IV.1.

WEAK LIGHT PHOTOINHIBITION OF PSII AND ITS LIGHT DEPENDENT RECOVERY

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INTRODUCTION

Irradiation of leaves, chloroplasts or algal cells at high quantum fluxes causes photoinhibition of electron transport activities (reviewed in 1). Inactivation of the water-oxidizing complex (by NH_4OH or Tris) prior to illumination increases the sensitivity of PSII reactions such that very weak light is sufficient for inhibition (2). Several lines of evidence lead to the assignment of the site of damage on the oxidizing side of PSII: a) DCMU insensitive silicomolybdate photoreduction remains inhibited (4), b) atrazine binding properties of Q_B are unaltered (4), c) neither F_o nor F_max is affected, although the variable fluorescence rise-time increases (2), d) an artificial electron donor to PSII diminishes the variable fluorescence rise-time ~four-fold (2). Recovery (in leaf segments) from weak light photoinhibition requires light and 70S protein synthesis (2). Here we identify the chloroplast encoded protein whose synthesis is required for recovery from photoinhibition as D2 (3) and present a model which describes the inhibition process and its recovery.

METHODS

Wheat chloroplasts were extracted with NH_4OH in darkness and washed as in (4). Chloroplasts routinely showed 90-95% loss of V_O2. PSII assays were performed at quantum yield intensities as described in (4). Preparation of wheat leaf segments, their extraction with NH_4OH and all V_O2 assays are described in (2). Gel slicing, solubilization for electrophoresis and radiolabel counting are described in (4). Azido atrazine photoaffinity labelling was carried out as in (5). Other methods are included in figure legends.

RESULTS AND DISCUSSION

Radiolabeled amino acid incorporation during recovery of weak light photoinhibited leaf segments shows enhanced synthesis of only two thylakoid polypeptides (Figure 1, Peaks 1 and 2). Thylakoid polypeptides were separated by the two different PAGE conditions shown, the gels sliced and counted. The relative mobilities of the two polypeptides are quite different in the two conditions. The polypeptide of Peak 1 demonstrates a more rapid mobility than the polypeptide of Peak 2 in SDS-PAGE, while Peak 2 polypeptide runs more rapidly than Peak 1 polypeptide in LDS-PAGE. Regardless of these differences, azido [^{14}C] atrazine photoaffinity labelling identifies Peak 1 as the 32 kD herbicide-binding protein (Q_B protein). As stated above, however, we exclude from consideration the Q_B protein as the site of damage in weak light photoinhibition.

Western blot analysis of radiolabeled Peaks 1 and 2 (Figure 2) identifies the polypeptide of Peak 2 as the D2 protein (3). In separate experiments weak light photoinhibited leaf segments were allowed to recover in the presence of [\(^{3}H\)] leucine or [\(^{3}H\)] lysine. Since the Q_B protein contains no lysine residues, no incorporation of [\(^{3}H\)] lysine is shown by Peak 1 in the fluorograph portion of Fig. 2, while Peak 2 does demonstrate incorporation. Despite the presence of CH (cycloheximide), the lysine-rich 33 kD extrinsic protein of PSII shows some incorporation of [\(^{3}H\)] lysine. Both Peaks 1 and 2 demonstrate incorporation of [\(^{3}H\)] leucine. Careful alignment of prelabeled standards in all gel lanes allows us to identify Peak 2 as the D2 protein on the basis of the Western blots shown in Figure 2.

Figure 2. FLUOROGRAPHY AND WESTERN BLOT ANALYSIS OF PEAKS 1 AND 2

NH\textsubscript{3}OH extracted and washed leaf segments were photoinhibited, then further illuminated (8 h) in the presence of 500µg/ml CH and either [\(^{3}H\)]-leucine or [\(^{3}H\)]-lysine to effect recovery. AV\textsubscript{O}_{2} values of 280 and 240 O\textsubscript{2}/Chl/h were obtained for samples recovering in the presence of leucine and lysine, respectively. Thylakoid membrane polypeptides were analyzed by SDS-PAGE (10-20%). One section of the gel was fixed, then treated with Enhance (New England Nuclear), dried and fluorographed (2 week exposure shown). The remainder of the gel was used for electrotransfer of proteins onto nitrocellulose filters. The resultant blots were decorated with the indicated antibodies linked to goat-anti-rabbit-horse radish peroxidase conjugate. The fluorograph and individual blots were positioned in the figure by alignment of electrotransferred [\(^{14}C\)]-methylated MW markers.