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PHOTOSYSTEM II REACTIONS IN LIPOSOMES RECONSTITUTED WITH CHOLATE-EXTRACTED THYLAKOIDS AND A MANGANESE-CONTAINING PROTEIN

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INTRODUCTION

The mechanism of oxygen evolution in green plants is not well understood. Despite experimental evidence that there is a "charge accumulator" that accumulates four positive charges before oxygen is released from water, and that manganese may play a key role in O_2 evolution [1], little is known about the chemical intermediate involved in water splitting. Spector and Winget [2] have recently purified a manganese-containing protein (MW = 65,000) from spinach. They combined this protein with preformed liposomes, containing cholate-extracted thylakoids, and observed restoration up to 85% of the original O_2 evolution activity. In addition, the protein functioned specifically in photosystem II. In this communication, we report several characteristics of liposomes containing the cholate-extracted thylakoids (to be referred to as depleted photosomes) and of liposomes containing the cholate-extracted thylakoids and the manganese protein (to be referred to as complete photosomes). The complete photosomes showed a normal chlorophyll *a* fluorescence transient, a normal pattern of O_2 evolution per flash in a series of flashes, almost normal steady-state O_2 evolution rates, and a normal 515 nm absorption change. In contrast, the depleted photosomes showed no variable chlorophyll *a* fluorescence, no O_2 evolution either in continuous light or with light flashes, and an unusual light-induced absorption

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decrease in the carotenoid region. We interpret these results to indicate that the depleted photosomes lack electron flow on the donor side of photosystem II, whereas complete photosomes catalyze photosystem II electron transport from water. Whether restoration of oxygen evolution to the depleted photosomes is due solely to the addition of a specifically required manganese-containing protein (redox enzyme), or due rather to the re-establishment of electron flow by some indirect changes in the physical characteristics of depleted photosomes brought by the addition of the manganese protein, cannot be answered from our present experiments.

MATERIALS AND METHODS

The depleted and complete photosomes were prepared as described elsewhere [2]*. The stock preparations contained 2.1 mg soybean phospholipid (asolectin) : 4 mg total protein : 1 mg chlorophyll. Samples were thawed before use and diluted to a chlorophyll concentration of 4 μg/ml for fluorescence and absorption measurements or to $\sim$ 20 μg/ml for O_2 measurements.

Oxygen evolution in continuous light was measured with a Clark electrode, and in flashing light with a Joliot electrode. Chlorophyll *a* fluorescence transient was measured as described by Malkin et al. [3], and 515 nm light-induced absorption change by a single beam spectrophotometer.

RESULTS AND DISCUSSION

Steady-state O_2 evolution rates

Fig. 1 (solid line) shows a trace of O_2 evolution in continuous saturating white light for complete photosomes suspended in 10 mM phosphate buffer and 2 mM $MgCl_2$ (pH 7.2); the rate of O_2 evolution in the samples used in this study was 160 μmol O_2 /mg Chl/h with 2 mM ferricyanide as an electron acceptor. The depleted photosomes, on the other hand, showed no O_2 evolution (dashed curve, Fig. 1).

O_2 evolution in flashing light

Fig. 2 shows O_2/flash in a series of light flashes (duration 0.6 μs; time between flashes 1 s) for complete (o) and depleted (×) photosomes. There was absolutely no O_2 evolution in the depleted photosomes and an almost normal flash pattern in complete photosomes.

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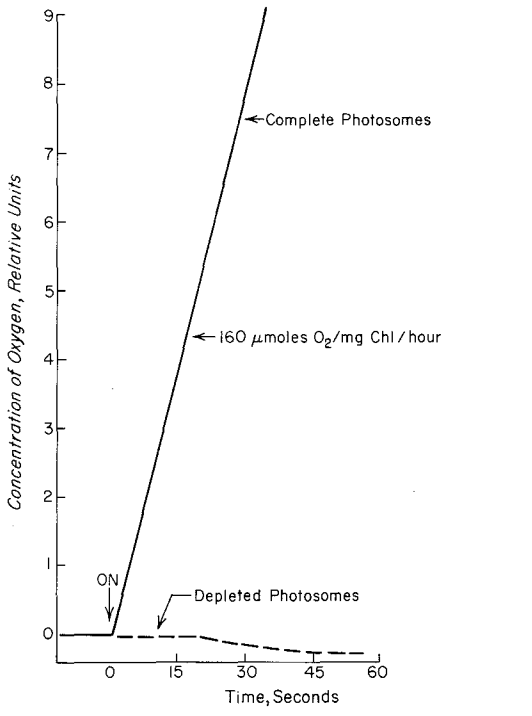
* The complete and depleted photosomes were prepared by two of us (M.S. and G.D.W.) at the University of Cincinnati, Ohio, and mailed by air freight in dry ice (−80°C) to Stanford, California, where the experiments were done.



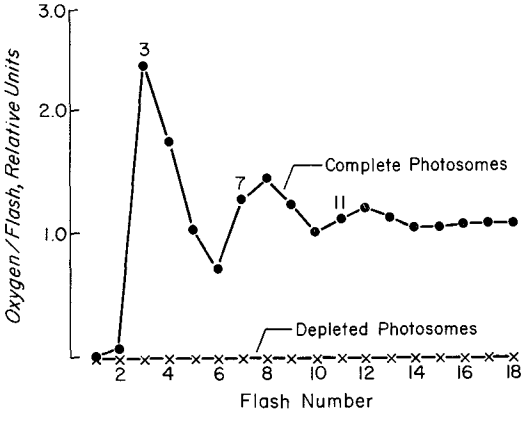
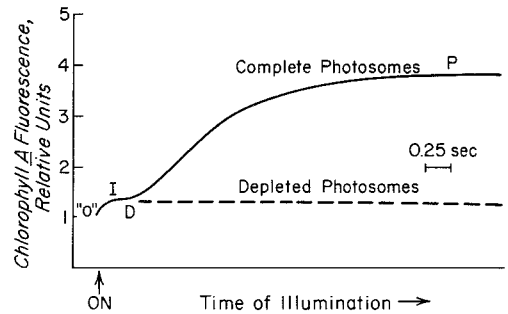


Fig. 1. Oxygen evolution as a function of time of illumination in complete photosomes measured by a concentration electrode (solid line). Suspension medium: Na phosphate buffer, 0.01 M (pH 7.2). The trace for the depleted photosomes shows the absence of O_2 evolution (dashed line). Electron acceptor, 2 mM ferricyanide; calculated rate of O_2 evolution in complete photosomes, without the addition of an uncoupler of phosphorylation, was 160 μmol/mg Chl/h.

Fig. 2. O_2 evolution per flash as a function of flash number in a series of flashes in complete photosomes (●) and depleted photosomes (X). Note the absence of O_2 evolution in depleted photosomes. The complete photosomes show results similar to chloroplasts.



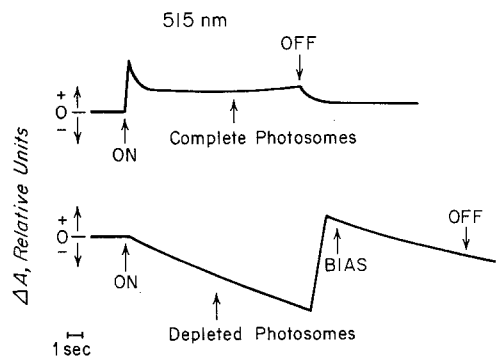


Fig. 3. Chlorophyll *a* fluorescence transients in complete (solid line) and depleted (dashed line) photosomes. For definition and meaning of symbols O, I, D and P, see Papageorgiou [4]. Note the absence of variable fluorescence (D to P rise) in depleted photosomes. Complete photosomes show results similar to chloroplasts.

Fig. 4. Light-induced absorbance changes at 515 nm in complete photosomes (upper curve) and in depleted photosomes (lower curve). Note the absence of absorbance increase in depleted photosomes; instead, a large absorbance decrease is observed. Complete photosomes show results similar to chloroplasts.

Chlorophyll a *fluorescence transients*

Fig. 3 shows that the complete photosomes have the usual fluorescence transient (O, I, D, P; see Papageorgiou [4] for definitions). The depleted photosomes have no variable fluorescence. This suggests that electron donation is absent in these preparations as they give results identical to those obtained with heat-treated chloroplasts (cf. ref. 4).

515 nm absorption change

Fig. 4 shows that the complete photosomes have a light-induced absorbance increase at 515 nm just as do chloroplasts. However, depleted photosomes show only an absorbance decrease at 515 nm. A spectrum of this decrease

suggests that carotenoids are bleached by the light in these preparations (data now shown).

Absorption spectra, emission spectra and action spectra of chlorophyll *a* fluorescence of both complete and depleted photosomes are approximately the same (data not shown), suggesting that there are no significant changes in the excitation energy transfer and in the pigment content and composition after the mild cholate treatment as used by Spector and Winget [2].

In conclusion, depleted photosomes behave as if a component on the electron donor side of photosystem II has been removed, whereas the complete photosomes behave as if this component has been replaced. This indicates that the 65,000 MW manganese protein is involved in electron transport on the donor side of photosystem II.

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