to exist in this temperature range. The peak wavelength ultimately reached 706 nm at 77 K.[6]

The temperature-dependent absorption spectra were also studied on isopentane and methylcyclohexane solutions. The features of the spectral change are essentially the same as those observed for the 3-methyl-

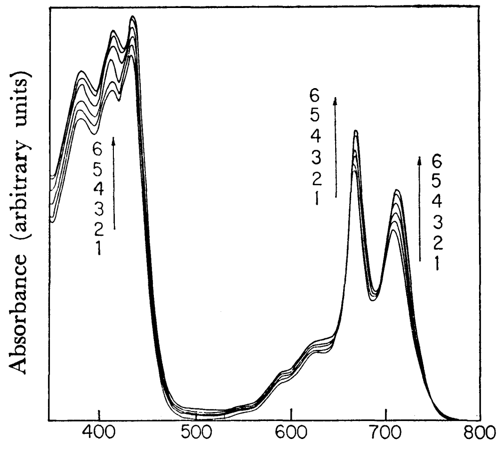

Fig. 5. Temperature-dependent absorption spectra of 3-methylpentane solution of Chl a (the same solution as that in Fig. 4) in the temperature range 180—95 K. 1): 180 K, 2): 160 K, 3): 140 K, 4): 120 K, 5): 100 K, 6): 95 K.

pentane solution; the 663 nm band vanishes at 160 K and a subsequent red shift of the 700 nm band was also observed below 160 K.

Different temperature-dependent absorption spectra were observed for the 3-methylpentane solution of Chl a prepared by omission of the methanol treatment. Typical examples are shown in Figs. 4 and 5. Figure 4 shows the spectral changes in the temperature range 260—180 K. The absorption spectrum at room temperature has an absorption peak at 663 nm with a shoulder at 675 nm. With decreasing temperature, the intensity at 663 nm decreases with a slight shift to 667 nm and a new absorption band appears around 706 nm with an isosbestic point at 678.5 nm. Below 180 K, however, the 667 nm band does not vanish but the intensities of both 667 nm and 706 nm bands increase accompanying slight red shift with decreasing temperature as shown in Fig. 5; their ultimate peak wavelengths reached 668 and 709 nm at 77 K. These results present a striking contrast to those obtained with the Chl a solutions prepared using methanol treatment. These differences will be discussed in the next section.

Discussion

Two Kinds of Hydrated Monomeric Chl a. Fong and Koester⁵⁾ reported that Chl a in a hydrocarbon mixture could be transformed into a 702.5 nm absorbing Chl a as a major species at 121 K. Wasielewski *et al.*⁸⁾ found that bis(chlorophyllide)ethylene glycol diester in carbon tetrachloride forms a species absorbing at 697 nm at room temperature when the solution was bubbled with wet nitrogen gas. This fact implies that water molecules play an important role in the formation of the ≈700 nm absorbing Chl a. Shipman *et al.*⁹⁾ studied the temperature dependence of the absorption spectra of 9.4×10^{-2} mol/dm^3 Chl a solutions in toluene containing 1.5 mol ethanol per mol Chl a and reported the formation of the 702 nm absorbing Chl a as a single species at 175 K. These results indicate that monosolvated Chl a, Chl a·H_2O and Chl a·C_2H_5OH can be converted into the ≈700 nm absorbing species at low temperatures. The structure of the Chl a dimer has been considered to be C_2-symmetrical.¹⁰⁻¹²⁾

The present results show that Chl a in 3-methylpentane has only one absorption peak at 695 nm in the red-band region at 160 K. Since the 695-nm peak grows with decreasing intensity of the absorption at 663 nm from 240 to 160 K, the 663-nm absorbing species is regarded as a precursor of the 695-nm absorbing species. The spectral shift from 695 to 706 nm observed at 160—77 K may be due to some configuration changes of the dimeric Chl a in glassy matrices by cooling. It should be noted that the peak wavelengths reported for the dimeric Chl a are ranged from 695 to 710 nm depending upon the solvent and temperature.

In the present study, we have reasonably concluded that the 695-nm absorbing species at 160 K is a single species from the temperature dependence of the absorption spectra in which clear isosbestic points are observed.

We sometimes observed that Chl a solutions prepared without the methanol treatment show essentially the same temperature dependence as that shown in Figs. 1—3. These observations imply that a trace amount of methanol, which might remain in the samples prepared by using the methanol treatment, is not responsible for the formation of the 695 nm absorbing species at 160 K. The methanol treatment is considered to provide a simple and reproducible procedure for preparing the Chl a solutions which produce 695-nm absorbing species at low temperatures.

The results on the Chl a solutions in 3-methylpentane prepared with and without the methanol treatment seem to indicate that there would be at least two kinds of monomeric Chl a in the hydrocarbon solution: one does form the dimeric Chl a (S706) and other does not. Katz *et al.*¹⁾ examined IR spectra in the OH-stretching region for a nonpolar solution of Chl a containing water, and suggested the presence of two kinds of Chl a species: $\mathrm{Mg}\cdots\mathrm{O}<^{\mathrm{H}}_{\mathrm{H}}$ and $\mathrm{Mg}\cdots\mathrm{O}<^{\mathrm{H}}_{\mathrm{H}\cdots\mathrm{O}<^{\mathrm{H}}_{\mathrm{H}}}$.

On the basis of their suggested species for the hydrated Chl a, the present results may be interpreted by assuming that the solutions not treated with methanol contain both monohydrated and dihydrated species, the latter of which does not form dimeric Chl a at low temperatures. Supposedly, the methanol treatment used for the preparation of the solutions serves to adjust the water content appropriate for the dimer formation. Since the water content is considered to be not always reproducible for the solutions without the methanol treatment, the complete or nearly complete dimer formation was only sometimes observed for these solutions. In fact, addition of trace amount of water into the methanol treated solutions was found to give similar spectral changes to those shown in Figs. 4 and 5.

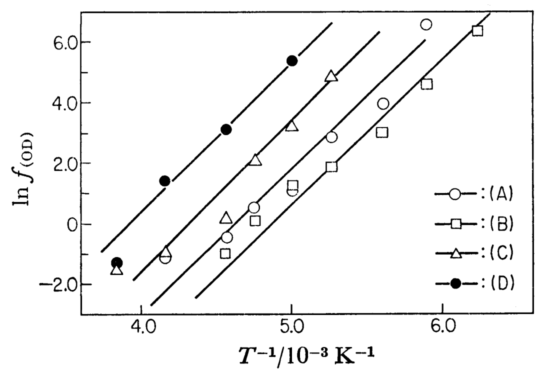

Fig. 6. Plots on $\ln f$(A) *vs.* T^{-1} for Chl a solutions. (A): 3-Methylpentane, (B): methylcyclohexane, (C): isopentane. A plot (D) was obtained for 3-methylpentane solution of Chl a prepared without methanol treatment.

Enthalpy and Entropy Changes for the Dimer Formation. The spectral changes shown in Figs. 1 and 2 are interpreted in terms of two step equilibria:[5]

$$(\text{Chl a})_2 + 2\text{H}_2\text{O} \rightleftharpoons 2\text{Chl a}\cdot\text{H}_2\text{O} \rightleftharpoons (\text{Chl a}\cdot\text{H}_2\text{O})_2.$$

The complicated features of the spectral change observed in the temperature range 300—240 K are considered to result from the above two equilibria in the solution. As the temperature decreases below 240 K, the equilibrium shifts toward the right-hand side. The temperature dependence of the spectrum observed between 240 and 160 K is regarded as being due to the equilibrium between Chl a·H_2O (M) and (Chl a·$H_2O)_2$ (D). The equilibrium constant, K, is defined as

$$K = [\text{D}]/[\text{M}]^2. \quad (1)$$

Below 160 K, M is completely transformed into D, and therefore,

$$2[\text{D}] + [\text{M}] = 2[\text{D}]_0, \quad (2)$$

where $[\text{D}]_0$ is the concentration of D at 160 K. From Eqs. 1 and 2,

$$K = [\text{D}]/4([\text{D}]_0-[\text{D}])^2. \quad (3)$$

Using the molar extinction coefficient of D at 700 nm (ε) and an optical pathlength (l), one obtains

$$K = \varepsilon l A_\text{T}/4(A_{160}-A_\text{T}) = \varepsilon l\cdot f(\text{A})/4, \quad (4)$$

where A_{160} and A_T are the absorbances at 700 nm at 160 K and T K, respectively.

Equation 4 is transformed into

$$\ln f(\text{A}) = [\Delta S/R - \ln(\varepsilon l/4)] - \Delta H/RT. \quad (5)$$

Plots of $\ln f$(A) *vs.* T^{-1} for the isopentane, 3-methylpentane, and methylcyclohexane solutions are shown in Fig. 6; the data obtained with the 3-methylpentane solutions prepared without the methanol treatment are also plotted for comparison. The enthalpy changes (ΔH) are identical within the experimental errors for these solutions, 41.0±0.8 kJ mol^{-1} (9.8±0.2 kcal mol^{-1}). From the intercept and ε (9.6×10^4 mol^{-1} dm^3 cm^{-1}), the entropy changes (ΔS) are calculated to be 113±8, 118±8, and 128±8 J K^{-1} mol^{-1} for the isopentane, 3-methylpentane, and methylcyclohexane solutions, respectively. The linear plot D in Fig. 6 implies that the monohydrated Chl a existing in the solution forms dimers independently of the coexisting dihydrated species.

The enthalpy change obtained for the dimer formation is double the enthalpy change (4—6 kcal mol^{-1}) estimated for the formation of one hydrogen bond of the type OH···O.[13] This result is in accord with the assumption that a dimeric Chl a has two hydrogen bonds between magnesium atoms and carbonyl groups of two Chl a molecules.

Pheophytins are known to dimerize in hydrocarbon solutions at low temperatures.[14] An estimated enthalpy change of 18 kJ mol^{-1} for the formation of the dimeric pheophytins is about one half that obtained for Chl a. This value also supports the view that two hydrogen bonds serve as principal interaction force between two Chl a molecules in the dimeric Chl a. Slightly different values for the entropy change may be ascribed to somewhat different configurations of the dimer structure in different solvents.

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