

SHORT COMMUNICATION

ASCORBATE PHOTOOXIDATION BY CHLOROPLASTS ISOLATED FROM SENESCENT AND PARAQUAT TREATED LEAVES

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Abstract—The ability of chloroplasts isolated from senescent and paraquat treated flax cotyledon leaves to photooxidise ascorbate in the presence or absence of DCIP was investigated. A progressive lack of requirement for DCIP to mediate this photooxidation during these two treatments is suggested to be indicative of a gradual disruption of thylakoid membranes *in vivo*.

VERNON and ZAUGG¹ demonstrated that NADP reduction could be restored to chloroplasts made inactive through ageing or DCMU treatment, by the addition of DCIP (2,6-dichlorophenol indophenol) maintained in the reduced state by ascorbate. In the absence of DCIP the rate of NADP reduction was considerably retarded. Davenport and Dodge² showed that the requirement for DCIP could be replaced by plastocyanin after isolated pea chloroplasts had been subjected to ultrasonic disintegration. Further experiments² with chloroplasts heated at 55° for 2–3 min indicated that, in a system of more gentle disruption where bound plastocyanin was not released, ascorbate was as effective as the ascorbate DCIP couple in restoring NADP reduction. Vernon *et al.*³ found that NADP reduction in a Triton X-100 treated chloroplast fraction was still considerably dependent upon the presence of DCIP in addition to ascorbate, although it had been shown previously that the DCIP requirement could be released after treatment of pea chloroplasts with digitonin.⁴ It was concluded in a previous investigation² that ultrasonic and heat treatments may cause some conformational changes in the chloroplast thylakoid structure enabling ascorbate to donate directly to the electron transfer chain. In this investigation we have extended this idea by assessing the ascorbate photooxidizing ability of chloroplasts isolated from flax cotyledon leaves which were undergoing natural senescence, or herbicide treatment. When chloroplasts were isolated from young, newly expanded flax cotyledon leaves 7–10 days after sowing the level of ascorbate photooxidation with DCIP was about 20% of the control. During the following 30 days senescence, the requirement for DCIP progressively diminished (Fig. 1); thus, just before the cessation of photosynthetic activity the rate of ascorbate photooxidation without DCIP had changed to 90% of the control. Similarly in chloroplasts isolated from paraquat treated leaves a rapid change occurred from 20% of the control level after 10 hr treatment to over 90% by 30 hr. It is well known that during the course of

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¹ L. P. VERNON and W. S. ZAUGG, *J. Biol. Chem.* **235**, 2728 (1960).

² H. E. DAVENPORT and A. D. DODGE, *Phytochem.* **8**, 1849 (1969).

³ L. P. VERNON, E. R. SHAW and B. KE, *J. Biol. Chem.* **241**, 4101 (1966).

⁴ A. D. DODGE, Unpublished (1965).

⁵ R. HILL and D. A. WALKER, *Plant Physiol.* **34**, 240 (1959).

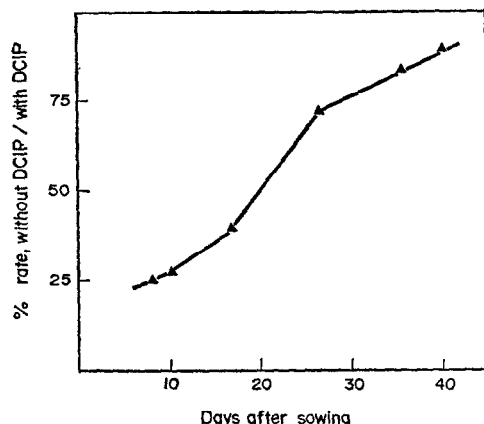


FIG. 1. ASCORBATE PHOTOOXIDATION BY CHLOROPLASTS ISOLATED FROM COTYLEDON LEAVES OF FLAX SEEDLINGS.

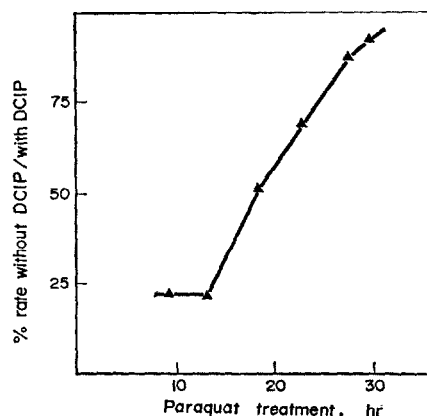


FIG. 2. ASCORBATE PHOTOOXIDATION BY CHLOROPLASTS ISOLATED FROM PARAQUAT TREATED FLAX COTYLEDON LEAVES.

senescence, the obvious leaf pigment changes which occur are a reflection of the dramatic changes in chloroplast structure.^{6,7} Likewise, flax cotyledon leaves treated with paraquat under conditions identical to this experiment showed considerable chloroplast disruption after 20 hr paraquat treatment.⁸ (Fig. 2).

These experiments are further evidence for the idea that the ability of isolated chloroplast fragments to catalyse the photooxidation of ascorbate in the absence of DCIP, is an indication of the intactness of the chloroplast thylakoids. Furthermore, this appears to be the case, irrespective of whether the damage is sustained after chloroplast isolation, or progressively within the leaf cell by natural or artificial means.

EXPERIMENTAL

Flax (*Linum usitatissimum*) seeds were sown on moist vermiculite and grown under continuous illumination of 1250 lx from 'day glow' neon tubes. The seedlings were left to grow on the vermiculite until the cotyledon leaves were fully senescent. For the paraquat treatment, 7-day-old cotyledon leaves were removed and floated on 1×10^{-4} M solutions of the herbicide in crystallizing dishes. These were illuminated by light of 2250 lx at 24°.

Chloroplasts were isolated from the senescent leaves until photochemical activity had ceased at about 40 days after sowing. With the paraquat treatment this point was reached after about 30 hr. The chloroplast isolation procedure was based on that of Hill and Walker,⁵ and ascorbate photooxidation was measured as an oxygen uptake by using an oxygen electrode (Rank Bros.). Illumination was provided by a 500-W lamp, with the reaction chamber maintained at 18°. Standard reaction mixtures of 4 ml contained, in addition to 0.3 ml chloroplast preparation (in μ mol) Tris-HCl buffer pH 8.0 100; Ascorbate 40; CMU 0.1; Benzylviologen 0.01; KCN 0.1; and DCIP as required 0.2.

⁶ M. SHAW and M. S. MANOCHA, *Can. J. Bot.* **43**, 747 (1965).

⁷ R. BARTON, *Planta* **71**, 314 (1966).

⁸ N. HARRIS and A. D. DODGE, *Planta* (1972), in press.

Key Word Index—*Linum usitatissimum*; Linaceae; chloroplasts; ascorbate photooxidation; leaf senescence; paraquat treatment.