Inhibition of Microsomal Aldrin Epoxidation by Diquat and Several Related Bipyridylium Compounds

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The bipyridylium compounds, diquat and paraquat, are quick-acting, contact herbicides with moderate acute toxicity to animals (CALDERBANK 1968, HOWE and WRIGHT 1965). Chloroplasts catalyze the formation of relatively stable, charged free radicals; and bipyridyl phytotoxicity is associated with disruption of photosynthesis (BUCHEL 1972, DODGE 1971).

Microsomal mixed-function oxidases are extremely important in the biotransformation of lipophilic chemicals, and this ubiquitous enzyme system requires NADPH and molecular oxygen for maximum catalytic activity. The microsomal electron transport system shuttles reducing equivalents from NADPH to the terminal oxidase, cytochrome P-450, which is a carbon monoxide binding hemoprotein (PARKE 1968).

Interactions between microsomal enzymes and bipyridyls have received little attention, GAGE (1968) reported that diquat (0.1 mM) and paraquat (1.0 mM) stimulate a carbon monoxide insensitive, microsomal NADPH oxidase, and further he demonstrated formation of diquat and paraquat free radicals under anaerobic conditions.

The present investigtion was conducted to determine whether diquat and paraquat can inhibit microsomal oxidation.

Materials and Methods

Microsomal fractions were prepared from 20% (w/v) homogenates of rat liver in 1.15% KCl (w/v) using an initial centrifugation of $10{,}000g_{max}$x15 min and a final centrifugtion of $100{,}000g_{max}$x60 min. Microsomal pellets were resuspended in 1.15% KCl and the suspension incubated at 37°C in open erlenmeyer flasks which contained the following: phosphate buffer, pH 7.4 (50 mM), glucose 6-phosphate (G-6-P) (mM), NADP (0.1 mM), KCl (2.7 mM), and G-6-P dehydrogenase (2-5 EU). Diquat dibromide (VII) and related compounds (Table) were added in water. Rates of aldrin epoxidation were measured as reported (KRIEGER and WILKINSON 1970), and values are means of three experiments.

Results and Discussion

Addition of either diquat (mM) or paraquat (mM) to incubation media resulted in essentially complete inhibition of microsomal

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aldrin epoxidation. Subsequently it was established that the molar concentration of diquat (VII) to give 50 per cent inhibition (I_{50}) was 6.6 x 10^{-6}M. Experiments on the relationship between herbicidal potency, chemical structure, and oxidation reduction potential with bipyridyls have shown that phytotoxicity is restricted to compounds with redox potentials (E_0) between -0.3 and -0.5 v (CALDERBANK 1968).

The redox properties of the microsomal electron transport system are not well defined, but acceptors such as cytochrome c (+0.22 v) and methylene blue (+0.01 v) are well-known inhibitors of microsomal oxidations. To define better the mechanism of diquat inhibition the inhibitory potency of a series of compounds with redox potentials (BLACK and SUMMERS 1971) ranging between -0.18 and -0.55 v was measured. The most effective inhibitor (I) had a redox potential of -0.18 v and the least potent (IX) had a redox potential of -0.55 v (Table).

TABLE

Potency of diquat and a series of related compounds as inhibitors of microsomal aldrin epoxidation.

Compound	E_0 (volts)	I_{50} (molar)
3,4-benzo-6,7-dihydrodipyrido[1,2-a:2',1'-c]pyrazinediium dibromide (I)	-0.18	4.5×10^{-7}
3,4-benzo-7,8-dihydro-6H-dipyrido[1,2-a:2',1'-c]-[1,4]diazepinium dibromide (II)	-0.21	6.6×10^{-6}
Dipyrido[1,2-a:2',1'-c]pyrazinediium dibromide (III)	-0.27	6.6×10^{-7}
6-Phenyl-6,7-dihydrodipyrido[1,2-a:2',1'-c]pyrazinediium dibromide (IV)	-0.28	3.8×10^{-6}
12,13-Dimethyl-5H-6,7-dihydro-[1,4]-diazepino[1,2,3,4-lmn]1,10-phenanthrolinium dibromide (V)	-0.31	1.8×10^{-5}
1,1'-dibenzyl-4,4'-bipyridinium dichloride (VI)	-0.35	1.1×10^{-4}
6,7-dihydrodipyrido[1,2'a:2',1'-c]-pyrazinediium dibromide (VII)	-0.35	6.6×10^{-6}
1,1'-Dimethyl-4,4'-bipyridinium dichloride (VIII)	-0.45	7.0×10^{-4}
7,8-Dihydro-6H-dipyrido[1,2-a:2',1'-c]-[1,4]diazepinium dibromide (IX)	-0.55	2.2×10^{-2}
Potassium bromide	---	$> 2.5 \times 10^{-2}$
Potassium chloride	---	$> 2.5 \times 10^{-2}$

Means from 3 experiments using female rat liver microsomes (1-2 mg protein/incubation).

Diquat (VII) and other bipyridyls probably inhibit microsomal oxidation due to their disruption of the microsomal electron transport system.

Some of the bipyridyls are very potent inhibitors as demonstrated by the fact that under identical incubation conditions the I_{50} for the well-known inhibitors SKF 525-A and sesamex are 5.2 x 10^{-5}M and 1.5 x 10^{-4}M respectively.

To determine whether a simple electro-chemical interaction was responsible for inhibition, diquat was preincubated with each of the components of the incubation medium, but such a procedure did not change the degree of inhibition. Epoxidation, aniline hydroxylation, dihydroisodrin hydroxylation, and p-chloro-N-methylaniline demethylation can be inhibited by low concentrations (10^{-6}-10^{-5}M) of diquat (KRIEGER and LEE, unpublished) and it is likely that such inhibition is associated with disruption of the microsomal electron transport system which includes cytochrome P-450. In collaboration with R.A. Neal of Vanderbilt University, biphasic reduction of diquat and paraquat (VIII) has been demonstrated under anaerobic conditions, and these rate profiles contrast with the monophasic curves obtained by GAGE (1968). The hypothesis that bipyridyls inhibit due to interference with microsomal electron transport is being further tested in efforts to describe the mode of action of these potent inhibitors of microsomal mixed-function oxidases.

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