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Photoreduction of Parathion by Spinach Chloroplasts

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Parathion, one of the representative organophosphorus insecticides, served as an electron acceptor (or a Hill oxidant) of photosynthetic electron transport system in spinach chloroplasts. This reaction was shown to require spinach ferredoxin as a reductant and to produce hydroxylaminoparathion as a major photoreduction product.

In the previous papers, we have reported that parathion (O,O-diethyl-O-*p*-nitrophenyl phosphorothioate) is reduced to aminoparathion by spinach homogenate fortified with $NADP^+$, G-6-P and FAD[2] and that this reduction proceeds *via* hydroxylaminoparathion.[3] In 1965, Wessels reported the mechanism of reduction of organic nitro compounds, especially 2,4-dinitrophenol, by spinach chloroplasts.[4] According to his results, ferredoxin-$NADP^+$ reductase mediates the electron transfer from NADPH to ferredoxin in the dark, and the reduction of nitro compounds by reduced ferredoxin proceeds nonenzymatically. Wessels also demonstrated that several aromatic nitro and nitroso compounds are reduced to the corresponding amino analogs by illuminated chloroplasts. In this respect, it is interest to determine if parathion serves as an electron acceptor for the photosynthetic electron transport system. This paper describes that parathion is reduced mainly to hydroxylaminoparathion by illuminated spinach chloroplasts and that spinach ferredoxin serves as a reductant.

Experimental

Materials——Technical grade of parathion (more than 98.0% pure) was used throughout this investigation. Nicotinamide-adenine dinucleotide phosphate ($NADP^+$), flavin mononucleotide (FMN), phenazine methosulfate (PMS) and spinach ferredoxin were purchased from Sigma Chemical Co. Other chemicals were reagent grade.

Procedures for Parathion Photoreduction——Once-washed chloroplasts were prepared from spinach leaves by the method of Whatley and Arnon.[5] The chlorophyll was determined by the method of Arnon.[6] Unless otherwise specified, the reaction mixture contained 0.4 ml of chloroplast suspension (equivalent to 200 μg of chlorophyll), 50 μg of ferredoxin, 0.2 ml of 0.5M Tris-HCl buffer (pH 7.8), and 0.1 μmole of parathion in a final volume of 3 ml. The reaction was carried out in Thunberg-type cuvettes under nitrogen gas. Cuvettes were illuminated with an 150 W tungsten lamp at room temperature and the reaction was terminated after 20 min by turning off the light. Two ml of the reaction mixture was pipetted into a test tube containing 2 ml of *n*-hexane and 0.1 ml of Tollens' reagent. After vigorous shaking and centrifugation, portions of *n*-hexane phase were subjected to gas-liquid chromatography.

Gas-Liquid Chromatography——Parathion and its reductive products were analyzed by gas-liquid chromatography under the following conditions: apparatus, Shimadzu Gas Chromatograph (Model GC-1C); detector, flame thermionic detector coated with potassium chloride (temp. at 230°); column, 10% DC-11 on Uniport B (80—100 mesh), 0.8 m in length, 4 mm in calibre and temperature at 185°; carrier gas, nitrogen at a flow rate of 50 ml/min; hydrogen gas flow rate, 30 ml/min; air pressure, 0.8 kg/cm^2; injection port temperature, 250°.

1) Location: *a) Aobayama, Sendai; b) 1-18-1, Kamiyoga, Setagaya, Tokyo.*
2) T. Suzuki and M. Uchiyama, *Eisei Kagaku*, **20**, 93 (1974).
3) T. Suzuki and M. Uchiyama, *J. Agr. Food Chem.*, **23**, 281 (1975).
4) J.S.C. Wessels, *Biochim. Biophys. Acta*, **109**, 357 (1965).
5) F.R. Whatley and D.I. Arnon, "Methods in Enzymology," Vol. 6, ed. by S.D. Colowick and N.O. Kaplan, Academic Press, New York and London, 1963, pp. 308—318.
6) D.I. Arnon, *Plant Physiol.*, **24**, 1 (1949).

Under these conditions, retention times of parathion, aminoparathion and nitrosoparathion were 2.6, 2.1 and 1.4 min, respectively. Since hydroxylaminoparathion was undetectable by gas-liquid chromatography under the conditions employed, it was quantitatively converted to nitrosoparathion by treating the reaction mixture with Tollens' reagent as mentioned above. The amount of each organophosphorus compound was determined by reference to each calibration curve prepared by using the authentic sample of parathion and the chemically prepared samples of aminoparathion and nitrosoparathion.

Results and Discussion

The mixture of parathion and washed chloroplasts supplemented with ferredoxin was illuminated under anaerobic condition. The reaction products were identified by the method described in our previous paper.[3] Fig. 1 shows the gas chromatogram of the reaction products. From acidified medium, only a trace amount of nitrosoparathion was detected as a photoreduction product in the *n*-hexane extract (a). When the reaction medium was treated with Tollens' reagent, however, a marked amount of nitrosoparathion and a small amount of aminoparathion were detected (b). Washing of the *n*-hexane of b) with 1N HCl resulted in a disappearance of the peak of aminoparathion which is basic, but not that of nitrosoparathion (c). These results indicate that hydroxylaminoparathion is major photoreduction product and that aminoparathion is minor one.

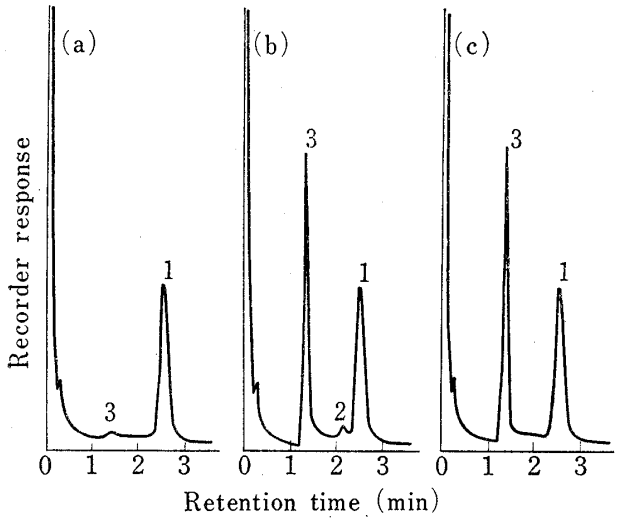

Fig. 1. Gas Chromatograms of Photoreduction Products of Parathion by Spinach Chloroplasts

a) Two ml of reaction medium was acidified by adding 0.5 ml of 1N HCl and followed by extraction with *n*-hexane.
b) Two ml of reaction medium was treated with 0.1 ml of Tollens' reagent and followed by extraction with *n*-hexane.
c) One ml of *n*-hexane phase of b) was washed with 0.5 ml of 1N HCl.
Peak-1, 2 and 3 correspond to parathion, aminoparathion and nitrosoparathion, respectively.

As can be seen from Table I, parathion was not reduced at all in the dark, but under illumination a rapid decrease of parathion was observed in proportion to increases of hydroxylaminoparathion and aminoparathion. Accordingly it is clear that the above mentioned nitro reduction is a photochemical reaction by chloroplasts. Table I aslo shows that spinach ferredoxin is essential for parathion photoreduction. Under aerobic condition, only a slight decrease of parathion and a limited formation of hydroxylaminoparathion were recognized.

In order to elucidate a role of ferredoxin in parathion photoreduction, the effects of some electron acceptors for photosystem I and of enzyme inhibitors were investigated. The results are summarized in Table II. Addition of $NADP^+$, which is a physiological Hill oxidant in chloro-

Table I. Parathion Photoreducing Activities of Washed Spinach Chloroplasts under Various Conditions

Ferredoxin added (μg)	Condition	n moles of parathion		
		Disappeared	Reduced to HAP[a]	Reduced to AP[a]
0	anaerobic, light	4.0	0.0	0.0
25	anaerobic, light	41.3	34.4	3.6
50	anaerobic, light	61.0	51.2	3.8
50	anaerobic, dark	0.0	0.0	0.0
50	aerobic, light	7.8	trace	0.0

a) HAP, hydroxylaminoparathion; AP, aminoparathion

plasts, resulted in a strong inhibition. This inhibition may be considered as evidence that $NADP^+$ competes with parathion for the electron from reduced ferredoxin. The above assumption was verified by the experimental facts that *p*-hydroxymercuribenzoate (*p*-HMB), which showed a little effect on parathion photoreduction at a concentration of 10^{-5}M, overcame the inhibitory effect of $NADP^+$. The same concentration of *p*-HMB inhibits ferredoxin-$NADP^+$ reductase without effect on the Hill activity of chloroplasts.[7] Therefore it is undoubted that ferredoxin-$NADP^+$ reductase is not involved in parathion photoreduction.

Table II. Effect of Some Chemicals on Parathion Photoreduction by Spinach Chloroplasts

Expt.	Addition (μmoles)	n moles of parathion		
		Disappeared	Reduced to HAP[a]	Reduced to AP[a]
I	——	72.0	62.5	6.2
	$NADP^+$ (0.5)	4.0	trace	0.0
	p-HMB (0.03)	64.5	49.4	4.9
	$NADP^+$ (0.5) and *p*-HMB (0.03)	78.8	62.5	7.2
	nitrite (1.0)	73.5	63.0	6.0
	KCN (3.0)	70.2	58.8	5.7
II	——	66.0	56.2	4.5
	PMS (0.1)	4.0	0.0	0.0
	FMN (0.1)	28.0	23.8	3.2

a) HAP, hydroxylaminoparathion; AP, aminoparathion

Although nitrite reductase is also known to be ferredoxin-dependent enzyme in chloroplasts, it is easily lost during the washing process of chloroplasts.[8] In some experiments we have observed that, when the nitrite reductase activity of chloroplasts used here was assayed by the method of Paneque, *et al.*,[9] nitrite is not photoreduced even in the presence of ferredoxin. Furthermore, parathion photoreduction was not affected by nitrite and potassium cyanide, which are known as a substrate and a inhibitor, respectively, for nitrite reductase. These facts suggest that this enzyme also does not participate in parathion photoreduction.

PMS and FMN, which are catalysts of cyclic electron flow around photosystem I, inhibited the reaction. The inhibitory effect of these cofactors seemed to be due to their acceptance of electrons from the primary electron acceptor of photosystem I.

Treatment of chloroplasts for 3 min at 50° destroyed the ability to reduce parathion, but the activity of heated chloroplasts was completely restored by adding 2,6-dichlorophenolindophenol and ascorbate as an artificial electron donor system for photosystem I.[10] Since such a mild heating is effective in selectively destroying the water oxidation step in the photosynthetic electron transport chain,[11] the photoreduction of parathion seems to occur *via* a Hill reaction with water as an electron donor and with ferredoxin as a final electron carrier. This reaction system may be noteworthy as one of the metabolism of nitro-containing organophosphorus insecticides in photosynthetic organisms.

7) San Pietro A., "Methods in Enzymology," Vol. 6, ed. by S.D. Colowick and N.O. Kaplan, Academic Press, New York and London, 1963, pp. 439—445.
8) J.M. Ramirez, F.F. del Campo, A. Paneque, and M. Losada, *Biochim. Biophys. Acta*, **118**, 58 (1966).
9) A. Paneque, J.M. Ramirez, F.F. del Campo, and M. Losada, *J. Biol. Chem.*, **239**, 1737 (1964).
10) Experimental data will be presented in our next paper (T. Suzuki and M. Uchiyama, *Chem. Pharm. Bull.* (Tokyo), **23**, No. 10 (1975)).
11) T. Yamashita and W.L. Butler, *Plant Physiol.*, **43**, 2037 (1968); S. Katoh and San Pietro A., *Arch. Biochem. Biophys.*, **128**, 378 (1968).

Various types of ferredoxin are recognized to be widely distributed in various organisms, *e.g.* anaerobic or aerobic bacteria, photosynthetic bacteria, algae and higher plants, and to be involved in many types of biological reactions. In this respect, it is supposed that the nitro-containing pesticides are reduced depending on ferredoxin by various organisms in the environment.