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AGGREGATION OF CHLOROPHYLL A IN AQUEOUS DIMETHYL SULFOXIDE

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In aqueous dimethyl sulfoxide(DMSO) solution, chlorophyll a (chl a) aggregated to give several colloidal forms; these were spectroscopically characterized as A-683, A-695, and colorless one. The water/DMSO ratio was the determining factor of the type of form obtained. The spectrum of A-683, which formed in 30-50 v/v % aqueous DMSO solution, was conspicuous bacause of its very high and narrow absorption band at 683±1 nm. The spectroscopic transition among these aggregated forms of chl a in aqueous DMSO is described.

It has long been recognized that the main red absorption band of the chlorophyll *in vivo* is bathochromic-shifted and broadened.[1] These spectral properties are attributed to chlorophyll-chlorophyll,[2] chlorophyll-water,[3] and chlorophyll-protein[4] interactions. The computer decomvolution of such spectra using Gaussian shaped components has led to the concept of different absorbing forms of chl a *in vivo*.[5] It is important to synthesize a model system corresponding to the several aggregated forms of chl a in order to reach a clear understanding of the state of chlorophyll *in vivo*. There have been a number of studies on the aggregation of chlorophyll a in organic or aqueous organic solutions.[6] In general, these studies have been unsuccessful in the predominant formation of a distinct component. In a preceding paper,[7] we reported on the characteristic aggregation of chl a in the presence of water-soluble macromolecules.

The present work describes the preparation and spectral properties of new kind of aggregates of chlorophyll a formed in aqueous DMSO solution. The water/DMSO ratio is pointed out as the determining factor of the type of aggregate and of the spectroscopic transition among these aggregated forms of chl a.

Chl a extracted and purified[8] from fresh green spinach was dissolved in DMSO of GR grade reagent furnished by Wako Pure Chemical Industries Ltd. Small amount (0.3 ml) of chl a DMSO solution(2.5×10^{-4}M) was added to 4.7 ml of an aqueous DMSO solution(3.4×10^{-2}M Tris buffer solution, pH 7.2). The mixture was stirred to give a homogeneous solution and the aggregation of chl a was monitored spectrophotometrically in the 350-800 nm region.

The absorption spectra of the mixture varied with time until equilibrium was established. Figure 1 shows a typical example of the time-dependent absorption spectra of 50 v/v % aqueous DMSO solution of chl a after mixing. The growth of the absorbance at 684 nm was observed at the expense of that at 667 nm. Such a marked spectral change of the region of red band(Q-band) compared with that of

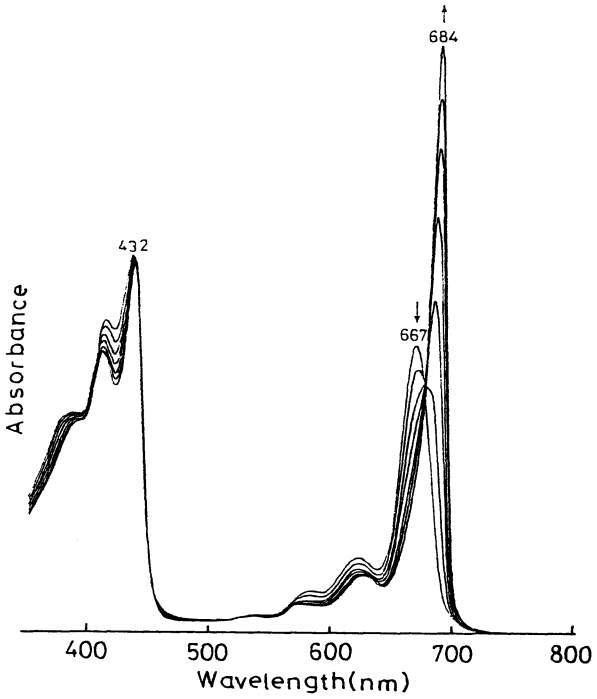

Fig. 1. Time-dependent absorption spectra of the 50 v/v % aqueous DMSO solution of 1.5×10^{-5} M chl a at 25°C. (Time=0, 30, 60, 90, 120, 150, and 210 min).

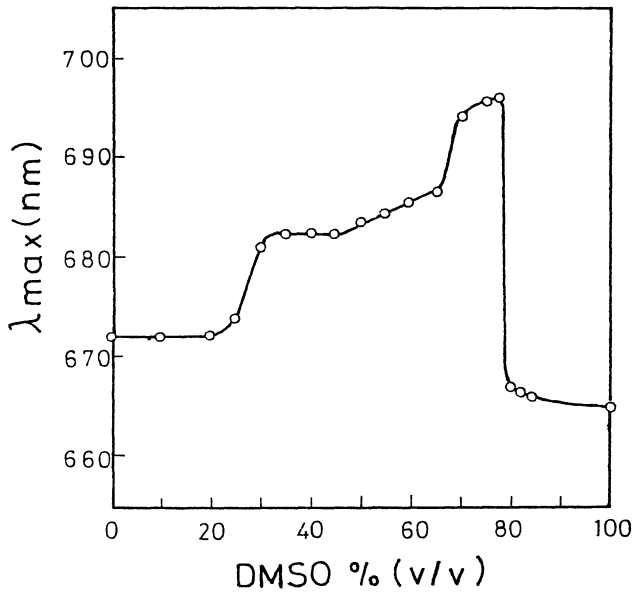

Fig. 2. Plots of DMSO %(v/v) *vs.* wavelength at red absorption maxima of chl a in the solutions when equilibrium is approximately reached at 25°C.

blue band(Soret band) suggests the aggregation of chl a in the 50 v/v % aqueous DMSO solution. The 684 nm band was conspicuous because of its very high band-height and narrow bandwidth. It is interesting to note that the red band was higher than the blue one. This sharp band seems to consist of one spectral component.

The absorption spectra of chl a aggregates in aqueous DMSO solution change stepwise with the water/DMSO ratio in aqueous DMSO solutions. Figure 2 shows plots of DMSO %(v/v) *vs.* wavelength at the red absorption maxima of chl a in aqueous DMSO solutions when equilibrium is reached at 25°C. It is clear that the discontinuous change of the wavelength can not be explained by the averaged polarity of the mixed solvents. Below 20 %(v/v), the 672 nm absorbing band was observed, which was similar to the typical 672 nm colloid.[6a] In the range of 30-50 %(v/v) DMSO, a very high and narrow band absorbing at 683±1 nm (A-683) was appeared as shown in Figure 1. In 70-75 v/v % aqueous DMSO solution, the red absorption maximum was located at 695 ±2 nm(A-695). As compared with A-683, the red band of A-695 was rather broad suggesting that it contains some additional spectral components. The spectra of chl a in the vicinity of 80 v/v % DMSO solution showed a spectral character similar to the colorless form which was found by Dijkman[6d] in a water-methanol mixture. In such a reaction mixture containing the colorless form, microcrystals of chl a aggregate could be observed under microscope. In a solution of

high DMSO content above 80 %(v/v), the dissolved form[6a] appeared(λ_{max} 665 nm), which corresponded to that in 100 v/v % DMSO solution. Such a complicated solvent effect may arise from the competition between chl a and DMSO(or water) for the coordination to Mg and for the hydrogen bonding to carbonyl group of chl a.

The conversion of monomeric colloid at 669±3 nm to other forms(A-683, A-695, and colorless form) was observed in the range of 30-80 DMSO %(v/v) as seen in Figure 2. After the reaction from monomeric colloid to A-683 was stabilized, the species A-683 can be transformed into A-695 by decreasing water content from 50 to 30 v/v % by the addition of DMSO. However, the reverse reaction (A-695 → A-683) was impossible by increasing water content from 30 to 50 v/v % by the addition of water. All of the forms, A-683, A-695, and colorless form, can be converted to the dissolved form(665 nm) by increasing the concentration of DMSO in their solutions up to 84 % or more. When small amount of dioxane(3 v/v %) was added into the aqueous DMSO solution containing the species A-683 or A-695, the spectrum was converted to that of chl a-dioxane colloid.[6a] It is suggested that the interactions of chl a with DMSO is weaker than those with dioxane and that A-683 or A-695 is transformed to the chlorophyll a-dioxane adduct, which has the structure $-(\text{chl}\cdot\text{dioxane})_n-$ represented by Leblanc and Chapados.[9]

Aqueous DMSO solutions of chl a described above were spreaded on thin polyethylene films followed by evaporation of the solvents *in vacuo* at elevated temperature. The spectral features in the solid chl a aggregates obtained were found to be almost identical with each other in spite of difference in initial water content. A red band was observed at 695±2 nm, suggesting that all the chl a aggregates formed in aqueous DMSO of various water content was transformed to A-695 by the evaporation of solvent. Upon exposure to DMSO vapor, a solid film of chl a cast from acetone solution was also converted to the one similar to the 695 nm absorbing species. The preparation of a solid form of A-683 is unsuccessful as yet.

The IR spectrum of the 695 nm absorbing species markedly differs from those of the chl a aggregates already known;[3e] absence of the coordinated keto absorptions at 1652 and 1662 cm^{-1}(assigned to C=O···Mg) and at 1638 cm^{-1}(assigned to C=O···HO(H)Mg), and appearance of the free keto C=O absorption at 1684 cm^{-1}(red shifted a little from usual free keto C=O at 1695 cm^{-1}) and of ester C=O at 1730 and 1734 cm^{-1}(slightly splitted and red shifted from usual one at 1736 cm^{-1}). The solid chl a sample did not show any absorption band in the O-H stretch region (3800-3000 cm^{-1}) but showed the absorption bands at 1305, 1055, and 965 cm^{-1} presumably arising from DMSO. These IR data suggest the formation of chl a-DMSO adduct without appreciable interactions of chlorophyll-chlorophyll or of chlorophyll-water. It has been known that chlorophyll-water adducts, $(\text{chl}\cdot H_2O)_2$ and $(\text{chl}\cdot 2H_2O)_n$, have their visible absorption maxima in more longer wavelength region(700 and 743 nm, respectively).[3]

The facts described in the present paper suggest that the species formed in 30-80 v/v % aqueous DMSO solution is discrete and new kind of aggregates of chlorophyll a incorporated by DMSO instead of water. It seems that water is essential for the formation of A-683 or A-695 but is not a component of the species. A-683 and A-695 may be used as a synthesized model for *in vivo* chlorophyll a forms. In order to establish the structure of them in more detail, fluores-

cence and NMR spectroscopic studies are now in progress.

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References

1) V. N. Lubimenko, *Rev. Gen. Bot.* 39, 347 (1927).
2) a) K. Sauer, J. R. L. Smith, and A. J. Schults, *J. Am. Chem. Soc.* 88, 2681 (1965). b) K. Ballschmiter, K. Truesdell, and J. J. Katz, *Biochim. Biophys. Acta* 184, 604 (1969). c) L. L. Shipman, T. M. Cotton, J. R. Norris and J. J. Katz, *J. Am. Chem. Soc.* 98, 8222 (1976).
3) a) J. J. Katz, K. Ballschmiter, M. G. Morin, H. H. Strain, and R. A. Uphaus, *Proc. Natl. Acad. Sci. U. S. A.* 60, 100 (1968). b) F. K. Fong, *Proc. Natl. Acad. Sci. U. S. A.* 71, 3692 (1974). c) F. K. Fong and V. J. Koester, *Biochim. Biophys. Acta* 423, 52 (1976). d) L. L. Shipman, T. M. Cotton, J. R. Norris, and J. J. Katz, *Proc. Natl. Acad. Sci. U. S. A.* 73, 1791 (1976). e) T. M. Cotton, P. A. Loach, J. J. Katz, and K. Ballschmiter, *Photochem. Photobiol.* 27, 735 (1978). f) J. E. Hunt, R. D. Macfarlane, J. J. Katz, and R. C. Dougherty, *Proc. Natl. Acad. Sci. U. S. A.* 77, 1745 (1980).
4) a) J. S. Kahn and T. T. Bannister, *Photochem. Photobiol.* 4, 27 (1965). b) J. M. Anderson, *Biochim. Biophys. Acta* 416, 191 (1975). c) J. P. Markwell, J. P. Thornber and R. T. Boggs, *Proc. Natl. Acad. Sci. U. S. A.* 76, 1233 (1978). d) J. P. Thornber, J. P. Markwell, and S. Reinman, *Photochem. Photobiol.* 29, 1205 (1979). e) M. S. Davis, A. Forman, and J. Fajer, *Proc. Natl. Acad. Sci. U. S. A.* 76, 4170 (1979).
5) a) C. S. French, *Proc. Natl. Acad. Sci. U. S. A.* 68, 2893 (1971). b) C. S. French, J. S. Brown, and M. C. Laurence, *Plant Physiol.* 49, 421 (1972). c) K. Sugiyama and N. Murata, *Biochim. Biophys. Acta* 503, 107 (1978).
6) a) B. B. Love and T. T. Bannister, *Biophys. J.* 3, 105 (1963). b) A. F. H. Anderson and M. Calvin, *Arch. Biochem. Biophys.* 107, 251 (1964). c) R. L. Amster, *Photochem. Photobiol.* 9, 331 (1969). d) H. Dijkmans, *Eur. J. Biochem.* 32, 233 (1973). e) T. M. Cotton, A. D. Trifunac, K. Ballschmiter, and J. J. Katz, *Biochim. Biophys. Acta* 368, 181 (1974). f) K. Iriyama and M. Yoshiura, *Colloid & Polymer Sci.* 255, 133 (1977).
7) K. Uehara, Y. Nakajima, M. Yonezawa, and M. Tanaka, *Chem. Lett.* 1981, 1643.
8) K. Iriyama, M. Shiraki, and M. Yoshiura, *Chem. Lett.* 1979, 787.
9) R. M. Leblanc and C. Chapados, *Biophys. Chem.* 6, 77 (1976).

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