Photodynamic Damage to Isolated Chloroplasts: A Possible Model for *in vivo* Effects of Photosynthetic Inhibitor Herbicides

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The breakdown of chlorophylls, carotenoids, and linolenic acid together with the formation of malondialdehyde and ethane was followed in isolated pea chloroplast membranes. Breakdown was enhanced by light, oxygen, D_2O and rose bengal, but retarded by crocetin. The results are discussed in relationship to the role of singlet oxygen in promoting damage *in vivo*.

Introduction

The observed toxic effects of photosynthetic electron transport inhibitor herbicides such as phenylureas and triazines are magnified by treatment of plants under increased light intensity [1–5], or diminished if oxygen is removed [4]. It has been suggested that excitation energy absorbed by the chloroplast pigments is not utilized and hence singlet chlorophyll (^{1}Chl.) undergoes intersystem crossing to generate the longer lived triplet state (^{3}Chl.) [4]. ^{3}Chl may induce directly damage to cellular components such as unsaturated fatty acids of membranes in type I reactions, or by interaction with oxygen ($^3O_2$) generate singlet oxygen ($^1O_2$). This may lead *via* type II reactions to damage in lipids, proteins and nucleic acids [6]. The overloading of chloroplast pigment systems in the presence of herbicides is analogous to a system in which leaves are incubated in the absence of carbon dioxide [7] or low temperature [8] where similar phytotoxic symptoms are observed. Several workers [9, 10] have examined the longer term effects of incubating isolated chloroplasts in the presence or absence of photosynthetic electron transport inhibitor herbicides, and have proposed mechanisms of damage. Others have incubated chloroplasts in high light conditions and followed the inactivation of photosynthetic electron transport [11–16] or the induction of lipid peroxidation [17, 18]. In this investigation we have studied the destruction of isolated chloroplast thylakoid membranes with a range of treatment conditions and additions in an attempt to describe the type of reactions which might occur in inhibited chloroplasts *in vivo*.

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Materials and Methods

Chloroplast membrane preparations were made from subapical leaves of 14–21 day old pea (*Pisum sativum*, var. Meteor) seedlings [19]. Membranes were incubated in 50 mM phosphate buffer pH 7.6, with an initial level of around 50 µg chlorophyll ml^{-1} in a total volume of 25 ml. Flasks were constantly shaken at 25 °C with illumination of 400 µE m^{-2} s^{-1}. Anaerobic conditions were obtained by gassing sealed flasks with argon. Some incubations were undertaken with D_2O or with the addition of crocetin (final concentration 1 mM) prepared from Saffron by the method of Friend and Mayer [20]. Rose bengal DEAE-sephadex complexes were prepared and used as previously described [19]. The final concentration of rose bengal was around 0.1 mM. The preparation of lipid extracts and the assessment of malondialdehyde and linolenic acid was as described by Percival and Dodge [19, 21]. Ethane was measured by sampling the gas phase of sealed flasks and by use of a Pye Unicam GCD gas chromatograph, and quantified by reference to a standard curve. Chlorophylls were measured by the method of Arnon [22], and carotenoids that of Kirk and Allen [23].

Results and Discussion

Isolated chloroplast thylakoid membranes treated in the absence of an exogenous electron acceptor for 6 h showed a conspicuous light enhanced breakdown of pigments (Fig. 1) and linolenic acid, the major fatty acid of the membranes (Fig. 2, C and D). Fig. 1 shows that carotenoid pigment breakdown preceeded that of chlorophylls over the first few hours of incubation. Experiments with electron transport inhibitor herbicides and whole leaves, also showed a preferential breakdown of these pigments [10]. Carotenoids *in vivo* act both as a $^1O_2$ and ^{3}Chl quenching system [24], and thus appear to be overtaxed and preferentially destroyed. Figs. 1 and 2 show that the addition of the water soluble carotenoid pigment crocetin, known to be a quencher of $^1O_2$ [25], retarded the breakdown of both pigments and lipids. In addition the presence of D_2O buffer which enhances the lifetime of $^1O_2$ [26] also promoted breakdown.

Incubation of chloroplast thylakoids in light but under anaerobic conditions, showed that the absence of oxygen retarded, but did not totally prevent pigment and membrane breakdown. It is possible that the major mechanism of damage is *via* type II reactions in which $^1O_2$ directly reacts with membrane components such as linolenic acid with the formation of lipid peroxides, [27], and further breakdown products such as malondialdehyde (Fig. 2), and ethane (Fig. 3). However in the absence of oxygen, type I reactions could play a more important part in breakdown. In electron transport inhibited chloroplasts within a leaf cell, oxygen would be ubiquitously present which would favour type II reactions, however some incipient damage *via* type I reactions would be promoted by the continued presence of oxygen, as this would be required for the further propagation of lipid peroxidation reactions. Furthermore $^1O_2$ may be generated from lipid peroxides [28] as may also the toxic hydroxyl radical from ^{3}Chl [29].

Rose bengal is well known as a photosensitizer of $^1O_2$ generation [30]. It has been previously used in experiments in which damage is promoted in isolated membrane lipids [19], and whole leaf tissue [31]. In these experiments immobilised rose bengal was added to isolated chloroplast thylakoid mem-

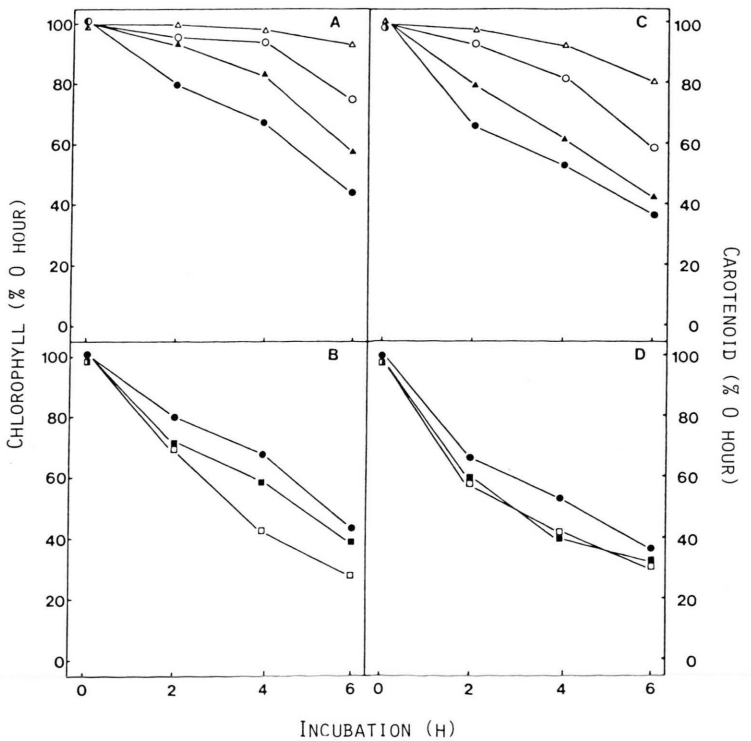

Fig. 1. A and B Chlorophyll loss from chloroplast membranes incubated in darkness △; anaerobically in light ○; light plus crocetin ▲; light ●; light with D_2O ■; light with immobilised rose bengal □. C and D, carotenoid loss from chloroplast membranes with similar treatments.

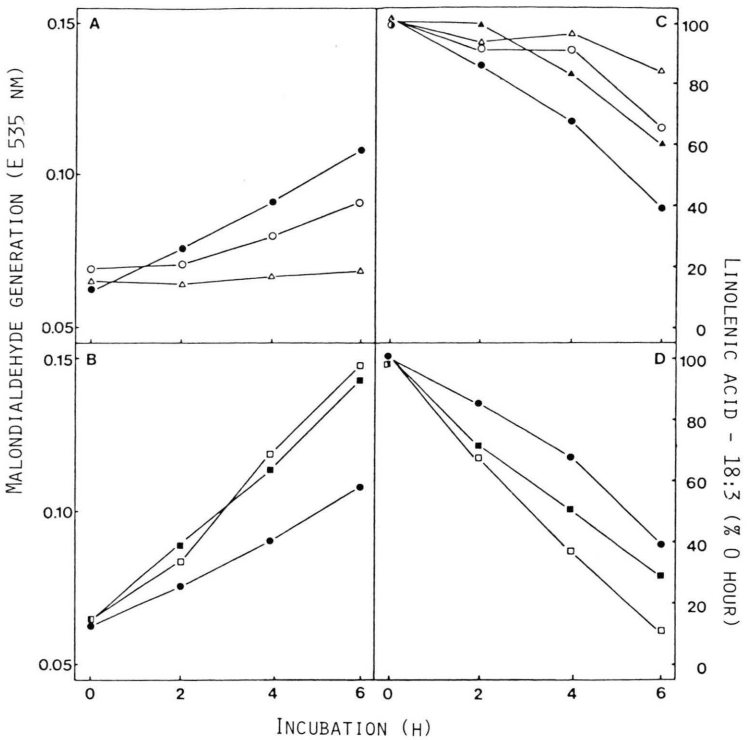

Fig. 2, A and B. Malondialdehyde formation (measured as optical density [*E*] at 535 nm) in isolated chloroplast membranes incubated in darkness △; anaerobically in light ○; light ●; light with D_2O ■; light with immobilized rose bengal □; C and D, loss of linolenic acid from isolated chloroplast membranes incubated as described for A and B in addition light plust crocetin ▲.

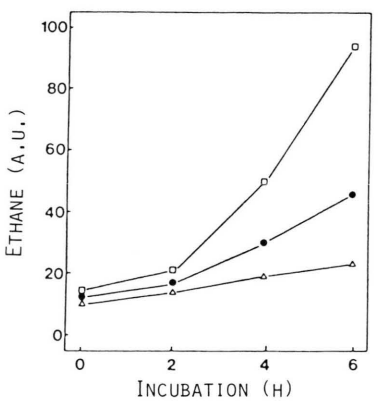

Fig. 3. Ethane generation from isolated chloroplast membranes incubated in darkness △; light ●; light with immobilized rose bengal □. (a. u. = arbitrary units.)

branes. In all instances its presence promoted the breakdown of pigments and lipids (Figs. 1 and 2) and in the case of ethane generation led to a considerably enhanced production of this gas after an initial lag period. This could indicate that the exogenous generator of $^1O_2$ was more effective as a damaging agent once the thylakoid membranes had been partially disrupted and destroyed.

Altogether these experiments indicate that excitation energy, which is not utilized in the promotion of electron flow, as could happen with herbicides *in vivo*, promotes pigment loss and membrane breakdown. Endogenous quenching systems are overtaxed and destroyed and the breakdown of lipids and pigments follows in parallel. These results provide good evidence for a possible role for $^1O_2$ in inducing membrane damage as well as ^{3}Chl by type I reactions. As membrane breakdown proceeds, the potential for the generation of these toxic species diminishes, but in photosynthetically inhibited leaves this is of no consequence. This could however act as a protective device in leaves treated under high light stress conditions [32].

Acknowledgement

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