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Solubilization of Chlorophyll a-Dioxane Complex in Water by Polyvinyl Alcohol

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The chlorophyll a (Chl a)-dioxane complex was solubilized in water by binding to polyvinyl alcohol (PVA), which resulted in Chl a-dioxane-PVA colloid. The absorption and fluorescence spectra of the aqueous solution of the Chl a-dioxane-PVA colloid were obtained. They were compared with the spectra of the Chl a in an aqueous dioxane (35%) solution and the Chl a-PVA complex in water.

Dioxane is well known to produce complexes with chlorophylls (Chls) which have characteristic absorption spectra.[1-6] The important features of the Chl-dioxane interaction model are that dioxane is bonded to the central magnesium atom and interacts with aggregates rather than single molecules of Chl, and the central magnesium atom has a coordination number of six in the complexed state.[5,6] The Chl-dioxane complexes have been investigated in aqueous dioxane solution[1-3] or the solid state.[4-6] As described in the present paper, however, we have succeeded in solubilizing the Chl a-dioxane complex in water (not containing dioxane) with the aid of PVA. Thus, the complexes will be able to be investigated in water medium which is the case *in vivo*.

Chl a was prepared from spinach leaves by the column chromatographic separations with DEAE-Sepharose CL-6B and Sepharose CL-6B.[7] PVA 117, manufactured by Kuraray Co., Ltd., with 98.5 mol-% saponification was separated into 5 fractions by the addition of 1-propanol into 1.5% aqueous solutions. The weight and number average molecular weights ($\bar{M}w$ and $\bar{M}n$) of the PVA fraction used were estimated to be 111400 and 78100, respectively by gel permeation chromatography; $\bar{M}w/\bar{M}n = 1.43$. The fractionation of PVA, however, may be not so crucial to the successful solubilization of Chl a-dioxane complex. Dioxane of a special grade reagent furnished by Kanto Chemical Co., Inc. was used without

further purification.

The procedure of solubilization of the Chl a-dioxane complex is as follows. Dioxane (0.5 ml) dissolving 0.1 mg of Chl a was added dropwise to 1.4 ml of an aqueous PVA (7.4%) solution with stirring. The solution was evaporated to a green film under a reduced pressure (30 mmHg) at room teperature. A small amount of water was added onto the film, and stirred gently until a green paste was formed. The paste was diluted with water to a given concentration of Chl a-dioxane complex binding to PVA (Chl a-dioxane-PVA colloid), and then filtered with a glass filter.

The Chl a-PVA complex[8,9] was prepared as follows. Diethyl ether (5 ml) dissolving 1 mg of Chl a was added to 5 ml of an aqueous PVA (10%) solution. After the mixture was shaken violently, diethyl ether was removed by evaporation under a reduced pressure (30 mmHg) with continuous shaking until no smelling of diethyl ether, and the aqueous solution of Chl a-PVA complex was prepared. It was finally filtered with a glass filter.

Absorption spectra at room temperature were measured with a double beam spectrophotometer, UVIDEC-510 (Japan Spectroscopic Co., Ltd.). Fluorescence-emission spectra (uncorrected) at room temperature were measured with a Hitachi Fluorescence Spectrophotometer 850; excitation wavelength was 430 nm and a band width, 5 nm.

Figure 1 shows the absorption spectrum of the Chl a-dioxane-PVA colloid in water, together with those of Chl a-PVA complex in water and Chl a in an aqueous dioxane (35%) solution. Chl a in an aqueous dioxane (35%) solution has a red peak at 680 nm and blue peaks at 390, 415, 430, and 440 nm. The red peak is very narrow; the half band width is 22 nm. The intensity ratio of blue peak to red peak, A_{440}/A_{680}, is 0.80. This spectral feature corresponds to that of the Chl a-dioxane complex reported by many investigators.[1-6] Therefore, it is thought that Chl a in an aqueous dioxane (35%) solution surely forms the Chl a-dioxane complex. The Chl a-dioxane-PVA colloid has a red peak at 683 nm and blue peaks at 390, 416, and 443 nm; the half band width of the red peak is 27 nm, and A_{443}/A_{683} = 0.91. This spectral feature corresponds with that of Chl a in an aqueous dioxane (35%) solution. On the other hand, the Chl a-PVA complex has an absorption spectrum different from that of Chl a in an aqueous dioxane (35%) solution. That is, it has a red peak at 672 nm and blue peaks at 390 and 421 nm. The red peak is very broad; the half band width is 52 nm. The ratio, A_{421}/A_{672} is 1.8.

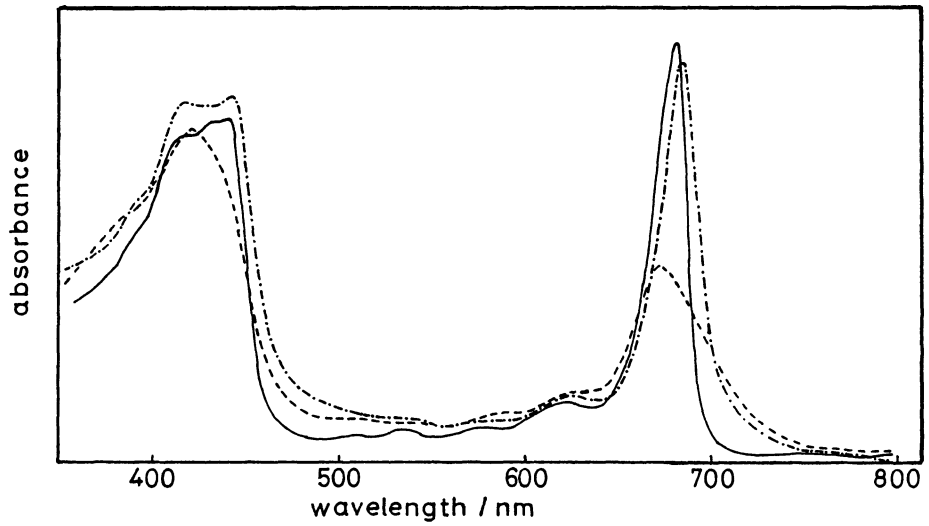

Fig. 1. Absorption spectra of the Chl a-dioxane-PVA colloid in water, the Chl a-PVA complex in water, and the Chl a in aqueous dioxane (35%) solution at room temperature. -·-·-: Chl a-dioxane-PVA colloid in water; molecular weight ($\bar{M}w$) of PVA is 111400, molar ratio PVA/Chl-a is 28, - - - -: Chl a-PVA complex in water; $\bar{M}w$ of PVA is 111,400, molar ratio PVA/Chl-a is 4.0, ———:Chl a in aqueous dioxane (35%) solution. Concentration of Chl a is 10 μg/ml in all cases.

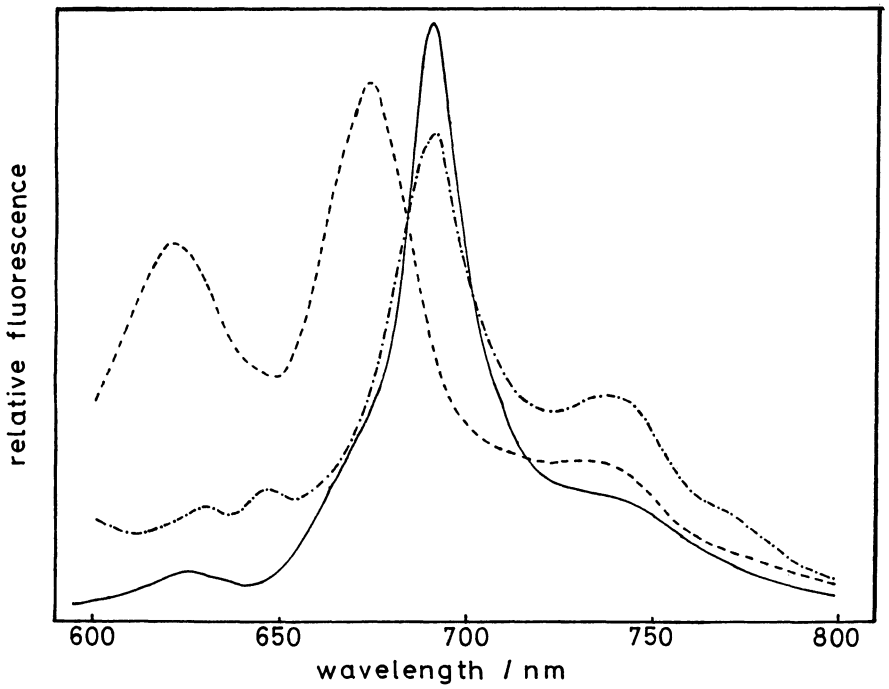

Fig. 2. Fluorescence spectra of the Chl a-dioxane-PVA colloid in water (-·-·-), the Chl a-PVA complex in water (- - - - -), and the Chl a in aqueous dioxane (35%) solution (———) at room temperature. The excitation wavelength was 430 nm. The samples and conditions are the same as indicated in the caption of Fig. 1.

Figure 2 shows the fluorescence spectrum of the Chl a-dioxane-PVA colloid in water, together with those of Chl a-PVA complex in water and Chl a in an aqueous dioxane (35%) solution. The fluorescence spectrum of the Chl a-dioxane-PVA colloid apparently corresponds to that of Chl a in an aqueous dioxane (35%) solution. They have a narrow fluorescence peak at about 692 nm. On the other hand, the Chl a-PVA complex has a broad fluorescence peak at 673 nm.

From the results described above, it is concluded that the Chl a-dioxane complex was solubilized in water by binding to PVA. In view of the narrow red peaks in absorption and fluorescence spectra of the Chl a-dioxane-PVA colloid, the Chl a involved in this colloid forms no other Chl a species than Chl a-dioxane complex. It is inferred that the association of the Chl a-dioxane complex with PVA contains the interaction between hydroxyl group of PVA and Mg of Chl a together with the hydrophobic interactions between the main chain of PVA and phytol group of Chl a.

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