QUANTITATION OF LABILE SULFIDE CONTENT AND P700 PHOTOCHEMISTRY IN SPINACH PHOTOSYSTEM I PARTICLES

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Treatment of spinach photosystem I particles with 2 or 4 M urea containing 5 mM ferricyanide produces a time-dependent conversion of labile sulfide to zero-valence sulfur in the membrane-bound iron-sulfur proteins. The integrity of the primary electron donor, P700, remains intact when measured as a chemical oxidized-minus-reduced difference spectrum. The effect on the light-induced oxidation of P700 is complex; the extent of the normally-fast P700 photo-oxidation correlates directly with the amount of labile sulfide remaining in the particle but a slow phase of photooxidation only becomes evident in increasingly depleted particles and shows no relationship with the amount of remaining labile sulfide. The data is taken as evidence for the participation of an iron-sulfur protein in the primary photochemistry of photosystem I in green plants.

INTRODUCTION

There are at present two likely candidates for the primary electron acceptor of photosystem I based on ESR* measurements of chloroplasts and photosystem I particles at low temperatures. The first is a membrane-bound iron-sulfur protein (termed ESR Center A (1); g = 2.05, 1.94 and 1.86) that becomes photoreduced at liquid helium temperatures and exhibits a direct (1:1) correspondence with $P700^+$ under various levels of reduction (2, 3, 4, 5). The second is a component (g = 2.08, 1.88 and 1.78) that becomes ESR-visible only when the above iron-sulfur protein (ESR Center A) is chemically reduced prior to illumination (6, 7). Its identity is unknown but recent findings suggest that it is not an iron-sulfur protein (8).

We have attempted resolution of this problem utilizing a biochemical approach that selectively inactivates membrane-bound iron-sulfur proteins

*Abbreviations: ESR - Electron Spin Resonance
DMSO - Dimethylsulfoxide

without destroying the integrity of the primary electron donor, P700. Destruction of the iron-sulfur cluster should parallel the decline in the amount of P700 capable of photooxidation were an iron-sulfur protein the reaction partner to P700. We find that treatment of purified photosystem I particles with 2 or 4 M urea and 5 mM ferricyanide produces a time-dependent decrease in labile sulfide content concomitant with loss in the ability of P700 to undergo photooxidation.

MATERIALS AND METHODS

Chloroplast photosystem I particles were prepared by Triton X-100 treatment of spinach thylakoid fragments followed sequentially by differential centrifugation, gel chromatography and ion exchange chromatography (9). The chlorophyll to P700 ratio in the resulting particle is 25 and the amount of non-heme iron and acid-labile sulfide to P700 averages between 8-10:1. The particles were stored frozen but dialyzed for 12 hours against 50 volumes of 25 mM Tris, pH 7.30, containing 1% Triton X-100 before use. Incubation in 2 M urea was performed by the addition of 1 volume of 8 M urea and 20 mM potassium ferricyanide in 25 mM Tris, pH 7.30, to 3 volumes of dialyzed particle. Incubation in 4 M urea was performed similarily except that additional solid urea was added to achieve the final concentration of 4 M. Argon was blown over the surface of the reagents for one hour prior to addition and incubations were performed for various times in total darkness at 4°C. Treatment was terminated by passage through Bio-Gel P-4 pre-equilibrated with 25 mM Tris, pH 8.4, containing 1% Triton X-100.

Acid-labile sulfide was determined by a modification of the methylene blue method (10). Acidification products of chlorophyll that were not removed by high speed centrifugation were partitioned into benzene and the aqueous methylene blue was salted into n-hexanol and determined spectrophotometrically. P700 was determined chemically as a reduced-minus-oxidized difference spectrum with a Cary Model 14 spectrophotometer. Sufficient ascorbate and ferricyanide was added to produce the maximum difference spectrum and the baseline was established immediately after the scan by the addition of ascorbate to the reference cuvette. P700 was determined photochemically at 697.5 nm with a Kok-design double beam spectrophotometer during illumination with blue light at a saturating intensity of 4×10^5 ergs/cm^2/sec (9). An ascorbate-dichlorophenolindophenol donor system was used at concentrations sufficient to minimize contribution of the measuring beam. Methyl viologen was used as a secondary acceptor only where noted. Chlorophyll was determined in 80% acetone.

RESULTS AND DISCUSSION

Treatment of photosystem I particles with either 2 or 4 M urea and 5 mM potassium ferricyanide produces a time-dependent loss in the amount of labile sulfide without affecting the integrity of the primary electron

donor, P700 (9). Because control levels of labile sulfide could be recovered in all treated particles by overnight treatment with 1 mM dithiothrietol, we believe that labile sulfide in the membrane-bound iron-sulfur proteins is being oxidized to covalently-bound zero-valence sulfur in a manner analogous to that first described by Petering et al. (11). The kinetics of loss in labile sulfide and photoactive P700 after treatment with 2 and 4 M urea and 5 mM ferricyanide are shown in Fig. 1. The loss in sulfide is paralleled by a loss in P700 determined photochemically. The treatment, however, promotes little or no loss of P700 determined chemically as a reduced-minus-oxidized difference spectrum.

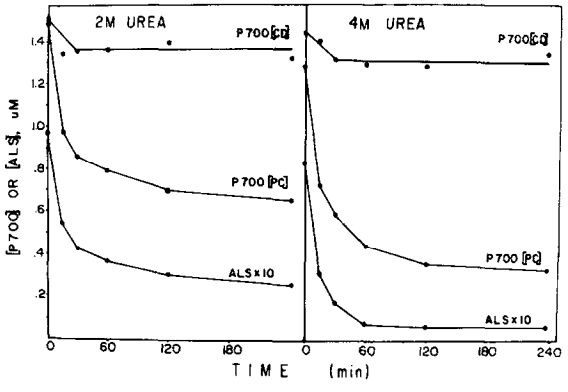

Figure 1. Effect of Urea and Ferricyanide on P700 and Labile Sulfide. P700 [CD], P700 determined by chemical difference spectroscopy; P700 [PC], P700 determined photochemically; ALS, Acid-Labile Sulfide. Chl = 45 µg/ml. Left: 2 M Urea and 5 mM Ferricyanide. Right: 4M Urea and 5 mM Ferricyanide.

The linear relationship between labile sulfide content and (total) P700 photochemistry (Fig. 2) suggests that the major fraction of P700 capable of undergoing photochemical oxidation is related to the amount of intact-iron-sulfur protein remaining in the particle. However, the best-fit line intersects the abcissa at a point removed from the origin, implying that a significant amount of P700 may not be functionally associated with an iron-sulfur center. Detailed inspection of the light-induced P700 oxida-

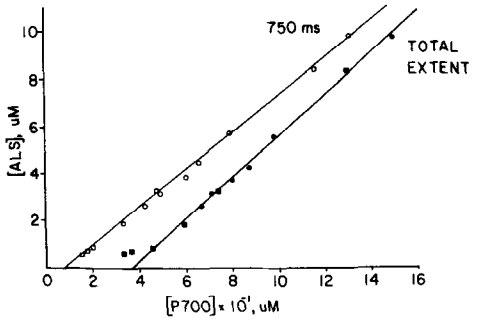

Figure 2. Amount of Labile Sulfide remaining in the particle vs. amount of P700 capable of light-induced oxidation. Total Extent, the total absorption change at 697.5 nm due to the photochemical oxidation of P700, 750 ms, extent of the absorption change 750 ms after illumination. Circles: 2 M Urea data from Fig. 1. Squares: 4 M Urea data from Fig. 1.

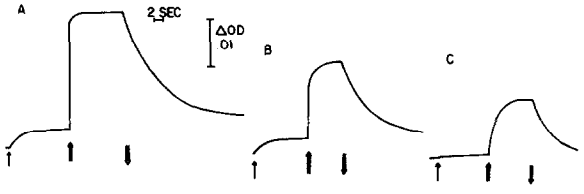

Figure 3. Kinetics of light-induced P700 oxidation at 697.5 nm. Single arrow, Measuring beam on; Double arrow, Actinic beam on and off. A: Control particle, Chl = 9.75 µg/ml. B: 2 M Urea and 5 mM Ferricyanide after 60 minutes, Chl = 12.7 µg/ml. C: 4 M Urea and 5 mM Ferricyanide after 60 minutes, Chl = 13.8 µg/ml.

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tion, moreover, reveals the existence of a slow phase superimposed upon the normally fast phase of P700 photooxidation in partially depleted particles (Fig. 3). We arbitrarily chose to define the fast phase as the instrumental time necessary to produce 92.5% of the full extent of P700 oxidation in the control particle; namely, 750 ms. The relative proportion of the fast contribution to the total extent of light-induced P700 bleaching in both treated and untreated particles is given in Table I. The contribution of the fast phase to the total extent of P700 photooxidation decreases with increased loss of sulfide. The inclusion of methyl viologen as a secondary acceptor is virtually without

TABLE 1

PROPORTION OF FAST (750 ms) CONTRIBUTION TO THE TOTAL EXTENT OF P700 PHOTOOXIDATION

TIME	% SULFIDE LOSS		FAST (750ms) EVENT; % OF TOTAL ABSORPTION CHANGE	
	2M Urea	4M Urea	2M Urea	4M Urea
0	0	0	92.5	92.5
15	44	60	81.0	62.5
30	55	77	76.5	58.2
60	62	89	75.5	48.2
120	67	91	70.5	47.4
240	73	93	64.7	46.7

All incubations include 5 mM Ferricyanide

effect on the relative contribution of fast and slow P700 kinetic phases in all classes of treated particles. It did, however, decrease the apparent extent of total P700 photooxidation but only because a larger amount was already oxidized by the weak measuring beam. The viologen dye presumably increased the efficiency of electron flow through P700 and its acceptor in weak light. The extent of the 750 ms event is also plotted against the amount of labile sulfide in Figure 2. The relationship is linear with a slope of 7.7 labile sulfide/P700 and the line intersects the abcissa very near the origin. Clearly, only the fast phase of P700 photochemistry appears to be associated with a protein containing labile sulfide. The slope of the line suggests either the demise of a single 8 iron, 8 sulfur protein containing two 4 iron, 4 sulfur clusters or may simply reflect the identical kinetic loss of two independent, but closely related, 4 iron, 4 sulfur proteins.

Either interpretation is consistent with the ESR data which show a strong interaction between the g = 1.86 line of ESR Center A and the 1.89 line of ESR Center B (g = 2.05, 1.92 and 1.89). This had led one group of investigators (1) to propose that the two ESR centers are so closely associated with each other that they interact during reduction. Further, the existence of 4 iron, 4 sulfur centers has already been inferred in a separate series of experiments that compare the ESR line positions of chemically reduced iron-sulfur centers in DMSO-treated photosystem I particles with chemically reduced, DMSO-treated soluble ferredoxins of known composition (12).

These findings suggest that the extent of the fast kinetic phase of P700 photooxidation is correlated linearily to the amount of labile sulfide remaining in the particle while the slower kinetic phase is not. The meaning of this slow phase is unclear. Freshly prepared photosystem I particles show a rapid light-induced P700 oxidation that is essentially complete after 750 ms of illumination. The slow kinetic phase of P700 photobleaching becomes apparent only after the particle has undergone treatment resulting in conversion of labile sulfide to zero valence sulfur. The destruction of the iron-sulfur centers may allow for an alternate (and perhaps non-physiological) pathway to function but which permits only a slow or inefficient rate of electron withdrawal from P700. The direct donation of electrons from P700 to molecular oxygen has not yet been ruled out and experiments are now in progress to test this proposal.

An alternative explanation of the data is that the urea-ferricyanide treatment may destroy the true (and still unidentified) primary electron acceptor at the same rate as iron-sulfur proteins are inactivated. However, the demonstration that loss of labile sulfide leads to a corresponding loss of P700 photochemistry under several different sets of conditions leads us to believe that the data genuinely reflect the participation of iron-sulfur proteins in the primary photochemistry of photosystem I in green plants.

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