CHEMISTRY LETTERS, pp. 1643-1646, 1981.

SPECTROSCOPIC STUDIES ON THE AGGREGATION OF CHLOROPHYLL *a* IN THE PRESENCE OF WATER-SOLUBLE MACROMOLECULES

Kaku UEHARA,* Yasuhiro NAKAJIMA, Masahiro YONEZAWA, and Makoto TANAKA

Department of Applied Chemistry, College of Engineering, University of Osaka Prefecture, Sakai, Osaka 591

The characteristic aggregation of chlorophyll *a* (chl *a*) was induced when a small amount of acetone solution of chl *a* was added to a diluted aqueous solution of a macromolecule such as poly(vinyl alcohol)(PVA), poly(vinyl pyrolidone)(PVP), or bovine serum albumin(BSA). The rate and state of aggregation were remarkably affected by the kind of polymer used. PVA fairly rapidly induced the aggregation to form aggregates having absorption maxima at 730 nm. On the other hand, BSA showed a tendency to conserve a typical 672 nm colloidal form.

The chlorophylls play a central role in the primary process of photosynthesis. Knowledge of the state of chlorophyll *in vivo* is essential for our understanding of its function. It was suggested that all the chlorophyll exists as chlorophyll-protein complexes in the chloroplast thylakoid membranes.[1] Therefore, the studies on the interaction of chl *a* with a natural or synthetic macromolecule seem to be interesting from this viewpoint. Recently, Inamura *et al.*[2] found that when chl *a* solid samples were dissolved in a concentrated aqueous solution of a water-soluble macromolecule, the aggregation states of solid chl *a* were conserved in the homogeneous aqueous solution regardless of the chemical nature of macromolecules used.

The aim of the present paper is to report that when a small amount of acetone solution of chl *a* was added to a diluted aqueous solution of macromolecules, the formation of chl *a* aggregates differing from those cited in the literature was induced. In our system, the rate and state of aggregation were remarkably affected by the kind of macromolecule used. It was suggested that affinity of chl *a* toward a macromolecule was an important factor for the aggregation of chl *a*.

Chl *a* was extracted from fresh green spinach and purified by the method of Iriyama *et al.*.[3] Purity of the chl *a* was checked by absorption spectroscopy and high-performance liquid chromatography.[4] Poly(vinyl alcohol), PVA, (molecular weight(MW), 22,000; saponification value, 99.0 mol-%) was kindly gifted from Nihon Gosei Kagaku Kogyo Co. Ltd.. Poly(vinyl pyrolidone), PVP, (MW, 40,000) and bovine serum albumin, BSA, (crystallized and lyophylized) were furnished by Wako Pure Chemical Industries Ltd. and Sigma Chemical Co., respectively. PVA was purified

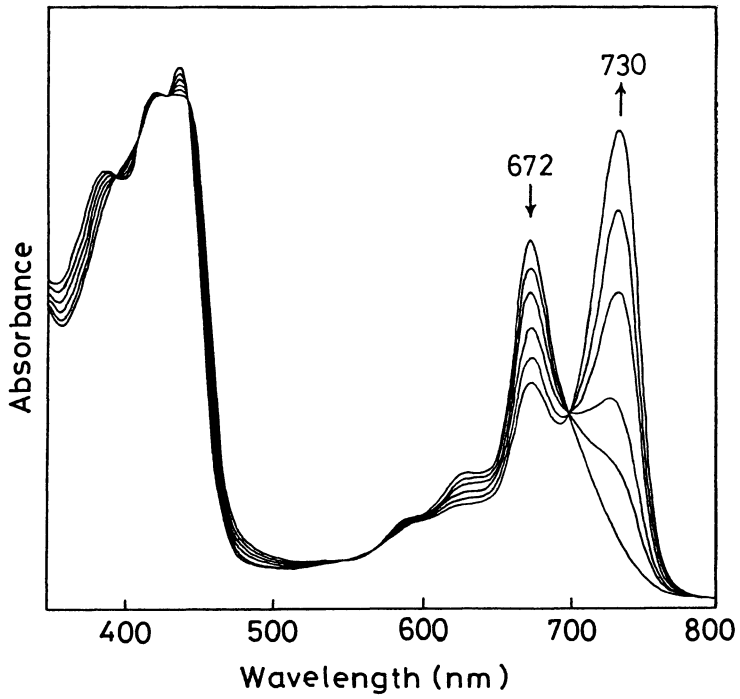

Fig. 1. Time-dependent absorption spectra of the system of 1.5×10^{-5}M chl *a*, 6 vol-% acetone, and 0.1 wt-% PVA aqueous solution at 15°C. (Time=0, 5, 10, 20, 30, 50 min).

as usual. PVP, BSA, and acetone of GR grade reagent were used without further purification. Small amount (0.3 ml) of chl *a* acetone solution (2.5×10^{-4}M) was added to 4.7 ml of the aqueous solution of macromolecule (0.1 wt-%, dissolved in 1×10^{-3}M phosphate buffer solution, pH 6.98) and stirred to give a homogeneous dispersion. Then the aggregation of chl *a* was monitored spectrophotometrically in the 350-800 nm region. In the absence of water-soluble macromolecule, chl *a* aggregates precipitated on the surface of cuvett, and no reproducible data were obtained.

The absorption spectra of the mixture varied with time until stable chl *a* aggregates resulted. Figure 1 shows a typical example of the time-dependent absorption spectra of a mixture of aqueous PVA and chl *a* acetone solution after mixing. The growth of the absorbance at about 730 nm was observed at the expence of that at 672 nm. Such a marked red shift of the red band (Q-band) and less spectral change in the region of blue band (Soret band) suggest the aggregation of chl *a* in the aqueous PVA solution. Though an isosbestic point is observed at 699 nm in Figure 1, 730 nm band may consist of three or more components with different band maximum because this band is rather broad compared with 662 nm monomeric band in acetone solution as shown in Figure 2.

Red shift of Q-band has been explained from the viewpoint of the chloropyll-chlorophyll or chlorophyll-ligand interactions.[5-7] It has been reported that *in vitro*, monomeric chl *a* has its principal absorption maximum in the 659-671 nm range[5], a special dimer of a monohydrated chl *a* (chl *a*·H_2O)$_2$ absorbs at 700 nm

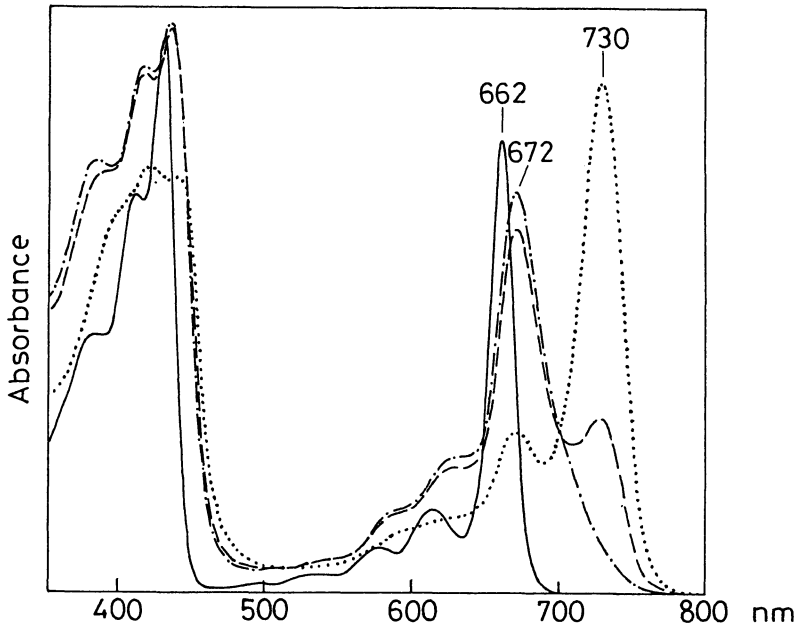

Fig. 2. Absorption spectra of chl *a* in 0.1 wt-% aqueous macromolecular solution containing 6 vol-% acetone when equiliblium is approximately reached (about 24 hrs later after mixing at 25°C).

———— chl *a* in acetone, — — — — chl *a* in PVP aqueous solution
—·—·— chl *a* in BSA aqueous solution, ····· chl *a* in PVA aqueous solution

and polymeric species of dihydrated chl *a* (chl $a \cdot 2H_2O)_n$ has a red absorption maximum at 743 nm.[6] It has been proposed that the calculated red absorption spectrum for the polymeric species of monohydrated chl *a* (chl $a \cdot H_2O)_n$ ranges from 693 to 721 nm as n is varied from 2 to infinity.[7] However, 730 nm absorbing species observed in our system did not correspond to any of them cited in the above literature. It may be attributed to oligomeric species of dihydrated chl *a* (chl $a \cdot 2H_2O)_n$, where n is smaller than that in 743 nm one.

Figure 2 shows the effect of water-soluble macromolecules on the aggregation of chl *a* in aqueous solution containing 6 vol-% acetone at 25°C. In the presence of PVP, the formation of the 730 nm absorbing band was very slow (Table 1) and its equilibrium constant was smaller than that in PVA. When BSA was used as a water-soluble macromolecule, the 672→730 nm conversion was no longer observed on standing at room temperature. The 672 nm absorbing band with a tail extending into the longer wave length region of the spectrum in the presence of BSA was similar to the typical 672 nm colloid[8] readily obtained by diluting an acetone solution of chl *a* with water. It was suggested that the aggregation in our system proceeded *via* two-stage mechanism; rapid formation of the 672 nm colloid in water followed by the 672 →730 nm conversion on the macromolecule. When a mixture of equivalent wt-% of BSA and PVP (or PVA) was used, aggregation behavior of chl *a* was similar to that in the

Table 1. The initial rate of the 672→730 nm conversion at 15°C

Macromolecule	ΔAbs(730 nm)/min
PVA	2.0×10^{-2}
PVP	4.6×10^{-5}
BSA	≃0

presence of BSA alone. And, a mixture of PVP and PVA showed similar effect as PVP alone. Thus, the order of the strength of the interaction with the chl *a* of 672 nm colloidal form was seen as follows: BSA > PVP > PVA. It is interesting to note that there is an anti-parallelism between the rate of the 672→730 nm conversion and the strength of interaction of the chl *a* with the macromolecules used. Such a notable tendency of BSA to depress the formation of higher aggregates and to retain chl *a* 672 nm colloidal form seems to be derived from its strong affinity toward the chl *a* . The effect of PVA on the 672→730 nm conversion may be accounted on the basis of the acceleration for the chl *a* hydration followed by aggregation and the depletion of the formation of aggregates whose wave length is more than 730 nm.

Detailed studies on the effect of macromolecules on the aggregation of chl *a* are now in progress.

References

1) J. P. Markwell, J. P. Thonber, and R. T. Boggs, *Proc. Natl. Acad. Sci. U.S.A.*, 76, 1233 (1979).
2) I. Inamura, K. Toki, T. Araki, and H. Ochiai, *Chem. Lett.*, 1980, 1481.
3) K. Iriyama, M. Shiraki, and M. Yoshiura, *Chem. Lett.*, 1979, 787.
4) K. Iriyama, M. Yoshiura, and M. Shiraki, *J. Chromatogr.*, 154, 302 (1978).
5) T. M. Cotton, A. D. Trifunac, K. Ballschmiter, and J. J. Katz, *Biochim. Biophys. Acta*, 368, 181 (1974).
6) F. K. Fong and V. J. Koester, *Biochim. Biophys. Acta*, 423, 52 (1976).
7) L. L. Shipman and J. J. Katz, *J. Phys. Chem.*, 81, 577 (1977).
8) B. B. Love and T. T. Bannister, *Biophys. J.*, 3, 99 (1963).

(Received September 16, 1981)