Inhibitory Effect of Parathion on the Photosynthetic Electron Transport System in Isolated Spinach Chloroplasts

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Pesticide chemicals usually have some broad specificity; thus they are commonly categorized as herbicides, insecticides, or fungicides, indicating the group of pests against which some selectivity of action has been demonstrated. It should not be assumed, however, that insecticides are necessarily harmless to plants. A number of organophosphorus insecticides have been shown to have a phytotoxic effect.

Our previous studies demonstrated that parathion (O,O-diethyl-O-p-nitrophenyl phosphorothioate) could be photoreduced dependently of ferredoxin by spinach chloroplasts (SUZUKI and UCHIYAMA, submitted-a) and independently of ferredoxin by heated chloroplasts with an artificial electron donor system (SUZUKI and UCHIYAMA, submitted-b). In the latter report, the possibility that parathion inhibits photosynthetic electron transfer was suggested.

In the present paper, attempts were made to confirm the above-mentioned possibility and to clarify the site and intensity of parathion inhibition. Consequently, it was found that parathion blocks the electron transfer from photosystem II (PS II) to photosystem I (PS I) and thereby inhibits Hill reaction in spinach chloroplasts.

EXPERIMENTAL

Once-washed spinach chloroplasts were prepared by the method of WHATLEY and ARNON (1963) and the chlorophyll content was determined by the method of ARNON (1949). Hill activity of chloroplasts was assayed in the presence or absence of parathion (technical grade, more than 98.0% pure) under the following conditions.

NADP photoreduction The reaction mixture in a quartz cuvette contained 1.0 μmole of NADP, 0.1 ml of washed chloroplast suspension (equivalent to 50 μg of chlorophyll), 50 μg of spinach ferredoxin (purchased from Sigma Chemical Co.), and 0.2 ml of 0.5M Tris-HCl buffer (pH 7.8) in a final volume of 3 ml. Cuvettes were illuminated at room temperature for 10 min with

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150 W tungsten lamp. Reduced NADP was determined by the increase in absorbance at 340 mμ against a blank which contained the complete reaction mixture but kept in the dark.

Ferricyanide photoreduction The reaction mixture in a quartz cuvette contained 5 μmoles of potassium ferricyanide, 0.1 ml of washed chloroplast suspension (equivalent to 25 μg of chlorophyll), and 0.2 ml of 0.5M Tris-HCl buffer (pH 7.8) in a final volume of 3 ml. Cuvettes were illuminated for 5 min. Upon termination of reaction, 0.2N sodium citrate, 0.01N ferric chloride in 0.1N acetic acid, and 0.1M o-phenanthroline were added, 0.1 ml each, into the reaction medium. After standing for 5 min, the optical density of the sample at 513 mμ was measured. A cuvette being handled in the same manner as the sample cuvette but kept in the dark served as a blank.

RESULTS

As can be seen from Table I, parathion inhibited the Hill reactions with NADP and ferricyanide as electron acceptors (Expt. A). At a concentration of 2.3 x 10^{-5}M, parathion caused an inhibition of approximately 45% on both photoreductions. Table I also shows that, when the electron transfer from PS II to PS I was blocked by adding o-phenanthroline and furthermore 2,6-dichlorophenol-indophenol (DCPIP) and sodium ascorbate were added as an artificial electron donor system for PS I in the reaction medium (Expt. B), the NADP photoreducing activity of chloroplasts was only slightly inhibited by parathion. This result indicates that parathion does not inhibit in the side of PS I, but in the side of PS II.

In order to clarify the site of action of parathion on the photosynthetic electron transport chain, NADP photoreducing activities of chloroplasts supplemented by various electron donor systems were assayed in the presence or absence of parathion. At a concentration of 10^{-4}M and above, p-phenylenediamine (PD) can serve as an electron donor for PS I (YAMASHITA and BUTLER 1968), like DCPIP. At lower concentration (10^{-5}M), it is an electron donor for PS II. Furthermore, p-hydroquinone (HQ) and diphenylcarbazide (DPC) are known to be electron donors for PS II (YAMASHITA and BUTLER 1969, VERNON and SHAW 1969).

The results were summarized in Table II. Although each electron donor itself affects more or less the Hill activity of chloroplasts, such an effect would not disturb the discussion for estimating the inhibition site of parathion. When any electron donor system was not added, parathion caused an inhibition of 61%. The in-

TABLE I

Effects of Parathion on Hill Activities of Chloroplasts

Expt.		NADP reduced (µmoles/mg Chl·hr)	Inhibition (%)	FeCy reduced (µmoles/mg Chl·hr)	Inhibition (%)
A	Control	34.4	—	84.4	—
	Parathion(2.3×10^{-5}M)	18.6	45.9	47.1	44.2
B	Control	20.2	—		
	Parathion(2.3×10^{-5}M)	19.2	4.9		

Expt. B was carried out in the presence of o-phenanthroline(0.1 mM), DCPIP(67 µM) and ascorbate(6.7 mM).

TABLE II

Effects of Parathion on NADP Photoreducing Activities of Chloroplasts with Various Electron Donor Systems

Electron donor system	NADP reduced (µmoles/mg Chl·hr) Control	NADP reduced (µmoles/mg Chl·hr) Parathion(2.7×10^{-5}M)	Inhibition (%)
—	29.9	11.6	61.2
67 µM DCPIP + 6.7 mM ascorbate	20.3	14.7	27.6
670 µM PD + 67 mM ascorbate	60.4	57.1	5.5
33 µM PD + 330 µM ascorbate	33.1	15.5	53.2
200 µM HQ + 330 µM ascorbate	30.2	10.1	66.6
0.5 mM DPC	19.7	6.0	69.5

hibitory effect was partially overcome by DCPIP-ascorbate and almost completely by a higher concentration of PD-ascorbate. On the other hand, a lower concentration of PD-ascorbate, HQ-ascorbate, and DPC could scarcely overcome the inhibitory effect of parathion.

From these results, which showed that the electron donors for PS II can not restore the Hill activity inhibited by parathion but those for PS I can do, it is concluded that parathion inhibition takes place between PS II and PS I, like the phenylurea, triazine and o-phenanthroline inhibitions.

Fig. 1 illustrates the ability of chloroplasts to reduce NADP in the presence of various concentrations of parathion. In this experiment, parathion at a concentration of approximately 2.2 x 10^{-5}M gives 50% inhibition.

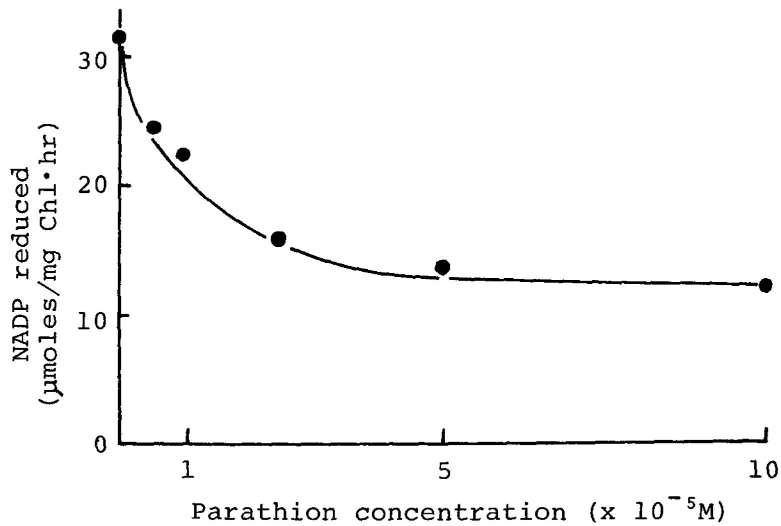

Fig. 1. Effect of Various Concentrations of Parathion on the NADP Photoreducing Activity of Chloroplasts

More than 5 x 10^{-5}M of parathion caused an only slight increase of inhibition. This fact may be due to the very low solubility of parathion in water. Actually, more than 10^{-4}M of parathion led to noticeable error in the measurement of optical density at 340 mμ because of turbidity of the reaction medium.

DISCUSSION

In the review concerning the interaction of pesticides with aquatic microorganisms, WARE and ROAN (1970) have described the effect of pesticides on photosynthesis in phytoplankton. Several workers have reported the effects of pesticides on the growth and survival of photosynthetic microorganisms (GREGORY et al. 1969, MOORE 1970, POORMAN 1973). With the exception of herbicides, however, reports of this type of research in higher plants have apparently not been published to date.

The results described here reveal that parathion blocks the electron transfer from PS II to PS I and consequently inhibits the Hill activity of chloroplasts. Other organophosphorus insecticides (paraoxon, sumithion, diazinon and disyston) caused 22-27% inhibitions at a concentration of 5×10^{-5}M. Although a conclusion can not be drawn due to an insufficient number of compounds tested, it is likely that the inhibitory effect on the photosynthetic electron transport system is of a general nature of organophosphorus insecticides.

The inhibitory effect of them is considerably weak as compared with that of DCMU (3-(3,4-dichlorophenyl)-1,1-dimethylurea) or Atrazine (2-chloro-4-(2-propylamino)-6-ethylamino-S-triazine) which are the potent and specific inhibitors of the Hill reaction and used as herbicides. It must be remembered, however, that parathion is still fairly active inhibitor as compared with other Hill reaction inhibitors; for example, o-phenanthroline gives a 50% inhibition at about 10^{-5}M, and that organophosphorus insecticides are now widely used and are now contaminating our environment.

Since the subatances which affect the photosynthesis in higher plants or microorganisms can affect the human life and the whole ecosystem as well, it is thought that their effect on the photosynthetic reactions should not be overlooked even if it is minor. It is necessary to investigate in detail the intensity and the mechanism of the inhibitory effect of a wide variety of organophosphorus insecticides on the photosynthetic reactions.

REFERENCES

ARNON, D.I., Plant Physiol. 24, 1 (1949)

GREGORY, W.W., REED, J.K. and PRIESTER,Jr. L.E., J. Protozool. 16, 69 (1969)

MOORE, R.B., Bull. Environ. Contam. Toxicol. 5, 226 (1970)

POORMAN, A.E., Bull. Environ. Contam. Toxicol. 10, 25 (1973)

SUZUKI, T. and UCHIYAMA, M., Chem. Pharm. Bull. (Tokyo) submitted-a

SUZUKI, T. and UCHIYAMA, M., Chem. Pharm. Bull. (Tokyo) submitted-b

VERNON, L.P. and SHAW, E.R., Plant Physiol. 44, 1645 (1969)

WARE, G.W. and ROAN, C.C., Residue Rev. 33, 15 (1970)

WHATLEY, F.R. and ARNON, D.I., Methods in Enzymology, Vol. 6, pp. 308-318, New York-London, Academic Press, 1963

YAMASHITA, T. and BUTLER, W.L., Plant Physiol., 43, 2037 (1968)

YAMASHITA, T. and BUTLER, W.L., Plant Physiol., 44, 435 (1969)