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CONSERVATION OF CHLOROPHYLL A AGGREGATES FORMED UNDER A SOLID CONDITION IN THEIR AQUEOUS SOLUTIONS. A NOTABLE ROLE OF SYNTHETIC AND NATURAL MACROMOLECULES

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Aggregation states of chlorophyll a (Chla) solid samples were found to be conserved in a homogeneous aqueous solution when dissolved in an aqueous solution of a water-soluble synthetic or natural macromolecule, e.g., PVA, PVP, PEG or BSA. The conservation of aggregation states was effective both for the monomeric (670nm) and microcrystalline (740nm) species. Furthermore, the states of Chla aggregate were stable for at least 1.5 years in the aqueous solution, as showing no significant spectral changes.

Aqueous solutions of chlorophyll a (Chla) have widely been prepared from solid samples with the aid of low molecular weight organic solvents or detargents. However, the former procedure has shown to form essentially unstable aggregation states of Chla: the red region spectra vary with time toward formation of higher aggregates.[1] The use of detargents also suggested to cause rearrangement of the aggregation states.[2] In both cases, there is no evidence that the Chla aggregation states formed in a solid condition were conserved in the resultant solutions. Since a variety of Chl aggregation states can generally be prepared and studied under water-insoluble solid conditions,[3] it is of particular value to bring them into a homogeneous aqueous solution conserving the aggregation state formed in a solid condition. This problem is now successfully solved by using a variety of water-soluble macromolecules from synthetic and natural sources as herewith reported.[4] In our system, the solubilized Chla aggregates are stabilized in water for quite a long time. The effect of macromolecules exerts almost independent of the chemical nature of macromolecules used. The role of macromolecules seems to be in a line of immobilization of chloroplast photosystems reported previously.[5]

According to the method of Iriyama et al.,[6] Chl's were extracted from fresh green spinach and isolated, then Chla and Chlb were separated by column chromatography on a sucrose column. Purity of the Chla was checked by absorption spectroscopy and thin-layer chromatography. Polyvinyl alcohol, PVA (PVA-117: molecular weight (MW), 77,000; saponification value, 98.5 mole-%) was a generous gift from Kuraray Co., Ltd. Polyvinyl pyrrolidone, PVP (MW, 40,000), polyethylene glycol, PEG (MW, 20,000) and bovine serum albumin, BSA, were furnished by Tokyo Kasei Kogyo Co., Ltd., Kishida Chemical Co., Ltd., and Armour Pharmaceutical Co., respectively. PVA, PVP and PEG were purified as usual, and BSA was used without further purification.

Our procedure for making an aqueous solution of Chl*a* is simply composed of successive three steps: (i) Solidification of Chl*a*, (ii) addition of concd. water-soluble macromolecular aqueous solution to dissolve, and then (iii) dilution with water. Two kinds of solidification techniques were employed, i.e., "dry" solidification and "wet" one. The "dry" system is designated because Chl*a* is solidified from the dried organic solutions, and "wet" system from the water-containing organic solutions. The abbreviations used here are summarised in Table 1, and these abbreviations are attached to the symbol Chl*a*. For example, Chl*a*(W-e) denotes the Chl*a* solid sample which is solidified from the "wet" ethyl ether solution.

Table 1. Abbreviations for Techniques of Chlorophyll Solidification.

	Organic Solvents			
	Ethyl ether	Petroleum ether	Carbon tetrachloride	Acetone
Dry	D-e	D-pet	D-cl	D-ace
Wet	W-e	W-pet	W-cl	W-ace

Ethyl ether, petroleum ether and acetone were dried over $CaCl_2$, and carbon tetrachloride over anhydrous Na_2SO_4 for 2 days followed by distillation (dry solvents). For preparing "wet" organic solvents, the water-insoluble solvents were shaken vigorously with water to saturation in a separating funnel and then separated from the water layer. Acetone of water content 2%(v/v) was used as its "wet" solvent.

Chl*a* was first dissolved in an organic solvent and filtered, then, solidified at room temperature as follows (step(i)): for dry samples, the filtrate was evaporated to dryness under a reduced pressure (30 mm Hg); for wet samples, the filtrate was dried under a stream of air. A concentrated aqueous macromolecular solution was added onto the solidified Chl*a* in a vessel, and stirred gently at room temperature until a homogeneous green solution was formed (step(ii)). The resultant solution was diluted with water to a given concentration and then filtered (step(iii)).

For taking absorption and IR spectra of the solid Chl*a*(D- and W-pet) samples prior to addition of macromolecular solutions, the samples were prepared by casting from the corresponding petroleum ether solutions on the thin polyethylene (PE) films (showing no absorption in the spectral ranges) followed by careful evaporation.

Figures 1 and 2 are comparisons of absorption spectra of the Chl*a*(D- and W-pet) in various aqueous macromolecular solutions with those of solid Chl*a*(D- and W-pet) and normal Chl*a* ether solution as references. In the macromolecular solutions of Chl*a*(D-pet) and Chl*a*(W-pet) red bands of Chl*a* were observed at about 670 and 740 nm, respectively. The spectral features in aqueous macromolecular solutions were found to be almost identical with those observed for the corresponding casted solid samples. The nature of macromolecules used (PVA, PVP, BSA) exerted no significant effect on the spectral properties. PEG also showed essentially similar results.

Figure 3 shows the IR spectra of solid Chl*a*(D- and W-pet) samples. The

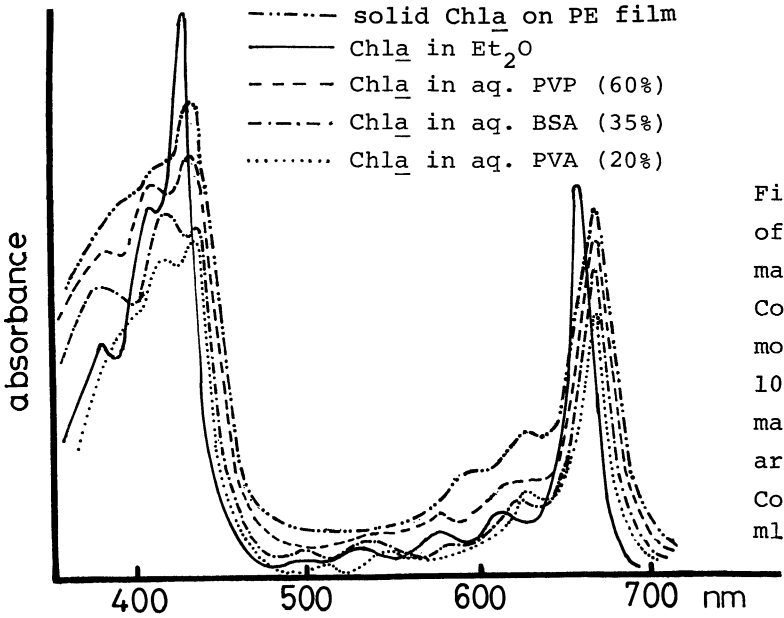

Fig.1. Absorption spectra of Chla(D-pet) in aqueous macromolecular solutions. Concentration of macromolecules at step (iii): 10%(w/w). Concentrations of macromolecules at step (ii) are noted in the Figure. Concentration of Chla: 9 µg/ml-aq. macromol. solution.

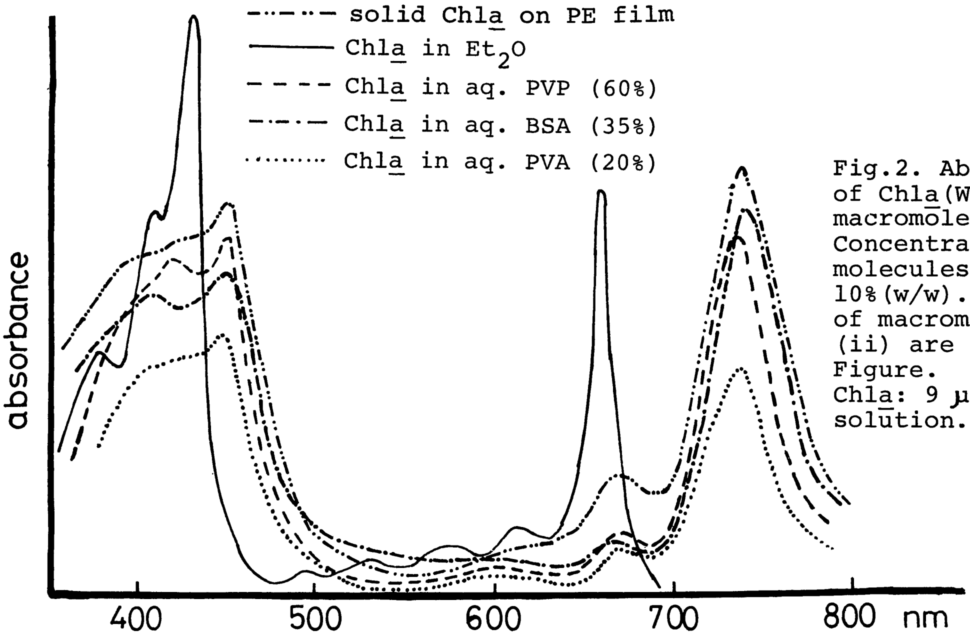

Fig.2. Absorption spectra of Chla(W-pet) in aqueous macromolecular solutions. Concentration of macromolecules at step (iii): 10%(w/w). Concentrations of macromolecules at step (ii) are noted in the Figure. Concentration of Chla: 9 µg/ml-aq. macromol. solution.

results clearly indicate that the "dry" system sample has mostly monomeric Chla species, while the "wet" system sample has predominantly microcrystalline $(\text{Chl}\underline{a} \cdot 2H_2O)_n$ species.[7] This conclusion is derived straightforward from a comparison of our spectra with the assignments made by Katz et al., for keto C=O--Mg (1652 and 1662cm^{-1}), keto C=O--HO(H)Mg (1638cm^{-1}) and free keto C=O (1695cm^{-1}) stretchings.[8]

Thus, from our results of UV and IR spectra, it is evident that the aggregation states of the Chla in the solidified condition are essentially retained in their aqueous macromolecular solutions.

As expected, the conservation of the aggregation states in the macromolecular solutions is shown to be concentration-depending; the higher the concentration of aqueous macromolecular solution at step (ii), the more effective conservation is

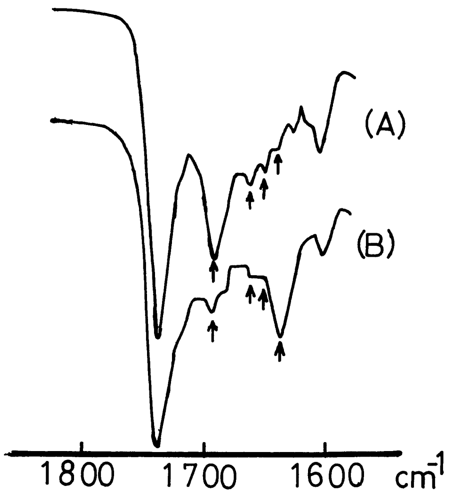

Fig. 3. IR spectra of A: Chl<u>a</u>(D-pet) and B: Chl<u>a</u>(W-pet). Casted on PE film

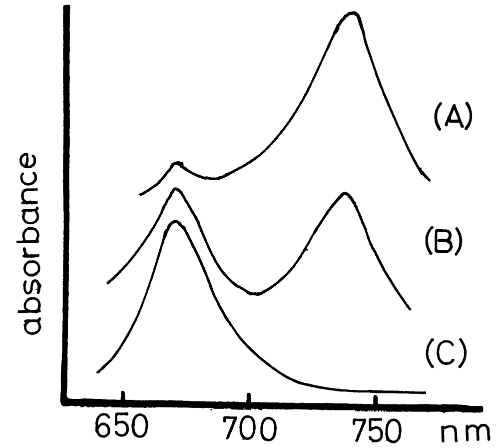

Fig. 4. Concentration dependence of absorption spectra of Chl<u>a</u>(W-pet). First dissolved in PVP aqueous solutions (A) 60%, (B) 30%, (C) 10%(w/w), and then diluted with water to 10%(w/w).

attained, as illustrated in Figure 4. The stability of the aggregation states of Chl<u>a</u> in the macromolecular solutions of a proper concentration was confirmed by (i) the 740/670nm intensity ratio remains almost unchanged during at least 1.5 year's storage in a refrigerator, and (ii) the 740nm band is almost fully retained even on further dilution with water to 0.1% (wt-macromolecule/wt-aq. solution) after aqueous Chl<u>a</u>(W-type)-macromolecular solution was once prepared.

The phenomena reported here are essentially common for D- and W-e, D- and W-cl, and D- and W-ace systems. Further detail of interaction of the macromolecules with Chl<u>a</u> will be published elsewhere.

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