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NEW PREPARATION METHODS OF CHLOROPHYLL/WATER-SOLUBLE MACROMOLECULAR COMPLEXES

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Three new methods for preparing the chlorophyll/water-soluble macromolecular complexes are described herein. Each method has been found to possess notable characteristic advantages as compared with the former method previously reported by us: (1) operation is very quick and easy, (2) the complexes containing a variety of Chl-a species can be obtained, (3) the Chl-a/polymer ratio can be raised, (4) reproducibility is good, and etc.

We[1, 2] previously prepared the chlorophyll/water-soluble macromolecular complexes, as artificial model compounds of the chlorophyll-proteins in the green plants. The amphiphilic nature of the water-soluble macromolecules, polyethylene glycol(PEG), polyvinylpyrrolidone(PVP), polyvinyl alcohol(PVA) and bovine serum albumin(BSA) was found to play an important role in interacting with Chl-a to keep it in a monomeric or polymeric hydrated state and in dissolving their complexes in water. The preparation method of the complexes was composed of three successive steps.[1, 2] By altering the steps (1) and (2), the new methods, (A), (B),and (C), have been developed.[3] Chl-a, PEG, PVP, PVA and BSA used are the same as in our previous work.[1, 2] Lecithin from soybeans was used.

Method (A) is composed of four successive steps: (1) preparation of Chl-a/polymer(poly)/organic solvent(org. solv.)/water solution, (2) evaporation, (3) addition of a small amount of water, and (4) dilution. Step (1):——organic solvent containing Chl-a was added dropwise to aqueous polymer solution (pH 6.7) with stirring, resulting in the compositions as shown in Table 1. In the case of PVA, the operation was done very slowly during the evaporation process of organic solvent in aqueous solution, because PVA tends to precipitate easily by the addition of organic solvents. Step (2):——the solution thus prepared was evaporated to dry Chl-a-polymer film under reduced 30 mmHg (A-1 series in Table 1) or 10^{-4} mmHg (A-2 series) pressure at room temperature. Step (3):——a small amount of water (pH 6.7) was added onto the film, and stirred gently at room temperature until a homogeneous green paste was formed. In the case of PVA stirring was done at 100 °C. Step (4):——the paste was diluted with water to a given concentration of the aqueous Chl-a/macromolecular complex solution and then filtered through a glass filter.

Method (B) is composed of four steps: (1) preparation of Chl-a/poly/org. solv. solution; steps (2)-(4), are the same as those of method (A). This method requires that the polymer be dissolved in the organic solvent without the aid of water. At

step (1), organic solvent containing Chl-a was added to poly/org. solv. solution, resulting in the composition as shown in Table 1.

Method (C) is composed of four steps: (1) preparation of Chl-a/lecithin/org. solv. solution, (2) evaporation, (3) addition of aqueous concentrated macromolecular solution, and (4) dilution. Step (1):——lecithin and Chl-a were dissolved in an organic solvent successively. Step (2):——the solution was evaporated into Chl-a-lecithin paste under reduced 30 mmHg pressure at room temperature and this green paste was stirred gently. Step (3):——an aqueous concentrated macromolecular solution, the concentration of which is indicated in Table 1, was added to the Chl-a-lecithin paste, and then stirred gently at room temperature until a homogeneous green paste was formed. Step (4):——the resultant paste was diluted with water to a given concentration of the aqueous Chl-a/lecithin/macromolecular complex solution and then filtered through a glass filter.

The decomposition of Chl-a during the preparing processes of the Chl-a/macromolecular complexes may be relatively small in view of the protecting action of the polymers.[1, 2] We ascertained that the absorption spectra of Chl-a extracted from the complexes showed no changes in comparison with those before complexing.

The composition of raw materials, molar ratio Chl-a/poly (molecular weights of polymers: PEG=20000, PVP=40000 and PVA=77000) and red peaks with their intensity ratio, for the Chl-a-macromolecular complexes prepared by methods (A), (B) and (C) are shown in Table 1. It should be noted that the ratio would be further raised, when no precipitate of Chl-a existed on filtration.

The Chl-a/poly molar ratio of the complexes prepared by method (A) was in the range of 0.05 to 1.28. Up to about 0.3 by the former method, the method (A) is more effective in elevating the ratio. There appeared a large number of wavelengths of red peaks in the range of 672 to 760 nm. Many such red peaks seem to be classified into three Chl-a groups; Chl-a(672), Chl-a(675-677),and Chl-a(715-760).

The Chl-a(672) species, corresponding to the Chl-a(670) species in our previous papers,[1, 2] can be ascribed to a monomeric Chl-a species.[1, 2, 4-6] Strictly speaking, this species is the sum of contributions from several different forms of Chl-a, judging from the broad Q band the half bandwidth of which was 32-37 nm. Chl-a(675-677) may be due to the aggregation species of Chl-a[5] or the preferential occurrence of the monomeric Chl-a forms which interact with polymer in a different way from that in the case of Chl-a(672).[7, 8] Chl-a(715-760) is inferred to be largely a crystalline form of hydrated Chl-a, the stoichometry of which may be $(\text{Chl-a}\cdot H_2O)_n$ proposed by Ballschmiter et al.[9] or $(\text{Chl-a}\cdot 2H_2O)_n$ by Brace et al.[10] The wide range of wavelengths may reflect the degree of association with the larger n giving the longer wavelength.

The red bands also largely depended on the technique of evaporation in step (2). In the case of A-2 series, the diversity of wavelengths of red peaks was depressed and Chl-a(672) prevailed over Chl-a(675-677) and Chl-a(715-760) as compared with A-1 series. Exclusion of water by evaporating at 10^{-4} mmHg is considered to increase Chl-polymer interactions which may produce more Chl-a(672) species. Furthermore, the complexes containing Chl-a(692) and Chl-a(700) were obtained, when dioxane was applied as an organic solvent in method (A).[3] They will be also reported in the near future.

Table 1. Composition of raw materials, and molar ratio Chl-a/polymer and red peaks of Chl-a-macromolecular complexes

Method	Composition of raw materials						Chl-a-macromolecular complex	
	Polymer (mg)	Chl-a (μg)	organic solvent (ml)		water (ml)	lecithin (μg)	Chl-a/poly	red peaks (nm) and their intensity ratio
A-1	PEG 75	3000	MeOH	2.25	0.68		0.05	743/672 = 0.19
	"	"	EtOH	1.50	"		"	743/672 = 0.25
	"	"	1-propanol	0.23	"		0.11	735/672 = 0.28
	"	"	acetone	0.60	"		0.14	736/675 = 0.75
	PVP 75	1670	MeOH	0.75	"		0.95[a]	715/676 = 0.49
	"	"	EtOH	0.65	"		0.41	715/676 = 0.39
	"	"	1-propanol	0.60	"		0.16	736/672 = 0.31
	"	"	acetone	1.50	"		0.20	736/672 = 0.41
	PVA 75	1310	MeOH	1.80	"		0.92[a]	725/677 = 0.89
	"	"	EtOH	0.90	"		0.83	753/672 = 3.50
	"	-	1-propanol	0.40	"		0.77[a]	760/672 = 3.26
	"	-	acetone	0.20	"		1.28[a]	727/677 = 1.03
	BSA 443	-	MeOH	1.00	9.00		0.20	740/715/672 = 0.38/0.40/1
	"	-	acetone	"	"		0.68	718/672 = 0.73
A-2	PEG 90	260	MeOH	0.40	0.91		0.07[a]	716/672 = 0.54
	"	"	acetone	0.80	"		"	672
	PVP 80	500	MeOH	1.00	0.92		0.21[a]	"
	"	"	acetone	2.00	"		"	"
	PVA 100	400	MeOH	0.12	1.05		0.35[a]	"
	BSA 330	6670	EtOH	1.00	10.0		1.45	"
B	PEG 80	1220	MeOH	6.42			0.29	672
	"	2140	chloroform	6.42			0.50	"
	PVP 80	1570	MeOH	6.42			0.29	"
	"	1540	EtOH	6.42			0.83[a]	"
C	PEG 30[b]	1500	ethyl ether	10	0.08	7500	0.39	672
	PVP 60[b]	"	"		0.12	"	0.16	"
	"	"	petr. ether	10	"	"	0.18	"
	"	"	dioxane	10	"	"	0.58	"
	PVA 126[b]	"	ethyl ether	10	2.39	"	0.11	"
	"	"	petr. ether	10	"	"	0.20	"
	PEG 622[c]	559	ethyl ether	10	1.40	559	0.001	740/672 = 1.00
	"	"	petr. ether	10	"	"	"	742/672 = 1.00
	PVP 1250[c]	"	ethyl ether	10	0.90	"	0.002	745/672 = 1.42
	"	"	petr. ether	10	"	"	"	745/672 = 1.66
	PVA 2340[c]	"	ethyl ether	10	12.5	"	"	740/672 = 1.00
	"	"	petr. ether	10	"	"	"	740/672 = 1.25

a) No precipitate of Chl-a existed on filtration.

b), c) Molar ratio Chl-a/poly in raw materials is 0.9 and 0.02, respectively.

The Chl-a/poly molar ratio of the complexes prepared by method (B) was in the range of 0.29 to 0.83, and this method is also effective in elevating the ratio. Interestingly, only Chl-a(672) appeared in all cases of method (B). It was unaffected by the technique of evaporation in step (2). The half bandwidths of the 672 nm peaks were in the range of 26-30 nm. Thus, method (B) is more effective in elevating the homogeneity in Chl-a forms in complexes than method (A). These notable characteristics of method (B) may be due to stronger hydrophobic nature of surroundings of Chl-a, caused by the depletion of water during steps (1) and (2). Method (B) is very quick and easy to perform, and excellent in reproducibility.

The Chl-a/poly molar ratio of the complexes prepared by method (C) was in the range of 0.001 to 0.58. The red peaks of complexes depended only on the Chl-a/lecithin weight ratio and Chl-a/poly molar ratio of raw materials, and not on the type of polymer or organic solvent. The peak at 672 nm for Chl-a/lecithin = 0.2 and Chl-a/poly = 0.9, and the two peaks at 672 nm and 740-745 nm for Chl-a/lecithin = 1.0 and Chl-a/poly = 0.02. Thus, by controlling the composition of raw materials, one can freely obtain a desired intensity ratio of red peaks. The weight ratio of Chl-a/lecithin may be the controlling factor rather than the molar ratio of Chl-a/poly. The half bandwidth of the 672 nm peak was 22 nm. Therefore, the Chl-a(672) species in the complexes prepared by method (C) is considered to be almost a single form of monomeric Chl-a.[11] The porphyrin ring of Chl-a may be located at the boundary of a lecithin bilayer, while the phytol tail buried in the hydrocarbon core of the bilayer.[12, 13] Such a Chl-a-lecithin complex is inferred to be further bound to the macromolecular chain, resulting in the Chl-a-lecithin-macromolecular complex. This complex was perfectly dissolved in water as well as the complexes prepared by the other methods described herein.

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References

1) I. Inamura, K. Toki, T. Araki, and H. Ochiai, Chem. Lett., 1980, 1481 (1980).
2) I. Inamura, H. Ochiai, K. Toki, S. Watanabe, S. Hikino, and T. Araki, Photochem. Photobiol., 38, 37 (1983).
3) I. Inamura, K. Toki, T. Araki, and H. Ochiai, Polymer Preprints, Japan, 33(8), 1947 (1984).
4) G. R. Seely and R. G. Jensen, Spectrochim. Acta, 21, 1835 (1965).
5) T. M. Cotton, A. D. Trifunac, K. Ballschmiter, and J. J. Katz, Biochim. Biophys. Acta, 368, 181 (1974).
6) L. L. Shipman, T. M. Cotton, J. R. Norris, and J. J. Katz, J. Am. Chem. Soc., 98, 8222 (1976).
7) M. Lutz, Biochim. Biophys. Acta, 460, 408 (1977).
8) K. Sugiyama and N. Murata, Biochim. Biophys. Acta, 503, 107 (1978).
9) K. Ballschmiter and J. J. Katz, J. Am. Chem. Soc., 91, 2661 (1969).
10) J. G. Brace, F. K. Fong, D. H. Karweik, V. J. Koester, A. Shepard, and N. Winograd, J. Am. Chem. Soc., 100, 5203 (1978).
11) N. Murata and N. Sato, Plant Cell Physiol., 19, 401 (1978).
12) A. Steinemann, N. Alamuti, W. Brodmann, O. Marschall, and P. Läuger, J. Membrane Biol., 4, 284 (1971).
13) F. Podo, J. E. Cain, and J. K. Blasie, Biochim. Biophys. Acta, 419, 19 (1976).

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