SHORT COMMUNICATIONS

Preparation and Properties of the Water-Soluble Chlorophyll-Dextran Complexes. Interaction between Chlorophyll and Dextran

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(Received February 15, 1988)

KEY WORDS Dextran / Chlorophyll / Complex / Interaction /
Solubilization / Stabilization / Gel Chromatography / Spectroscopy /

We devised a preparation method of water-soluble chlorophyll (Chl)-macromolecular complexes.¹⁻³ They were found valuable from the following viewpoints: solubilization of Chls in water,¹⁻⁶ stabilization of Chls or Chl aggregates,¹⁻⁶ industrial utilization such as Chl electrode film,⁷ and etc. The macromolecules used so far for preparing the complexes are classified as either synthetic linear macromolecules such as poly(vinyl alcohol) (PVA), poly(vinylpyrrolidone) (PVP) or poly(ethylene glycol) (PEG), and naturally-occurring globular proteins such as bovine serum albumin (BSA). The amphiphilic nature of these polymers was found important for interacting with Chls and dissolving the complexes in water.

Dextran is a naturally-occurring polysaccharide consisting of α -D-glucose, being a linear branched macromolecules. Therefore, it is interesting to ascertain whether this polymer can interact with Chl and form a water-soluble Chl-macromolecular complex as well as the other macromolecules described above. As described in the present paper, we recently prepared new water-soluble Chl *a* or *b*-dextran complexes, and their properties were investigated by gel chromatography, and absorption and fluorescence spectroscopies.

EXPERIMENTAL

Materials

Chl *a* and *b* were prepared from spinach leaves by column chromatographic separations with DEAE-Sepharose CL-6B and Sepharose CL-6B.⁸ Dextran (average molecular weight, 75000) was purchased from Wako Pure Chemical Industries, Ltd., and used without further purification.

Preparation of Chl-Dextran Complexes

The procedure for preparing Chl-dextran complexes was according to our method A-1³; at step (1), methanol (2 ml) containing 360 μ g of Chl was added to 2 ml of an aqueous dextran (7.2%) solution.

Gel Chromatography

Gel chromatography of the aqueous Chl *a*-dextran complex solution thus prepared was performed as follows. 2.6 ml of the complex solution (molar ratio dextran/Chl, 4.8; 5% Chl+dextran wt/solution vol) were applied onto a column (3.1 $\times$ 49 cm) of Sephadex G-100 equilibrated with distilled water, and eluted with distilled water at a flow rate of 1 ml min⁻¹. The content of Chl *a* involved in the eluted fraction (3 ml) was monitored by

measuring the absorbance of red peak, and the amount of dextran by weighing the fraction after evaporation, which contained in some cases a negligible content of Chl *a*.

Spectroscopy

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 wavelengths were 430 nm and 460 nm for Chl *a* and *b*-dextran complexes, respectively, and a band width was 5 nm.

RESULTS AND DISCUSSION

Figure 1 shows the elution pattern of gel chromatography of the aqueous Chl *a*-dextran complex solution. Chl *a* was observed in the range of fractions 34–43. The ratio dextran/Chl in the eluted fraction gradually increased as the fraction number grew, and the ratio in fraction 35 was determined to be about 1.1. Dextran was still eluted in the range of fractions 44–50 where Chl *a* was not observed. Such an elution pattern, similar to that of the

Figure 1. Elution pattern of gel chromatography with Sephadex G-100 of Chl *a*-dextran complex. 2.6 ml of the complex solution (molar ratio dextran/Chl, 4.8; 5% Chl + dextran wt/solution vol) were applied onto a column (3.1×49 cm) of Sephadex G-100 and eluted with distilled water at a flow rate of 1 ml min^{-1} .

Chl-PVP complex previously reported,² indicates that the Chl *a*-dextran complex would be larger than the unreacted dextran and that it eluted faster than dextran. It is inferred that, in the range of fractions 34–43, the unreacted dextran gradually increased as the fraction number grew. From the results of gel chromatography, it was verified that the interaction of dextran with Chls was quite strong enough to form the complex in the aqueous medium.²

Figure 2 shows the absorption spectra of the Chl *a* or *b*-dextran complexes in water, together with those of Chl *a* or *b* in diethyl ether at room temperature. The red peaks of the Chl *a* and *b*-dextran complexes were observed at 675 and 656 nm, which shifted to a longer wavelength by 17 and 14 nm as compared with those of Chl *a* and *b* in diethyl ether, respectively. The blue peaks of the Chl *a* and *b*-dextran complexes appeared at 425 and 463 nm, and shifted to a longer wavelength by 6 and 11 nm compared with those of Chl *a* and *b* in diethyl ether, respectively. The intensity ratios of blue peak to red peak were 1.5 and 2.5 for the Chl *a* and *b*-dextran complexes, respectively, where

Figure 2. Absorption spectra of the Chl *a* or *b*-dextran complexes in water, together with those of Chl *a* or *b* in diethyl ether at room temperature: ----, Chl *a*-dextran complex in water; fraction 35 (molar ratio dextran/Chl, 1.1), concentration of Chl is $2.8 \mu\text{g ml}^{-1}$; ····, Chl *b*-dextran complex in water; unfractionated sample (molar ratio dextran/Chl, 4.8), concentration of Chl is $10 \mu\text{g ml}^{-1}$; —, Chl *a* in diethyl ether; concentration of Chl is $10 \mu\text{g ml}^{-1}$; - · - · -, Chl *b* in diethyl ether; concentration of Chl is $2.5 \mu\text{g ml}^{-1}$.

Figure 3. Fluorescence-emission spectra of the Chl *a*-dextran complex in water at room temperature: —, fraction 35 (molar ratio dextran/Chl, 1.1); ---, fraction 41; ·····, unfractionated sample (molar ratio dextran/Chl, 4.8). Concentration of Chl is $10 \mu\text{g ml}^{-1}$ in all cases. The excitation wavelength was 430 nm.

as those of Chl *a* and *b* in diethyl ether were 1.7 and 2.8, respectively. These spectral features are similar to those of the water-soluble Chl-macromolecular (PVA, PVP, PEG, or BSA) complexes previously reported by us.^{1,2}

Figure 3 shows the fluorescence-emission spectra of the Chl *a*-dextran complex in water at room temperature. Fraction 35 showed the spectrum having a narrow peak in red region at 689 nm, which is similar to that of Chl *a* in diethyl ether. However, the unfractionated sample before application to the column had a broad peak at 735 nm, and the fraction 41 also had a broad peak at 698 nm. Further, the unfractionated sample of Chl *b*-dextran complex had a broad peak at 712 nm (data not shown). The change in the fluorescence spectra described above probably reflects difference in

interactions between Chl and dextran in the samples. Here, at least three factors should be considered: (1) there would exist unreacted dextran molecules surrounding the Chl-dextran complex; (2) each fraction would contain dextran of different molecular weight; and (3) there is the possibility of association of dextran interacting Chl. The mechanism of change in fluorescence spectra from sample to sample should be cleared up in future.

It is very interesting to separate perfectly the Chl-dextran complex from unreacted dextran using an appropriate column gel, and determine the number of Chl *a* or *b* bound to one dextran chain. Also, the molecular weights of the Chl-dextran complexes determined by an analytical ultracentrifugal measurement should directly provide valuable information as to size of complex structures. These investigations are now in progress.

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