than 230 nm becomes higher than that of DDC or DDC disulfide, and the shoulder spectra characteristic of SeT was observed in the wavelength region longer than 300 nm. In the FAB-MS spectra of the isolated selenotrisulfide, molecular ion peak, $(M+1)^+$, was observed at m/z = 377. Thus, almost pure yellow crystalline SeT was obtained by a very simple preparation method without further purification.

Molar ratio of DDC: selenious acid in the reaction under the investigated conditions was 2:1 on analysis by the continous variation method and the molar ratio method.

A balanced equation for the reaction may be written as follows:

$$4\,\text{R-SH} + 2\,H_2SeO_3 + 2\,CH_3OH \rightarrow$$
$$\text{R-S-Se-S-R} + \text{R-S-S-R} + H_2Se + 4\,H_2O + HCHO + HCOOH,$$

where R-SH, R-S-Se-S-R, and R-S-S-R represent DDC, DDC-SeT, and DDC disulfide, respectively. This equation, which includes some speculation, is based on the result described above and on an observation that an equimolar amount of DDC disulfide and DDC-SeT were simultaneously formed in the reaction mixture, as shown by HPLC analysis.

We isolated a crystalline DDC-SeT by a simple method and characterized it by UV spectra, elemental analysis, and FAB-MS spectra. Biological activities, including superoxide dismutase-like activity, of the isolated DDC-SeT are now under investigation.

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The Sequential Release of Three Extrinsic Polypeptides in the PSII Particles by High Concentrations of Trichloroacetate

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Photosystem II (PSII) particles capable of O_2 evolution contain D_1 protein, D_2 protein, cytochrome b_{559}, and three extrinsic polypeptides, with apparent molecular masses of 33, 23, and 17 kDa. This enzyme system also contains manganese, chloride, calcium, and additional chlorophyll-protein complexes CP-47 and CP-43 [1]. Upon illumination, it oxidizes water to oxygen and reduces plastoquinone to plastoquinol, and is thus referred to as water-plastoquinone oxidoreductase. Photosynthetic oxygen evolution of thylakoids or PSII particles can be inactivated by various treatments including: mild heat; hydroxyamine; high pH; 0.8 *M* Tris (pH 8.0), and high concentration of NaCl [2]. After such treatments, three extrinsic polypeptides of oxygen evolution complex (OEC) of M_r 33, 23, and 17 kDa are partially or totally released. Here, we report a new method for successive release of the three extrinsic polypeptides from PSII.

The preparation of PSII particles from spinach is a modification of the methods used in [3]. Thylakoids were suspended in MN reaction medium containing 15 m*M* NaCl, 10 m*M* $MgCl_2$, and 50 m*M* Mes-NaOH, pH 6.0. The suspension was incubated with Triton X-100 (Triton X-100: Chl, 25:1, w/w) for 30 min with stirring at 4 °C. The treated suspenion was centrifuged at 40000 *g* for 30 min. The pellets (5 mg Chl/ml) were resuspended with 20% glycerol and stored in liquid nitrogen until use. Analysis of polypeptides using SDS-PAGE was done as in [4]. Electrophoresis was performed at a constant current (9 mA) for 11 h at room temperature in a slab-gel apparatus (12×15, 1.5 mm thick), by using a stacking gel with 6% acrylamide and a resolving gel with 13.5% acrylamide containing 6 *M* urea. The gel was stained with Coomassie Brilliant blue R250.

Figure 1 is the SDS-PAGE result, displaying the polypeptide pattern of the PSII particles treated with different concentrations of trichloroacetate. The incubating medium contained 0.2 *M* sucrose, 7.5 m*M* NaCl, 25 m*M* Mes-NaOH (pH 6.0); incubation was done

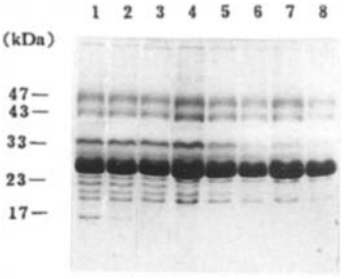

Fig. 1. SDS-PAGE patterns of PSII particles: effect of different concentrations of trichloroacetate on 17-, 23-, and 33-kDa polypeptides. *Lane 1* Control; *lanes 2–8* treated with 50, 100, 200, 400, 600, 800, 1000 m*M* trichloroacetate in dim light (3.2×10^4 erg cm^{-2} s^{-1}) at 0 °C and then diluted with MN reaction medium

in dim light (3.2×10^4 erg cm^{-2} s^{-1}) at 0 °C for 30 min. By changing the concentration of trichoroacetate, it is found that three extrinsic polypeptides of PSII, with apparent molecular masses of 17, 23, and 33 kDa, can be sequentially released. The 17-kDa band first disappeared (Lane 3) from the PSII particles treated with 100 m*M* trichloroacetate. The 17- and 23-kDa bands vanished (Lane 5) when the concentration of trichloroacetate reached 400 m*M*. All of the 17-, 23-, and 33-kDa bands disappeared as the concentration was >600 m*M* (Lanes 6–8). Thus, by using trichloroacetate to treat PSII particles, we have a novel method to sequentially release three extrinsic polypeptides of PSII.

SDS-PAGE results, showing that a trace of 33 kDa remains even after treatment with 1 *M* trichloroacetate, demonstrate that the 17- and 23-kDa polypeptides are amenable to easier extraction and the 33-kDa is a species with relatively higher binding characteristics. There could be two reasons for the sequential release of the three extrinsic polypeptides: their different locations and different interactions between each polypeptide and the PSII reaction center.

Unlike the treatment with 0.8 *M* Tris-HCl (pH 8.0) [5], the depletion of three extrinsic polypeptides of PSII is independent of light. The SDS-PAGE result of the polypeptide pattern of PSII particles treated with 1 *M* trichloroacetate in the dark is similar to that in dim light (Fig. 2). The depletion of the extrinsic polypeptides is also pH-independent. Figure 2 shows the SDS-PAGE analysis of the polypeptide pattern of trichloroacetate-treated PSII particles at pH 6.0, 7.0, and 8.0. Further comparison between different treatments will be necessary for the understanding of the relationships between oxygen evolution and the structure of water-plastoquinone oxidoreductase.

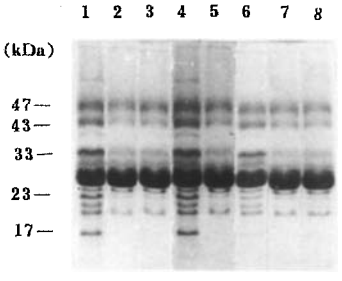

Fig. 2. SDS-PAGE patterns of PSII particles: dependence on light intensity and pH of trichloroacetate (1 *M*) treatment. *Lanes 1, 4, and 6* Control at pH 6.0, 7.0, and 8.0, respectively; *lanes 2, 5, and 7* treated in dim light at pH 6.0, 7.0, and 8.0, respectively; *lanes 3 and 8* treated in the dark at pH 6.0 and 8.0, respectively

The assay of 2,6-dichlorophenol indophenol (DCIP) induction was done with the Shimadzu UV-3000 spectrometer at 590 nm. The actinic light was obtained with a red cut-off filter transmitting wavelengths >645 nm. The illuminating intensity after the filter was 2×10^5 erg cm^{-2} s^{-1}; 0.4 *M* sucrose, 15 m*M* NaCl, and 50 m*M* Tris (pH 7.8), or 50 m*M* Mes (pH 6.0) were included in the reaction medium (3 ml). Chlorophyll concentration was 10 μg Chl/ml. Concentrations of DCIP and diphenyl carbazide (DPC) were 20 μ*M* and 1 m*M*, respectively. Figure 3 shows a comparison of the DCIP reduction rates between trichloroacetate-treated and -untreated PSII particles. In suspension medium without PSII particles, DPC shows no effect on DCIP reduction. The treatment of PSII particles with 1 *M* trichloroacetate totally blocks the $H_2O \rightarrow$ DCIP electron flow in the PSII particles. With the addition of 1 m*M* DPC, the DCIP reduction rate, however, was 42% (pH 6.0) or 80% (pH 7.8) of that in the different control level, indicating a good PSII photochemical activity after the treatment. The addition of 10 μ*M* DCMU (dichlorophenyl dimethyl urea) shows no influence on the recovered activity of DCIP reduction in the PSII particles, indicating that DCIP must accept electrons before the Q_B-binding site in our system. The most plausible candidate is Q_A. Since the treatment basically keeps the photochemical activity of the PSII particles, it can be used as a convenient means for future investigations on the mechanism of oxygen evolution and the interaction between the PSII reaction center and the oxygen-evolving abilities.

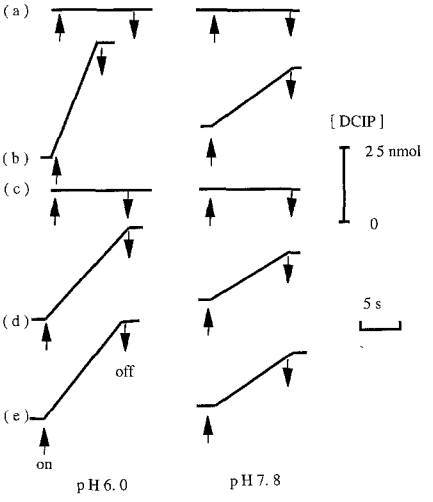

Fig. 3. Time courses of DCIP reduction measured in: a) suspension medium with 1 m*M* DPC and 20 μ*M* DCIP. b) Control PSII particles. c) PSII particles treated with 1 *M* trichloroacetate, d) with 1 m*M* DPC in c), and e), with 10 μ*M* DCMU in d)

We have reported earlier that the inhibitory effect of lower-concentration halogenated acetates on the electron acceptor side of PSII, particularly on electron transport from Q_A to Q_B, is correlated well with their hydrophobicity [6]. Results in this paper show an additional drastic effect on the donor side of PSII, without significant effect on the photochemistry of PSII measured by electron flow from DPC to DCIP. This donor side effect of chloroacetates becomes of immediate importance due to the recent discovery [7] of donor side effects of bicarbonate on PSII.

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