

Mechanism of Paraquat Action: Inhibition of the Herbicidal Effect by a Copper Chelate with Superoxide Dismutating Activity

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The treatment of flax cotyledons (*Linum usitatissimum*) with paraquat was shown to decrease the levels of chlorophyll and carotenoid pigments. The fatty acid content of chloroplast fragments isolated from treated tissue was determined and shown to be greatly decreased by paraquat treatment. The superoxide radical was demonstrated to play an important role in the phytotoxic action of paraquat by the use of a copper chelate of D-penicillamine, which has a high superoxide dismutating activity. The action of paraquat was inhibited by this compound. The role of superoxide is discussed with reference to the generation of more toxic species, such as singlet oxygen.

Introduction

The bipyridylium herbicides *e. g.*, paraquat and diquat, initiate their phytotoxic action by successfully competing with NADP for electrons emanating from the P 700 acceptor [1]. They are reduced univalently and undergo immediate reoxidation by molecular oxygen [2]. The superoxide anion and hydrogen peroxide are both formed during the autoxidation of the reduced compounds. It was originally believed that hydrogen peroxide was the initial toxic species responsible for the action of paraquat [3], more recently however, superoxide has been shown to be an earlier, more reactive intermediate [2]. Hydrogen peroxide is formed via both the enzymatic and nonenzymatic dismutation of superoxide. Superoxide dismutase (SOD) is present in the chloroplast [4] and is found associated with the thylakoid lamellae and free in the stroma [5, 6] and rapidly catalyses the harmless dismutation of superoxide [7]. Asada *et al.* [8] have calculated the concentration of SOD in the chloroplast to be about 10^{-5} M, which is sufficient to deal with steady-state concentrations of superoxide of about 10^{-9} M. However, the presence of paraquat can increase the superoxide concentration up to 10^{-8} – 10^{-7} M, which is in excess of the enzymatic defences and so will lead to cellular damage.

Earlier work showed that paraquat treatment caused the loss of chlorophyll and disruption of the

tonoplast and plasmalemma membranes [9]. In the present study, the role of superoxide in the herbicidal action of paraquat has been investigated with the aid of a low molecular weight copper chelate of D-penicillamine (PA-Cu) [10, 11], which is known to have a high superoxide dismutating activity [12].

Materials and Methods

Flax seedlings (*Linum usitatissimum* var. Reina) were grown under continuous illumination of 5.25 W/m^2 on waterlogged vermiculite for seven days at $25 \pm 3^\circ \text{C}$. The cotyledons were removed and floated on solutions as indicated under the same conditions of temperature and illumination as described above. The final concentration of paraquat was 10^{-5} M and 50 units of PA-Cu were used per treatment. The addition of paraquat was delayed for 24 hours to allow PA-Cu to infiltrate the cotyledon tissue.

Chloroplast fragments for fatty acid analysis, were isolated from treated cotyledons by a modification of the procedure of Izawa and Good [13]. Total lipids were extracted with a chloroform-methanol mixture [14] and then refluxed with methanolic NaOH and boron-trifluoride-methanol to form methyl ester derivatives of the fatty acids [15, 16]. These were separated and identified using a Pye Unicam GCD chromatograph with 10% diethylene glycol succinate as the stationary phase. The column temperature was 190°C .

The carotenoid pigments of treated cotyledons were extracted and the levels determined according to the method of Bishop and Wong [17]. Chlorophyll was measured as described by Arnon [18].

Abbreviations: Paraquat, 1,1'-dimethyl-4,4'-bipyridylium dichloride; SOD, superoxide dismutase; PA-Cu, copper chelate of D-penicillamine.

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Results

The decrease in chlorophyll content is one of the most obvious symptoms of the phytotoxic action of paraquat. Chlorophyll breakdown in paraquat treated cotyledons was markedly reduced by PA-Cu (Fig. 1) during an illumination period of 72 hours. Similarly the breakdown of carotenoid pigments in the presence of paraquat was retarded by PA-Cu over the same time period (Fig. 2). Although the levels of all carotenoid pigments decreased following paraquat treatment, α - and β -carotenes were more markedly affected than the xanthophylls. In addition, the restraining effect of PA-Cu was most evident on the xanthophyll pigment levels. α - and β -carotenes were completely destroyed by paraquat treatment, but the additional presence of PA-Cu reduced the breakdown to 87%. In the case of the xanthophylls, 89% were destroyed by paraquat alone, but the decrease was only 26% in the presence of PA-Cu.

One of the early effects of paraquat treatment is the initiation of lipid peroxidation reactions which

CAROTENOID CONTENT OF FLAX COTYLEDONS

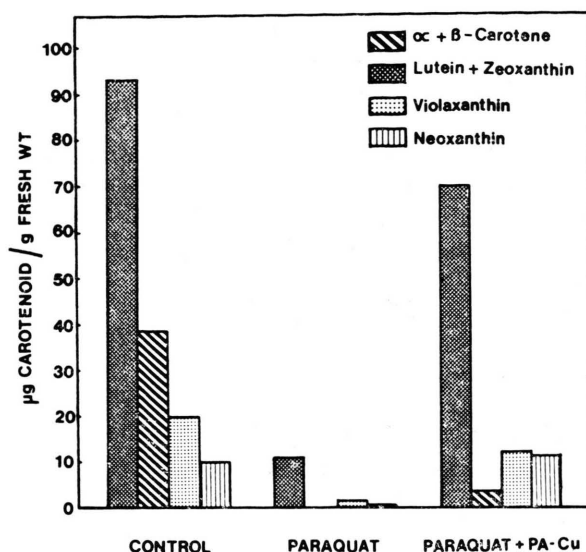


Fig. 2. Changes in carotene and xanthophyll levels of treated flax cotyledons. Incubation conditions are detailed in Materials and Methods.

CHLOROPHYLL CONTENT OF FLAX COTYLEDONS

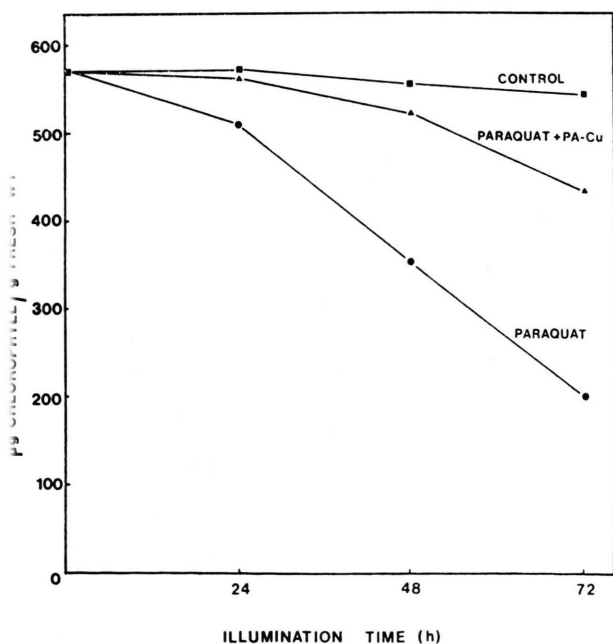


Fig. 1. Chlorophyll content of paraquat treated flax cotyledons in the presence and absence of PA-Cu. Incubation conditions were as described under Materials and Methods.

FATTY ACID CONTENT OF CHLOROPLASTS

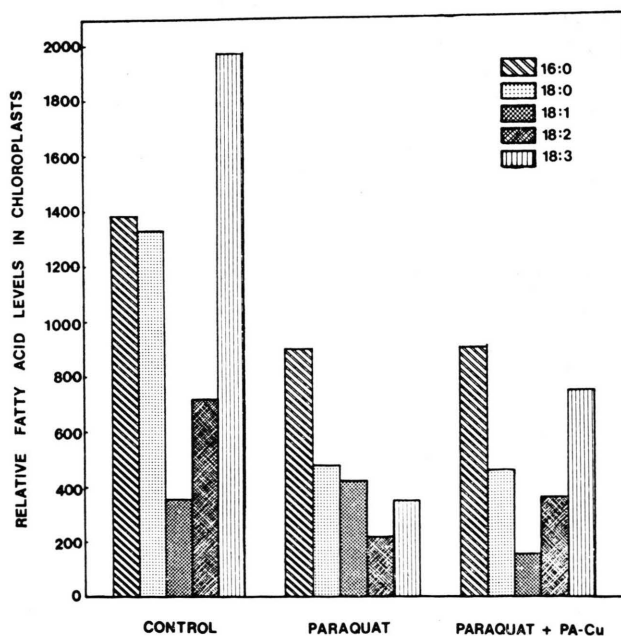


Fig. 3. Effect of paraquat on the fatty acid content of chloroplasts from paraquat treated flax cotyledons, in the presence and absence of PA-Cu. Experimental conditions are given in Materials and Methods.

primarily involve unsaturated fatty acids and lead to membrane damage and eventual loss of cell integrity. Fig. 3 shows the effect of paraquat in the presence and absence of PA-Cu, on the levels of some important chloroplastic fatty acids. After 72 hours of paraquat treatment, the greatest decrease was observed in the levels of linoleic and linolenic acids (18 : 2 and 18 : 3 acids, respectively). Paraquat treated flax cotyledons have been shown to release ethane [19, 20] and both these fatty acids probably act as substrates for this hydrocarbon gas [21–23]. In general, PA-Cu appeared to retard the breakdown of these fatty acids.

Discussion

The treatment of flax cotyledons with paraquat led to a breakdown of chlorophyll and carotenoid pigments and a decrease in the levels of fatty acids, notably linoleic and linolenic. A concomitant release of ethane has also been demonstrated [19, 20]. All these parameters of paraquat action were inhibited by PA-Cu. This showed that PA-Cu was able to enter the cotyledonary tissue and scavenge superoxide which appeared to play a role in each of the degradative processes. It is thought unlikely that superoxide itself possesses sufficient reactivity to abstract protons from unsaturated fatty acids in membranes to instigate lipid peroxidation reactions. It is probable that the importance of superoxide lies in its ability to give rise to more reactive species such as singlet oxygen, which have been

shown to initiate lipid peroxidation reactions [24, 25].

Chlorophyll and carotenoid photobleaching has been shown to occur through the agency of singlet oxygen, and β -carotene is known to be an effective quencher of this toxic oxygen species [26]. Singlet oxygen may be formed from the superoxide radical or via the decomposition of lipid hydroperoxides. Peiser and Yang [27] have provided *in vitro* evidence that chlorophyll is destroyed by alkoxy radicals without the direct involvement of oxygen. However, our results indicate that superoxide does play a role in the bleaching reactions, although this does not rule out the possibility that some bleaching is unrelated to lipid peroxidation reactions. Oxygen is probably more important in the initial stages of pigment breakdown, but its effect may be augmented by lipid radicals once fatty acid breakdown has been instigated.

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