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Paraquat in chloroplasts

Evidence has been presented by MEES[1] that the bipyridylium herbicides* kill green plant tissue by a photosynthetically mediated process. For maximum herbicidal activity chlorophyll, oxygen and light are all essential. Several workers with systems *in vitro* have demonstrated that the herbicides are reduced by isolated chloroplasts[2], catalyse photophosphorylation[3,4] and that hydrogen peroxide is produced in an illuminated chloroplast suspension in the presence of diquat[5]. In this investigation we have measured the amount of paraquat reaching the chloroplasts of sugar beet plants after treatment which is just sufficient to kill the plant. Paraquat has been shown to be metabolically stable in the plant, and the radioactivity we measure can be ascribed to paraquat itself[6].

Sugar beet plants (*Beta vulgaris*, var. Suttons Improved) which were grown on a 16-h day at about 22° were used when they had 6–8 mature leaves. Treatments were either through the petiole, by placing several leaves in small tubes with 0.5 ml solution containing 50–75 μg paraquat ion, or through the leaf blade when 50 μl paraquat solution containing 3.5 μg paraquat ion was spread over the leaf in 50 × 1-μl droplets. This latter application more nearly resembles agricultural spraying conditions. The treated material was kept in darkness for 24 h to enhance the uptake of chemical before the leaves were washed to remove unabsorbed material and the chloroplasts subsequently isolated.

Chloroplasts were isolated by the method of LEECH[7] in a sucrose–phosphate medium with glycerol to form a density gradient, by the polyethylene glycol method of CLENDENNING, BROWN AND WALLDOV[8], and after freeze-drying the leaf material by the nonaqueous procedure devised by STOCKING[9] using a density separation in heptane–carbon tetrachloride. The preparations were examined under the light microscope to check that contamination by other cell constituents did not occur. The presence of glutamic–oxaloacetic transaminase (EC 2.6.1.1) was used as an index of mitochondrial contamination of chloroplasts[10], but we did not find this method suitable for the freeze-dried nonaqueous preparations. Chlorophyll estimations were by the method of BRUINSMA[11]. A correction factor for the yield of chloroplasts from the leaves was made from the ratio of the chlorophyll content of the crude homogenate and of the final pellet. The amount of paraquat present in the isolated fractions was radioassayed after [^{14}C]paraquat application by resuspending the final pellet and collecting the material by filtration on a Millipore filter (5 μ pore size). The filter disc was combusted by the oxygen flask method and the $^{14}CO_2$ radioassayed by liquid scintillation counting.

Table I shows the influence of the isolation procedure on the quantities of herbicide measured in the chloroplasts. Paraquat is present in all preparations, but appears to have been washed out of the chloroplasts during aqueous separations due to its high water solubility. It has been reported that in sucrose containing buffers chloroplasts may swell and burst, releasing their contents[12]. The polyethylene glycol

Abbreviation: DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea.

* Paraquat and diquat are the common names for the 1,1′-dimethyl-4,4′-bipyridylium and 1,1′-ethylene-2,2′-bipyridylium cations which as the chloride and bromide, respectively, are the active principles of the herbicidal formulations 'Gramoxone' and 'Reglone', marketed by Plant Protection Ltd. Paraquat dichloride is also known as methyl viologen.

TABLE I

UPTAKE OF PARAQUAT INTO CHLOROPLASTS

For isolation techniques see text. Sugar beet leaves treated with 50 × 1-μl drops of paraquat solution containing 3.55 μg cation per leaf.

Isolation medium	*Dose absorbed by leaf* (%)	*Chlorophyll content of samples* (*mg*)	*Herbicide in chloroplasts* (μg)	*Dose in chloroplasts* (%)	*μg paraquat per mg chlorophyll*	*Moles chlorophyll** / *Moles paraquat*
Sucrose–phosphate	71.6	0.19	0.026	5.6	0.14	1500
	62.4	0.30	0.022	5.3	0.07	2800
	82.4	0.51	0.026	2.5	0.05	4100
	66.6	0.53	0.054	4.5	0.10	2000
Polyethylene glycol	83.0	0.73	0.11	14.0	0.15	1350
Heptane–carbon tetrachloride	75.8	0.68	0.79	10.5	1.16	175
	69.1	0.83	1.72	18.9	2.07	100

* Mol. wt. chlorophyll taken as 900.

TABLE II

ADSORPTION OF PARAQUAT TO CHLOROPLASTS

Chloroplasts were prepared from untreated leaves by the sucrose–phosphate density gradient technique[6]. Incubation mixtures were 1 ml chloroplast suspension (0.75 mg chlorophyll), 3 ml buffer and 1 ml paraquat solution to give final concentrations of 0.3 M sucrose, 0.067 M potassium phosphate (pH 7.3), 2 mM EDTA and 1.8 mM $MgSO_4$. The mixtures were incubated for 1 h at room temperature in either a darkened cupboard or normal daylight. The figures are corrected for a small amount of adsorption to the Millipore filter pad.

Paraquat concn. (μM)	*Paraquat adsorbed* (μg)	
	Light	*Dark*
1.9	0.20	0.22
3.8	0.32	0.36
7.6	0.61	0.44
15.2	0.79	0.90
30.4	1.53	1.59

method of isolation gave an intermediate paraquat content and the highest values were obtained using nonaqueous separation. To confirm that leaching did occur chloroplasts isolated in the sucrose–phosphate medium were treated with paraquat, and then washed by centrifugation. About 90% of the chemical was lost in three washings.

Although uptake of bipyridylium herbicides into whole leaves is affected by light (greater amounts are taken up in darkness[13]) the results in Table II suggest that uptake by isolated chloroplasts is not affected by light. There does not appear to be any binding of paraquat to chloroplast components and the uptake is directly proportional to concentration, in contrast to the irreversible binding effect shown for 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) by IZAWA AND GOOD[14].

We consider that only the results obtained by the nonaqueous isolation procedure give a true representation of the amounts of paraquat reaching the chloroplast; the values obtained suggest that the number of molecules of chlorophyll associated with one molecule of herbicide after a treatment just sufficient to kill lies in the range 100–200.

The size of the "photosynthetic unit" has not been fully resolved. Quantasomes of 230 chlorophyll molecules have been shown by electron microscopy[15] though the DCMU inhibition studies suggest one site of oxygen evolution for every 2500 chlorophyll molecules. One expects an equivalence in sites generating reducing potential to those generating oxygen, and as paraquat is thought to interact at this reducing site[16] it may be that not all the herbicide we have measured is associated with active sites. As the bipyridylium herbicides act catalytically by reversible reduction and subsequent reoxidation the situation is rather different from the urea herbicides which are considered to be active due to inhibition of the Hill reaction, where kinetic data of enzyme inhibition have been obtained[14].

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Action of insulin and triiodothyronine on energy-controlled pathways of hydrogen

Stimulation of biological oxidations by triiodothyronine has been shown by many investigators[1–3] and formerly was deduced from uncoupling effects[4,5], but during recent years was formulated on the basis of alterations of mitochondrial enzyme patterns[6,7]. However, as shown previously in our laboratory[8], the respiratory capacity of liver mitochondria was also markedly increased by insulin treatment of rats. A concomitant increase of several mitochondrial enzymes was observed but no change of P/O ratios as reported elsewhere[9–12]. The present report deals with major changes of mitochondrial contents of cytochromes and pyridine nucleotides produced by insulin or triiodothyronine. The contrary effects of both hormones on the respiratory system may operate as a switch for energy-controlled hydrogen flux.

Hyperthyreotic rats were obtained by daily treatment with 100 μg triiodothyronine per 100 g body weight. Insulin was injected in doses of 2×2 to 2×4 I.U. per day. All animals were kept under standard conditions and sacrificed after 4 days. Liver mitochondria were prepared and incubated as described previously[13]. Respiration, P/O ratios, and respiratory control were measured polarographically[14]. Enzyme activities were measured according to standard methods[15–17]. Mitochondrial pyridine nucleotides were determined enzymatically[18,19] and cytochrome content was measured by the method of KLINGENBERG[20,21], using a Phoenix dual-wavelength scanning spectrophotometer. All results are given on a protein basis.

As shown in Fig. 1, respiration with various substrates is stimulated by triiodothyronine and insulin as well. Respiratory rates are increased unspecifically by triiodothyronine; whereas, insulin, with a group of substrates, produced an increase of respiration in constant proportions. The rate of oxidation of α-glycerophosphate was not influenced by insulin, but rose to the 30-fold after triiodothyronine treatment[22].

The stimulation of respiratory capacity resembles increases of enzyme activities,