

Short Communication**Diphenylcarbazone: A Reagent for Photosystem 1 Activity in Chloroplast Fragments¹**

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We have shown earlier that diphenylcarbazine serves as an electron donor to photosystem 2 of chloroplasts (5, 6) and during the process becomes oxidized. During the course of these experiments, it was observed that solutions of diphenylcarbazone, the initial oxidation product of DPC,² were decolorized upon illumination with chloroplasts. This reaction, followed by measuring the absorbance decrease at 487 nm, is a ready assay for photosystem 1 activity but does not appear to be a simple photooxidation.

With subchloroplast fragments obtained by treatment of spinach chloroplasts with Triton X-100, the reaction requires the presence of added plastocyanin, is insensitive to DCMU, and proceeds with rates of the order of 2500 μ moles/hr·mg chl.

MATERIALS AND METHODS

The preparation of spinach chloroplasts and the methods used for fragmentation of such chloroplasts with Triton X-100 have been previously described (4). The subchloroplast fragments produced by this treatment are designated TSF-1 and TSF-2, indicating the Triton subchloroplast fragments 1 and 2 corresponding to photosystem 1 and 2, respectively. The TSF-2 fraction was further fractionated to produce TSF-2a particles by suspending the TSF-2 pellets (from the final centrifugation) in an ice cold solution of 0.5 M sucrose and 50 mM tris buffer, pH 8.0, of about the same volume as that from which the particles were originally sedimented. Suspension of the particles was accomplished with a glass homogenizer, after which the suspension was allowed to stand for 1 hr at 0 C and then centrifuged twice at 144,000g for 1 hr each time, discarding the pellets. The supernatant fluid, which contained about 1% of the chl, was decanted and passed through a 1-inch deep column (1 inch in diameter) of Bioglass 2500 (BioRad Laboratories, Richmond, California). The column was eluted with the same solution used to suspend the TSF-2 pellets. The deep green fraction eluted was then concentrated by partial freeze-drying to about one-third the volume. The small particles so prepared are designated TSF-2a particles.

Plastocyanin was prepared by a modification of the procedure of Katoh *et al.* (2). The DPCO used in this study was purchased from Eastman Kodak Company. It was recrystallized prior to use, yet still contained some DPC (1). The procedures and apparatus used for illumination of the reaction mixture have been previously described. The millimolar extinction coefficient for DPCO used for rate calculations was 1.8 at pH 7.8.

Table I. *Photoreaction of DPCO with Spinach Chloroplasts and Subchloroplast Fragment Containing Photosystem 1 Activity (TSF-1)*

The reactions were performed in 2.0 ml of 50 mM phosphate buffer, pH 7.8, which also contained 2.5 mM DPCO and 20 μ g of chl in the case of chloroplasts or 11 μ g of chl with the TSF-1 particles. DCMU and plastocyanin were added as indicated. Illumination was with red light at an intensity of 2×10^5 ergs/cm²·sec, using the apparatus previously described (4).

Experimental Material	Plastocyanin μ M	DCMU μ M	DPCO Reacting μ moles/hr·mg chl
Chloroplasts	0	0	460
Chloroplasts	2	0	480
Chloroplasts	2	10	475
TSF-1 particles	0	0	0
TSF-1 particles	0.08	0	484
TSF-1 particles	0.4	0	1,450
TSF-1 particles	1.0	0	2,500
TSF-1 particles	1.0	10	2,450

RESULTS AND DISCUSSION

The photoreaction of DPCO with spinach chloroplasts and subchloroplast fragments is a photosystem 1 reaction. This reaction proceeds at a high rate in the presence of the photosystem 1 fragments prepared from spinach chloroplasts through the use of Triton X-100 (4), which are designated TSF-1 particles. It appeared initially that this reaction represented a simple oxidation of the DPCO to the diphenylcarbadizone, coupled to the reduction of oxygen by the particle. When the reaction was performed in the absence of oxygen, however, it was inhibited by approximately 20%. Accordingly, it appeared the reaction is complex and could represent an oxidation of the

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² Abbreviations: DPC: diphenylcarbazine; DPCO: diphenylcarbazone; DCPIP: dichlorophenolindophenol.

DPCO as well as a dismutation of some DPCO. Such a dismutation has been reported by Shneyour and Avron (3).

Table I shows the distribution of DPCO photoactivity in spinach chloroplasts and TSF-1 fragments obtained by treatment of chloroplasts with Triton X-100. These data show the reaction is insensitive to DCMU, which indicates it is a photosystem 1 reaction. The TSF-1 particles used in this experiment were inactive without the addition of plastocyanin. Treatment of chloroplasts with Triton X-100 removes most of the plastocyanin, and the experiments of Table 1 show the requirement for this compound to couple DPCO to the subchloroplast particle. The reaction with TSF-1 particle in the presence of plastocyanin is much faster than is that with chloroplasts alone,

which probably reflects the ready accessibility of DPCO to the soluble plastocyanin, which then reacts with the TSF-1 particle.

The photoreaction of DPCO can be used as a ready assay for photosystem 1 activity in subchloroplast fragments, as shown in Figure 1. In this figure, the activities of three such fragments are compared. The data show the concentration of activity in the TSF-1 particle and its absence in the TSF-2a particle, the latter being a purified photosystem 2 particle (7). In the presence of added plastocyanin, this particle is devoid of activity, which indicates complete removal of contaminating photosystem 1 from this preparation. The usual TSF-2 fragment, obtained by the initial split with Triton X-100, still contains some photosystem 1 activity. This agrees with earlier data obtained with this particle, in which photosystem 1 activity was determined as NADP photoreduction in the presence of ascorbate and DCPIP with 0.1% Triton X-100 present in the reaction mixture to facilitate interaction of the reagents with the TSF-2 fragment (5).

The advantage of the present photoreaction to measure photosystem 1 activity lies in the stability of the reaction, the fact that it does not need the presence of added enzymes to couple to NADP, and the fact that the reaction is catalyzed at a high quantum efficiency (3). These considerations indicate that this reaction will be of value in further studies on the fractions obtained from chloroplasts by various fractionation techniques.

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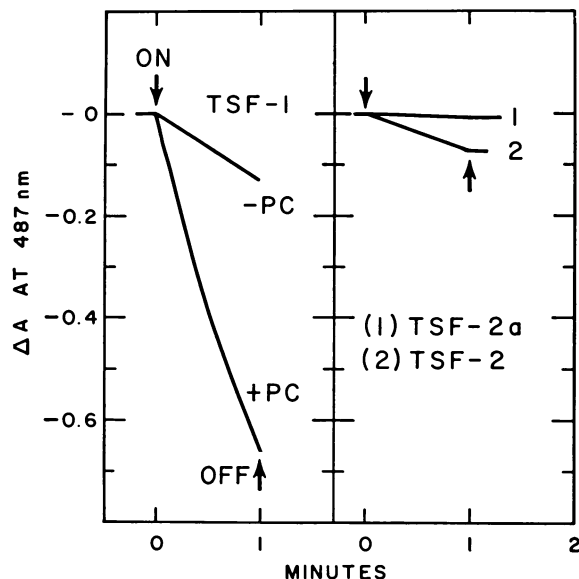


FIG. 1. Photoreaction of diphenylcarbazone catalyzed by chloroplasts and subchloroplast fragments. The reaction mixtures contained 2.5 mM diphenylcarbazone; 50 mM phosphate buffer, pH 7.8; and subchloroplast particles with the following chl concentrations in 2.0 ml of reaction mixture: TSF-1 (10 μ g), TSF-2 and TSF-2a (20 μ g). The experiments shown at the left were performed with or without the addition of 1.0 μ M plastocyanin as indicated. The experiments shown at the right with TSF-2 and TSF-2a fragments were performed with plastocyanin present at the same level. Illumination was with a tungsten lamp filtered through a Corning filter (No. 2403) to give red light of an intensity of 2×10^5 ergs/cm²·sec, using the apparatus previously described (4).