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QUINONE AND PHEOPHYTIN IN THE PHOTOSYNTHETIC REACTION CENTER II FROM SPINACH CHLOROPLASTS

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Prenylquinones and pheophytin *a* in a preparation of photosynthetic reaction center II from spinach chloroplasts were chemically determined. Each reaction center II had two molecules, each of plastoquinone-9 and pheophytin *a*, but practically no phylloquinone, α-tocopherylquinone or α-tocopherol.

Spectrophotometric studies [1–3] have suggested that the primary electron acceptor, Q, and the secondary electron acceptor, B, of the photochemical reaction center II are both plastoquinones. The secondary electron donor, Z (or D_1), next to P-680, has also been suggested to be a plastoquinone [4]. An intermediate electron acceptor before Q has been demonstrated to be pheophytin *a* [5]. To characterize the reaction center in more detail, chemical analysis is necessary to identify these electron carrier components. A highly purified preparation of the photochemical reaction center II from higher plant chloroplasts [6,7] is suitable for such study. Spectrophotometric analysis [8] indicates that this preparation contains one molecule each of P-680, Z (D_1), and Q per about 50 chlorophyll molecules. In this work, the reaction center II preparation was employed for the chemical determination of quinones and pheophytins.

The photochemical reaction center II was prepared from spinach chloroplasts using digitonin as described previously [7]. The chlorophyll *a*-to-*b* ratio in this preparation was 11 : 1, suggesting insignificant contamination by the light-harvesting chlorophyll *a*/*b* protein [9]. For the determination of prenylquinones, total lipids were extracted from the preparation according to Bligh and Dyer [10]. The quinones were analyzed by HPLC on a column of Lichrosorb SI 60, 5 μm (Merck) according to Lichtenthaler and Prenzel [11], using a high performance liquid chromatograph (Waters, 6000A) equipped with an ultraviolet detector (Shimadzu, SPD-2A). Elution of the quinones was monitored at 260 nm for plastoquinone-9, phylloquinone and α-tocopherylquinone, and at 290 nm for α-tocopherol. The amounts of the quinones were determined by measuring the areas under the elution bands and comparing them with those of standards. The chromatographic standards, such as phylloquinone, α-tocopherol, α-tocopherylquinone and plastoquinone-9, were obtained as described previously [12]. Concentrations of the standard solutions were determined using absorption coefficients of $E^{1\%}_{1cm} = 210$ at 255 nm for plastoquinone-9 in ethanol [13], 419 at 248 nm for phylloquinone in isooctane [14], 414 at 262 nm for α-tocopherylquinone in ethanol [13], and 74.7 at

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Abbreviations: DCIP, 2,6-dichlorophenolindophenol; DPC, 1,5-diphenylcarbazide; HPLC, high performance liquid chromatography.

TABLE I

QUINONE AND PIGMENT CONTENTS OF REACTION CENTER II PREPARATION

Values with deviations were obtained from three separate extractions from the same preparation.

Component	Molar ratio Component/50 chlorophyll
Plastoquinone-9	1.85 ± 0.05
Phylloquinone	0.04 ± 0.02
α-Tocopherylquinone	0.04 ± 0.02
α-Tocopherol	0.02 ± 0.01
Pheophytin *a*	2.1 ± 0.1
Carotenoids	10 ± 0.5

292 nm for α-tocopherol in ethanol [15].

For the determination of pheophytin and carotenoids, total pigments were extracted with acetone/water (85 : 15, v/v). The pigments were transferred to ethyl ether [16], leaving digitonin in the aqueous layer, and the ether solution was evaporated to dryness under reduced pressure. The pigments were dissolved in acetone and subjected to column chromatography with DEAE-Sepharose CL-6B [17]. Pheophytin *a* and carotenoids were eluted with acetone, and determined spectrophotometrically, using absorption coefficients, $E^{1\%}_{1cm}$, of 587 at 675 nm for pheophytin *a* [18] and 2500 at 450 nm for carotenoids [19]. The molecular weight of the carotenoids was assumed to be 540. Chlorophylls were eluted from the column with acetone/water (80 : 20, v/v), and spectrophotometrically determined according to Arnon [20].

Table I shows the contents of the prenylquinones and pigments in the reaction center II preparation. For 50 chlorophyll molecules, there were two molecules of plastoquinone-9, but virtually no phylloquinone, α-tocopherylquinone, nor α-tocopherol. One of the two plastoquinone-9 molecules is ascribed to Q and the other possibly to Z (or D_1). No plastoquinone-9 seems to be left for the secondary electron acceptor, B. In previous studies [21], the electron transport reaction from DPC to DCIP in the reaction center II preparation was little inhibited by a Photosystem II herbicide, atrazine, although the herbicide-binding protein, the apoprotein of B, was present [7]. These observations seem to suggest that the herbicide-binding protein had lost a major portion of the plastoquinone during preparation of the reaction center II, and therefore the electron transport became insensitive to the herbicides. Another possible attribution of plastoquinone-9 is that the two molecules are ascribed to Q and B, provided that Z (or D_1) differs from plastoquinone-9.

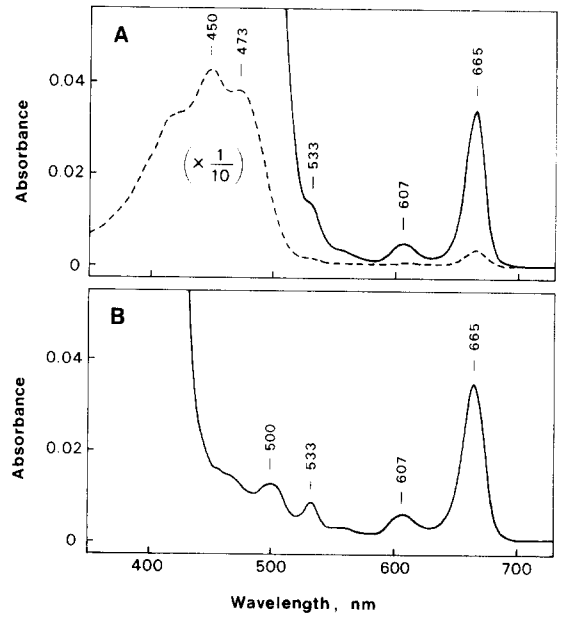

Fig. 1. Absorption spectra of a fraction eluted with acetone from DEAE-Sepharose CL-6B column chromatography. (A) Spectrum with no addition; (B) Spectrum measured 30 min after addition of 0.25 mg·ml^{-1} tetracyanoethylene. The concentrations of pheophytin *a* and carotenoids were determined to be 0.78 and 2.3 μg·ml^{-1}, respectively.

Pigments extracted from the reaction center II preparation were divided into two fractions by column chromatography with DEAE-Sepharose CL-6B; the one eluted with acetone contained carotenoids plus pheophytin *a* and the other eluted with acetone/water (80 : 20, v/v) contained chlorophylls. Fig. 1 shows the absorption spectra of the former fraction. Major peaks at 450 and 473 nm and a shoulder at 408 nm are ascribable to carotenoids, and peaks at 533, 607 and 665 nm to pheophytin *a*. Tetracyanoethylene, which removed the absorption bands of carotenoids in the blue region [18], disclosed absorption peaks at 500 and 533 nm (Fig. 1B). Absorption bands at 500, 533, 607 and 665 nm in Fig. 1B are characteristic of pheophytin *a*.

Quantitative analysis of the pigments indicated that there were about two molecules of pheophytin *a* and 10 molecules of carotenoids per 50 chlorophyll molecules in the reaction center II preparation (Table I). Since the ratio of chlorophyll to reaction center II is about 50 : 1 in this preparation [8,21], these findings suggest that two molecules of pheophytin *a* and 10 molecules of carotenoids are involved in the reaction center II complex. Klimov et al. [5] observed in their spectrophotometric analysis that one molecule of pheophytin *a* can be reduced by light per reaction center II. Probably, only one of the two pheophytin molecules is photo-reducible. It is noteworthy that the bacterial reaction center contains two molecules of bacteriopheophytin *a*, one of which can be reduced by light [22].

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References

1 Stiehl, H.H. and Witt, H.T. (1969) Z. Naturforsch. 24B, 1588–1598
2 Van Gorkom, H.J. (1974) Biochim. Biophys. Acta 347, 439–442
3 Pulles, M.P.J., van Gorkom, H.J. and Willemsen, J.G. (1976) Biochim. Biophys. Acta 449, 536–540
4 Nugent, J.H.A., Evans, M.C.W. and Diner, B.A. (1982) Biochim. Biophys. Acta 682, 106–114
5 Klimov, V.V., Klevanik, A.V., Shuvalov, V.A. and Krasnovsky, A.A. (1977) FEBS Lett. 82, 183–186
6 Satoh, K. and Butler, W.L. (1978) Plant Physiol. 61, 373–379
7 Satoh, K., Nakatani, H.Y., Steinback, K.E., Watson, J. and Arntzen, C.J. (1983) Biochim. Biophys. Acta 724, 142–150
8 Satoh, K. and Mathis, P. (1981) Photobiochem. Photobiophys. 2, 189–198
9 Satoh, K. (1979) Plant Cell Physiol. 20, 499–512
10 Bligh, E.G. and Dyer, W.J. (1959) Can. J. Biochem. Physiol. 37, 911–917
11 Lichtenthaler, H.K. and Prenzel, U. (1977) J. Chromatogr. 135, 493–498
12 Murata, N., Miyao, M., Omata, T., Matsunami, H. and Kuwabara, T. (1984) Biochim. Biophys. Acta 765, 363–369
13 Barr, R. and Crane, F.L. (1971) Methods Enzymol. 23, 372–408
14 Dunphy, P.J. and Brodie, A.F. (1971) Methods Enzymol. 18C, 407–461
15 Baxter, J.G., Robeson, C.D., Taylor, J.D. and Lehman, R.W. (1943) J. Am. Chem. Soc. 65, 918–924
16 Šesták, Z. (1971) in Plant Photosynthetic Production (Šesták, Z., Čatský, J. and Jarvis, P.G., eds.), pp. 672–701, Martinus Nijhoff/Dr. W. Junk Publishers, The Hague
17 Omata, T. and Murata, N. (1983) Plant Cell Physiol. 24, 1093–1100
18 Wilson, J.R., Nutting, M.-D. and Bailey, G.F. (1962) Anal. Chem. 34, 1331–1332
19 Liaaen-Jensen, S. and Jensen, A. (1971) Methods Enzymol. 23, 586–602
20 Arnon, D.I. (1949) Plant Physiol. 24, 1–15
21 Satoh, K. (1983) in The Oxygen-Evolving System of Photosynthesis (Inoue, Y., Crofts, A.R., Govindjee, Murata, N., Renger, G. and Satoh, K., eds.), pp. 27–38, Academic Press, Tokyo
22 Okamura, M.Y., Steiner, L.A. and Feher, G. (1974) Biochemistry 13, 1394–1403