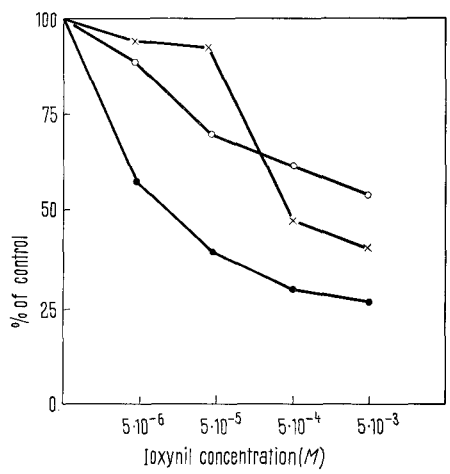
The Effect of Sulphydryl Agents on the Ioxynil-Inhibited Cyclic Photophosphorylation in Chloroplasts of *Vicia faba* L.

The role of sulphydryl groups as functional parts of enzyme systems is well known from studies of the inhibition of enzymic reactions by heavy metal derivatives and by many organo-halogen compounds[1,2]. In several instances it has been possible to reverse this inhibition, in full or in part, by the use of non-toxic sulphydryl agents such as cysteine and glutathione. Such agents offer a means of enzyme protection by binding the inhibitor through the interaction of their sulphydryl groups, thus leaving the enzyme sites intact. It has been shown that the herbicide ioxynil (4-hydroxy-3, 5-diiodobenzonitrile) inhibits the formation of ATP during cyclic photophosphorylation in chloroplasts of *Vicia faba* L., when the (eaction is catalysed by phenazine methosulphate rPMS)[3,4]. The first report of an enzyme containing a sulphydryl group involved in cyclic photophosphorylation, resulted from experiments with *p*-chloromercuribenzoate (PCMB)[5]. It was found that the inhibition of cyclic photophosphorylation by PCMB could be reversed if the reaction mixture was incubated with $10^{-3}M$ glutathione. Similar experiments have been carried out in this laboratory with the herbicide ioxynil. The results of these experiments are shown in the Figure.

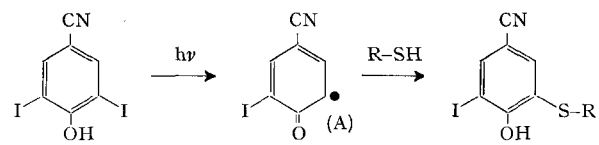
The reaction mixture contained the following components: Tris-HCl buffer, pH 7.5, 40 μ moles; $MgCl_2$ 2 μ moles; KH_2PO_4-K_2HPO_4, pH 7.5, 0.75 μ moles (containing approximately 1 μ curie of P^{32}); ADP 1 μ mole; PMS, 0.1 μ mole; chloroplast fragments containing 25 μg chlorophyll. Ioxynil was added as required. The final volume was 1.5 ml.

The tubes were illuminated by a 500 W bulb and incorporating a water filter to eliminate overheating. The reaction was terminated by the addition of 0.3 ml of trichloracetic acid (25% in $0.05M$ phosphate buffer) after an illumination period of 15 min. The radioactive ATP in the reaction mixture was measured following the precipitation of the excess inorganic phosphate by magnesia mixture (NH_4Cl-NH_4OH-$MgCl_2$, 2:1:2). It can be seen that in the presence of $5 \cdot 10^{-6}M$ ioxynil, cysteine lowers the inhibition of cyclic photophosphorylation by 30%, while a lowering of 40% is observed with glutathione. The cysteine curve closely parallels the control curve over the whole range of ioxynil concentrations, whereas a sharp dip in the glutathione curve is observed at ioxynil concentrations greater than $5 \cdot 10^{-5}M$. No clear explanation can be given for this observation at present, but it is likely that much higher glutathione concentrations are required to obtain relief of inhibition over the whole range of ioxynil concentrations.



Comparison of cysteine and glutathione as sulphydryl protectants of cyclic photophosphorylation catalysed by PMS. ●—● = no sulphydryl protectant; o—o = cysteine (10 μ moles); x—x = glutathione (10 μ moles).

The following explanation of a possible mechanism involved in the interaction of ioxynil with sulphydryls is based on previous chemical evidence for the photolysis of iodo-aromatic compounds[6] and in particular ioxynil[7].



This reaction may be repeated a second time with another mole of the sulphydryl agent. The mode of action of ioxynil appears to be complex, but two possibilities arise on the assumption of the generation of the free radical (A). In the first case, the free radical (A) could react with the sulphydryl groups of the enzymes involved in cyclic photophosphorylation; and secondly, the free radical could react with the electrons generated during the photolysis of water in photosynthesis. In the second case, the free radical could act as an electron sink. These processes acting together may be responsible for the observed inhibition of NADPH and ATP formation during photosynthesis[3,4]. However, such a mechanism remains a speculation until evidence is presented of the existence of an ioxynil-free radical in vivo during photosynthesis[8].

Zusammenfassung. Es wurde die Rolle von Cystein und Glutathion als Schutzmittel für Enzym-Sulfhydrylgruppen studiert. Zyklische Photophosphorylierung, durch PMS katalysiert und durch Herbizid Ioxynil gehemmt, kann durch Zugabe von 10 μM Cystein oder Glutathion wieder teilweise bis ständig hergestellt werden. Es wird eine Hypothese aufgestellt, wonach der Hemmungsmechanismus auf einer Radikal-Reaktion beruhe.

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