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VISIBLE ABSORPTION SPECTRA OF CHLOROPHYLL b IN METHANOL, DIOXANE AND/OR WATER SOLUTIONS

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Chlorophyll b (Chl-b) in methanol solution absorbing at 650 nm (Cb650) forms microcrystals absorbing at 680 nm after the addition of water. In methanol-dioxane-water solutions. Chl-b molecules form an aggregate absorbing at 672 nm (Cb672). Cb672 was obtained either by mixing a small volume of a methanol-dioxane solution of Chl-b with a large volume of water, or by mixing a large volume of methanol-water solution of the pigment with a small volume of dioxane.

INTRODUCTION

It has been known that in the red wavelength region chloroplasts have many absorption maxima derived from chlorophyll molecules. However, when purified chlorophyll a (Chl-a) and chlorophyll b (Chl-b) are dissolved in an appropriate solvent, one gaussian curve of the red absorption which means the existence of one chemical substance has ever been found for Chl-a and Chl-b respectively. Therefore, it has been suggested that Chl-a molecules in vivo exist in two1) or six2,3) different forms. On the other hand, Chl-b molecules exist in only one4) or two5) different forms. Such many forms of Chl-a in vivo have been presumed to arise from complex formation of chlorophyll with protein6) or to be simply due to different kinds of chlorophyll aggregation.7) There have been many reports on the aggregation of Chl-a in vitro. It has been known, for examples, that dioxane interacts with Chl-a to form Chl-a-dioxane adducts8-10) and its visible absorption spectrum is red-shifted relative to that of dimer (Chl-a_2) found in acetone-dioxane-water solution.8,9) Recently Dijkmans11) also reported the transformation between Chl-a aggregates appearing in solvents of water and methanol mixed with different ratio. In his paper, he reported the formation of the four species; Ca670, Ca710, Ca745 and colorless form. Little is known, however, about the properties of Chl-b in solutions compared with Chl-a. The difference of chemical structures between Chl-a and Chl-b is only one functional group at position C-3. Nevertheless, the function and location of Chl-b in chloroplast are different from those of Chl-a.

In this paper, we describe the transformation between Chl-b aggregates appearing in methanol, dioxane and/or water solutions.

EXPERIMENTAL

Chl-b was prepared from fresh spinach leaves in darkness as follows; A partially purified preparation of chlorophylls was separated from an original crude mixture of chlorophylls and other yellow pigments in aqueous methanol, according to the so-called "dioxane method" (K. Iriyama, N. Ogura and A. Takamiya; to be published elsewhere). Then Chl-b was separated by passing the product through a column of powdered sugar according to the method of Perkins and Roberts.[12] The same absorption spectrum of Chl-b as that of Comar and Zscheile[13] was obtained. The molar extinction coefficient as reported by Comar and Zscheile[13] was used for determination of the concentration of Chl-b. All solvents purchased were analytical reagent grade, and further purified by the usual methods. Water solution described below was 10^{-3}M phosphate buffer, pH8. Absorption spectra were measured with a Hitachi 356 Two-Wavelength Double Beam Spectrophotometer.

RESULTS AND DISCUSSIONS

In Fig.1 are shown spectra of Chl-b 1 hr after preparation (1) in methanol, (2) in a mixture of methanol and dioxane (10:90, V/V), (3) in a mixture of methanol, dioxane and water (50:10:40, V/V/V), and (4) in a mixture of methanol and water (60:40, V/V). The red absorption maximum of Chl-b in methanol was located at 650 nm; [Fig.1 (1)]. The shape and positions of major peaks in this spectrum are generally in agreement with the results of Mackinney.[14] After adding dioxane to a solution of Chl-b in methanol, the red absorption maximum was shifted from 650 nm to 648 nm as shown in Fig.1 (2). The red absorption maximum of Chl-b in dioxane solution was located at 643 nm; Cb643. The red absorption maximum of Chl-b in the mixture of methanol and dioxane changed reversiblly from 643 to 650 nm, as the methanol concentration was increased. Perhaps the spectral changes may be due to the solvent effect. The absorption spectrum of the species Cb672 absorbing at 672 nm [Fig.1 (3)] was obtained, whenever methanolic solutions of Chl-b were mixed with aqueous dioxane or solutions of Chl-b in aqueous methanol were mixed with a small amount of dioxane. Although it was reported by several authors[15,16] that a species (Ca670) absorbing at 670 nm was obtained after adding a small amount of water to a solution of Chl-a in methanol, no such a distinct shift was observable when a small amount of water was added to a methanolic solution of Chl-b. Ballschmitter and Katz[16] reported that Ca670 binds six water molecules per Chl-a molecule and has an absorption maximum at 670 nm. We also obtained the same observation. However, after adding water to a solution of

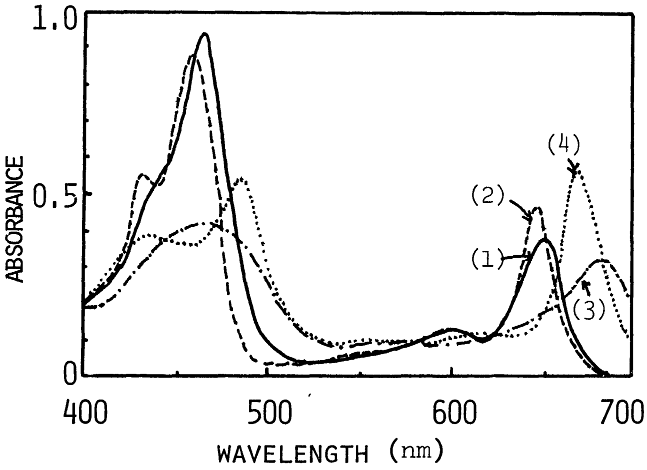


Fig.1. Absorption spectra of chlorophyll b. (1)———; chlorophyll b dissolved in methanol, (2)-------; after adding dioxane to (1), (3)—·—·—·; chlorophyll b dissolved in aqueous methanol, (4)··········; chlorophyll b in solvent containing methanol, dioxane and water.

Chl-b in methanol until the final volume proportion of water was 40 %, the red absorption maximum shifted from 650 nm to 680 nm; Cb680. [Fig.1 (4)]. After one day, we observed visible granules of Chl-b sedimented at the bottom of spectrophotometer cell. The absorption spectral changes (Cb650 → Cb680) as a function of time are shown in Fig.2. The formation of Cb680 was completed within one hour. Of interest are two isobestic points at 637 nm and 667 nm. The red absorption maximum of Cb680 was considerably broad and lower in height compared with that of Cb650. A colorless-form was observed about 6 hrs after preparation of a solution of Chl-b in a methanol-water mixture (60:40, V/V), as reported in the case of Chl-a by Dijkmans11): the absorption decrease could be due to an ordinary " sieve effect".17) This is consistent with the fact that on prolonged standing the colorless-form preparation yielded a sediment of visible granules of Chl-b as mentioned above. When the solution having the red absorption maximum at 680 nm was diluted with excess methanol, the maximum shifted to 670 nm. The reverse reaction was not possible.

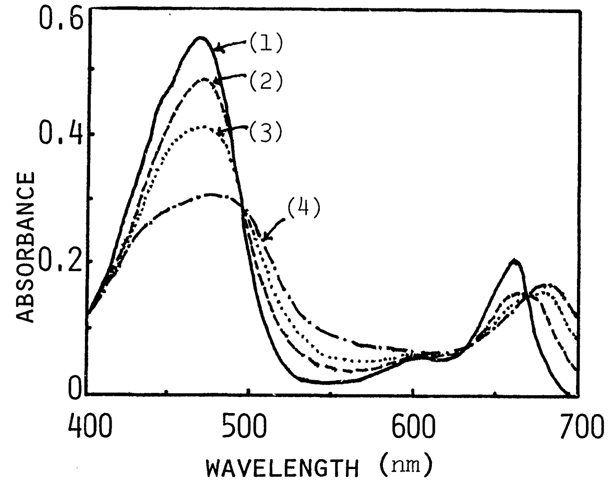
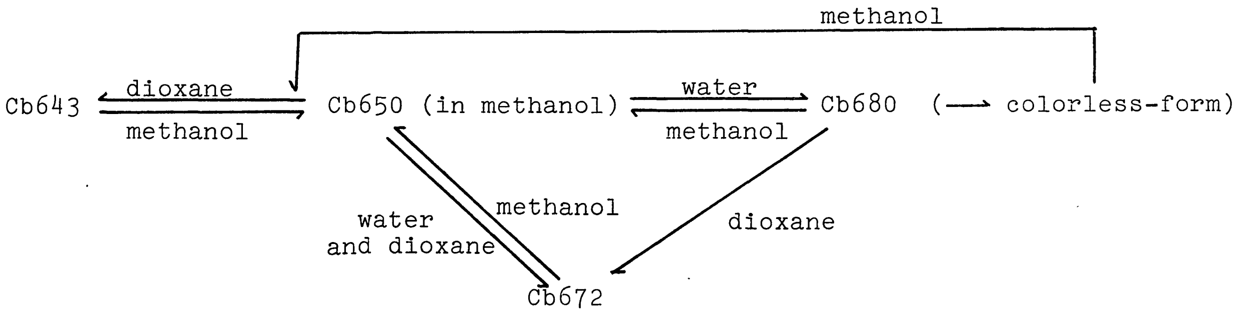


Fig.2. Absorption spectra of the transformation of chlorophyll b. Species Cb650 → Cb680 in 60 % methanol. (1) ——— ; preparation after 3 min, (2) --------; preparation after 22 min, (3) ·········; preparation after 30 min, (4) —·—·—·—; preparation after 60 min.

After adding excess methanol to the solution having a red absorption maximum at 672 nm, the maximum shifted to nearly 650 nm.

A plausible scheme for the various forms of Chl-b under the various solvent conditions was proposed on the basis of the above observations as follows;



As described above, Chl-b in solutions can form the various aggregated states. However it was reported by Thomas and Bretschneider4) that more than a single Chl-b complex was not observed *in vivo*. On the contrary, it has been decided that Chl-a *in vivo* exists in two1) or six2,3) different forms. It is very important subject to investigate the essential differences of the existence-form and functional role between Chl-a and Chl-b in chloropast.

The preparation and properties of aggregated state of Chl-a in aqueous dioxane (Ca680) were studied by Love and Bannister.8) In their paper it was reported that the formation of Ca685 requires the Mg atom. We also found that the formation of Cb672 requires the Mg atom.

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