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Photoreduction of $NADP^+$ by a chloroplast photosystem II preparation: effect of light intensity

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Recent work in this and other laboratories has demonstrated that, contrary to the favored Z scheme hypothesis, photosystem II (PS II) can photoinduce electron transfer from water to $NADP^+$, without the participation of photosystem I (PS I). One proposed explanation for this conflict between hypothesis and observation was that PS II can reduce $NADP^+$ but only at high light intensities. We report here findings at variance with this proposal. A PS II preparation made from spinach chloroplasts by the two-phase aqueous polymer partition method photoreduced $NADP^+$ without the involvement of PS I, at varying light intensities ranging from limiting to saturating.

Photosynthesis; Electron transport; Pheophytin

1. INTRODUCTION

The currently prevailing concept of photosynthetic electron transport in oxygen-evolving cells envisions a linear (noncyclic) electron flow from water to ferredoxin (and thence enzymatically to $NADP^+$) that requires the collaboration of two photosystems (PS I and PS II) joined by a chain of electron and proton carriers (the Z scheme). The essence of the Z scheme is that PS II generates the strong oxidant that oxidizes water but that only PS I can generate the strong reductant needed to reduce ferredoxin ($E_{m,7} = -420$ mV).

This thermodynamic restriction on PS II lost its force when pheophytin ($E_m \sim -610$ mV) was found to be the primary electron acceptor in PS II [1–4]. Nevertheless, the Z scheme was retained on the premise that although reduced pheophytin was thermodynamically competent to reduce ferredoxin, its physiological function was different: it served as intermediate electron carrier between the PS II reaction center chlorophyll (P680) and Q_A, the specialized plastoquinone that is the first stable electron acceptor in PS II [1–5].

The reduction of Q_A through the mediation of pheophytin entails a large expenditure of reducing power ($\Delta E_h \sim 600$ mV). A more economical expenditure of photochemically generated reducing power would result from reduction of ferredoxin by pheophytin ($\Delta E_h \sim 200$ mV). Such a role for pheophytin was envisaged in the alternative scheme for photosynthetic electron transport [6] in which PS II (renamed the oxygenic photosystem) drives the complete noncyclic electron transport from water to ferredoxin without the collaboration of PS I (renamed the anoxygenic photosystem). In the alternative scheme, the physiological role of PS I is limited to cyclic electron transport and phosphorylation. Except for regulatory connections, the two photosystems are viewed as being basically autonomous and as operating not collaboratively in series but synchronously in parallel [6].

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Abbreviations: PS, photosystem; Q_A, a specialized plastoquinone in PS II; diuron (DCMU), 3-(3,4-dichlorophenyl)-1,1-dimethylurea; DCIP, 2,6-dichlorophenolindophenol

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The Z scheme is supported by reconstitution experiments in which $NADP^+$ reduction required the inclusion of both PS II and PS I fractions when water [7] or a substituted PS II donor (diphenyl carbazide) was used [8,9]. However, in these reconstitution experiments the PS II and PS I fractions were isolated by detergent treatments of chloroplasts. Detergents modify membrane structure by releasing and exchanging with lipids and lipid-soluble components [10]. Native links between components may be lost and new links established.

Different results, consistent with the alternative scheme of electron transport, were obtained with inside-out vesicles enriched in PS II and prepared from chloroplasts by a non-detergent, aqueous two-phase partition method, more likely to protect membrane integrity [11]. The inside-out vesicles supplemented with plastocyanin, ferredoxin and ferredoxin-$NADP^+$ reductase required $NADP^+$ with electrons originating from water, under experimental condition that excluded the participation of PS I [12].

More recently, subchloroplast oxygen-evolving PS II preparations were found to photoreduce pheophytin under anaerobic conditions; reduced pheophytin was in turn oxidized, without the collaboration of PS I, by low-potential acceptors (benzyl viologen and methyl viologen) [13]. Of special interest were concordant experiments under anaerobic conditions with algal cells that included a *Chlamydomonas reinhardtii* mutant lacking PS I [14]. This mutant, which in effect represents a PS II specimen free from preparative artifacts and PS I contamination, was also able to photoinduce electron transport from water to pheophytin and thence to $NADP^+$ or to benzyl and methyl viologen [14].

Klimov et al. [14] interpreted their findings as being consistent with the alternative scheme of photosynthetic electron transport from water to $NADP^+$ but they also suggested that this scheme probably operates only at high light intensities when Q_A accumulates in the reduced state. We undertook, therefore, to investigate the validity of this proposed limitation. We report here that inside-out vesicles enriched in PS II photoreduced $NADP^+$ with electrons from water, without collaboration of PS I, at light intensities ranging from limiting to saturating.

2. METHODS

Chloroplasts were isolated from freshly harvested spinach leaves (*Spinacia oleracea*, var. Marathon) grown in a greenhouse in nutrient solution culture [15]. Inside-out vesicles enriched in PS II were prepared by the aqueous polymer two-phase (dextran-polyethylene glycol) partition method [11,12], with minor modifications [16]. Chlorophyll was estimated [15] and $NADP^+$ photoreduction was measured [17] as described. Spinach ferredoxin and plastocyanin were prepared by conventional methods. The A_{278}/A_{398} ratio of oxidized plastocyanin was 2.0.

3. RESULTS

The difference between PS II preparations made with detergents and without detergents in their capacity for oxygenic photoreduction of $NADP^+$ is shown in fig.1. The PS II preparation made with detergents (BBY in fig.1, inset) was totally inactive in $NADP^+$ reduction, either in the presence or absence of plastocyanin. In contrast, the PS II-enriched inside-out vesicles gave an appreciable rate of oxygenic $NADP^+$ reduction that, in agreement with earlier findings [12], depended on plastocyanin, up to a saturating concentration of about 3 μM.

To test the possibility that the oxygenic photoreduction by the inside-out vesicles was due to the still remaining small PS I component, electron flow from water was totally inhibited by diuron (DCMU) and a PS I electron donor (ascorbate/DCIP) included in the reaction mixture. Fig.1 shows only a small $NADP^+$ reduction via PS I that cannot account for the observed $NADP^+$ photoreduction by electrons from water. Moreover, this small PS I activity was not influenced by plastocyanin concentration (fig.1). Externally added plastocyanin, when reduced by ascorbate, is an effective donor to P700 if P700 is accessible [19]. Thus, it appears that the small PS I component was not accessible to plastocyanin and was not a factor in the oxygenic reduction of $NADP^+$.

Fig.2 shows that the oxygenic reduction of $NADP^+$ by inside-out vesicles occurred over a wide range of light intensities. The rate of $NADP^+$ reduction was proportional to light intensity, and began to level off as light was approaching saturation. The low rate of $NADP^+$ reduction by the small PS I component remained the same at all light intensities (fig.2).

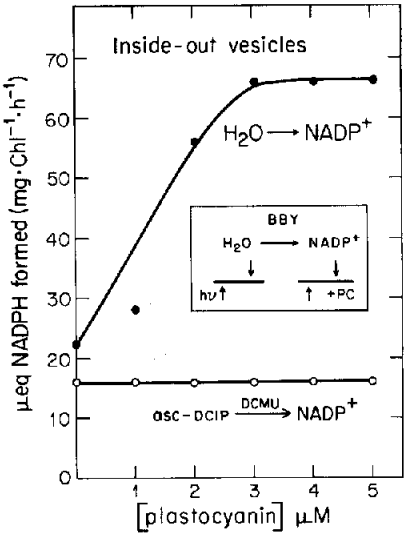

Fig.1. Effect of plastocyanin on photoreduction of $NADP^+$ by inside-out vesicles enriched in PS II and by a PS II preparation (BBY, [18]) isolated with detergents (inset). Reaction mixtures contained 50 mM (*N*-morpholino)ethanesulfonic acid (Mes) buffer at pH 6.7, 5 mM $MgCl_2$, 10 μM spinach ferredoxin, 2 mM $NADP^+$, 50 μg chlorophyll per ml and a saturating amount of ferredoxin-$NADP^+$ reductase. Other additions, where indicated: diuron (DCMU), 10 μM; 1 μM DCIP; 10 mM ascorbate (asc); and in inset, 3 μM plastocyanin (PC). The reaction mixtures were illuminated at room temperature in open to air cuvettes (2 mm light path) by 650 nm light. Arrows pointing up, light on; arrows pointing down, light off.

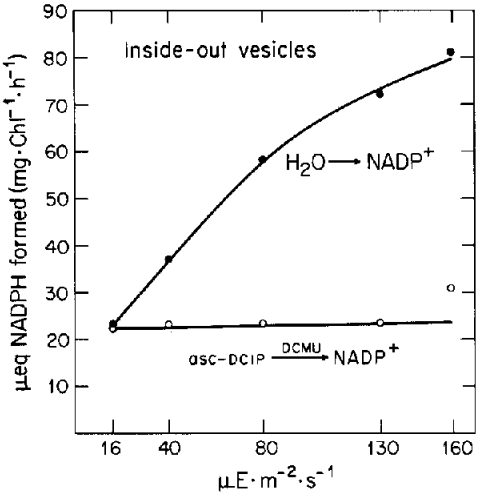

Fig.2. Effect of light intensity on photoreduction of $NADP^+$ by inside-out vesicles enriched in PS II. Other experimental conditions as in fig.1 except that chlorophyll concentration was 20 μg/ml.

In summary, we found no experimental support for the suggestion that oxygenic photoreduction of ferredoxin-$NADP^+$ by PS II can occur only at high light intensity [14]. It occurred at light intensities ranging from limiting to saturating.

4. DISCUSSION

The important discovery of pheophytin as the primary electron acceptor from P680 has removed the thermodynamic barrier to $NADP^+$ reduction by PS II. Photoreduction of $NADP^+$ (and hence ferredoxin) by PS II, via pheophytin, was observed not only with subchloroplast PS II preparations but also with cells of a *Chlamydomonas* mutant lacking PS I [14]. However, such activity by PS II was deemed to be a special case possible only at high light intensity when Q_A accumulates in the reduced state [14].

This investigation does not support this view but is in accord with the alternative concept of photosynthetic electron transport that envisions, with no limitation of light intensity, photoreduction of ferredoxin/$NADP^+$ by PS II, without the involvement of PS I [6,12].

New support for the alternative concept of PS II as the generator of a strong reductant has come recently from evidence of photoinduced production of molecular hydrogen by subchloroplast preparations enriched in PS II [20]. Like photoreduction of $NADP^+$ by PS II [14], photoproduction of H_2 by PS II was also observed with intact cells of *Chlamydomonas reinhardtii* mutants lacking PS I [21]. Both instances involve electron transfer from photoreduced pheophytin ($E_m \sim -610$ mV) to an acceptor with a redox potential of ~ -400 mV, i.e., ΔE of ~200 mV.

Past research in photosynthesis provides examples of findings that were out of harmony with dominant contemporary concepts but have in time prevailed and led to advances in knowledge [22–24]. There can be no certainty now that oxygenic photoreduction of ferredoxin/$NADP^+$ by PS II will be added to this category. However, the existing evidence for the alternative hypothesis of photosynthetic electron transport is already incompatible with, and in Popper's terminology 'falsifies' [25], the Z scheme hypothesis whose postulates merit continued investigation.

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