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Inhibition of Housefly Glutathione S-Transferase by Catecholamines and Quinones

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The cytoplasmic glutathione S-transferases play an important role in the detoxication and elimination of toxic and undesirable foreign compounds (CHASSEAUD 1973, MOTOYAMA and DAUTERMAN 1974, YANG 1976). Considerable progress has been made in the purification and characterization of these enzymes from insects and mammals (CHASSEAUD 1973, HABIG et al. 1974, MOTOYAMA and DAUTERMAN 1974,1977), however, few specific inhibitors have been reported (CLARK et al. 1967, OHL and LITWACK 1977). Studies with crude enzyme preparations from insects are hampered by the presence of various kinds of contaminating endogenous compounds (MOTOYAMA et al unpublished results, WILKINSON and BRATTSTEN 1972). In this communication we are reporting that catecholamines and related compounds are inhibitors of glutathione S-transferases from insecticide-resistant and susceptible houseflies.

MATERIALS AND METHODS

The chemicals used were obtained commercially from the following sources: reduced glutathione (GSH), mushroom tyrosinase, D-, L-, DL-dopa, dopamine, N-acetyl dopamine, 2-methyl-1,4-naphthoquinone (menadione) and 2-methyl-3-phytyl-1,4-naphthoquinone (vitamin K_1) from Sigma Chemical Company; 1,4-naphthoquinone, 5-hydroxy - 1,4-naphthoquinone (juglone), 5-hydroxy-2-methyl-1,4-naphthoquinone (plumbagin) and 9,10-anthraquinone from Aldrich Chemical Company; 1,4-dihydroxynaphthalene, 1,4-dihydroxy-9,10-anthroquinone and 1,2-dichloro-4-nitrobenzene (DCNB) from Eastman Organic Chemicals; p-benzoquinone and 1,4-dihydroxybenzene from Fisher Scientific Company.

Partially purified enzyme preparations from the Rutgers insecticide-resistant (62-fold purified) and the CSMA-susceptible strains (202-fold purified) of houseflies were used. The specific activities of the preparations were 11.0 and 3.0 µmoles DCNB conjugated/min/mg protein respectively (MOTOYAMA and DAUTERMAN 1977). The glutathione S-transferase assay, based upon the method of BOOTH et al. (1961), was as follows: the

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required volumes of 0.1 M Tris-HCl buffer (pH 9.0) and the enzyme were mixed, and the cuvettes were held at 37°C for 3 min for temperature equilibration. The inhibitor was then added, mixed and further incubated for an additional 3 min. Fifteen µmoles of GSH and 3 µmole DCNB were then added, and the increase in absorbance at 344 nm was monitored using an Aminco DW-2 spectrophotometer in the split beam mode. The reaction mixtures in both cuvettes were identical except that the enzyme in the reference cuvette was replaced with an equal volume of buffer. The extinction co-efficient of 10.0 mM^{-1} cm^{-1} for S-(2-chloro-4-nitrophenyl)-glutathione (ASKELOFF et al. 1975) was used to estimate enzyme rates. Different inhibitors were added at the specified concentrations in either distilled water, ethanol or acetone. Appropriate control experiments were performed to diminish the carrier-effects on enzyme activity. In all the experiments, the final reaction volume was 3.0 ml. All assays were duplicated.

RESULTS AND DISCUSSION

It may be seen from the data given in Table 1 that all of the catecholamines listed other than N-acetyldopamine caused a slight inhibition of housefly transferase at a concentration of 1×10^{-4}M, but an increase in inhibition was observed when tyrosinase was incorporated into the reaction medium. No stereospecific difference in transferase inhibition was noticed in the case of dopa. Tyrosinase did not increase transferase inhibition in the case of N-acetyldopamine, and quantitatively, the level of inhibition was essentially the same as that observed with dopa and dopamine in the presence of tyrosinase.

The above results suggested that quinones produced by oxidation of catcholamines by tyrosinase might be inhibitors of glutathione S-transferase. Therefore, the inhibitory activity of a series of quinones and related compounds was studied (Table 2). These data clearly indicate that the compounds with naphthalene nucleus were the most potent while those with either a benzene or an anthracene nucleus were less active. Under the experimental conditions used, no obvious difference in inhibitory potency was observed between quinones or the corresponding dihydroxy compounds. In addition to the compounds shown in Table 2 a large series of quinones and dihydroxy compounds were tested and the importance of the 1,4 position was clearly apparent (MOTOYAMA et al. unpublished observations).

TABLE 1

The Effect of Oxidation of Catcholamines on the Inhibition of Glutathione S-Transferase[a]

Catecholamine(1×10^{-4}M) ± Tyrosinase (10 µg)	% Inhibition
L-Dopa	30.2
L-Dopa + Tyrosinase	80.0
D-Dopa	38.4
D-Dopa + Tyrosinase	85.6
DL-Dopa	46.3
DL-Dopa + Tyrosinase	77.5
Dopamine	38.4
Dopamine + Tyrosinase	68.8
N-acetyldopamine	64.4
N-acetyldopamine + Tyrosinase	75.0

[a]Partially purified enzyme from CSMA strain was used. The procedure and incubation conditions are described under Materials and Methods.

TABLE 2

Inhibition of Glutathione S-Transferase by Benzene, Naphthalene and Anthracene Derivatives[a]

Compound (1.0×10^{-4}M)	% Inhibition
1,4-benzoquinone	64.4
1,4-dihydroxybenzene	55.9
1,4-naphthoquinone	94.8
1,4-dihydroxynaphthalene	93.8
9,10-anthraquinone	21.0
1,4-dihydroxy-9,10-anthraquinone	78.0
2-methyl-1,4-naphthoquinone (menadione)	84.7
2-methyl-3-phytyl-1,4-naphthoquinone (vitamin K_1)	53.5
5-hydroxy-1,4-naphthoquinone (juglone)	96.3
5-hydroxy-2-methyl-1,4-naphthoquinone (plumbagin)	92.8

[a]Partially purified enzyme from CSMA strain was used. The incubation conditions and assay procedure are under Materials and Methods.

The poor inhibition observed with 9,10-anthraquinone may therefore be attributed to steric factors causing relative inaccessibility of the reactive ketonic groups to the enzyme molecule. The insertion of hydroxyl groups at the 1,4-position of the anthraquinone structure resulted in an increase in inhibition. The presence of a large alkyl group, as in the case of Vitamin K_1, results in a loss of potency while no change is apparent when a hydroxyl group is present at the 5 position of naphthoquinone. When both the 2-methyl and the 5-hydroxyl groups are present, the inhibition observed is essentially the same as with the parent naphthoquinone.

The I_{50} values calculated for p-benzoquinone and 1,4-naphthoquinone using partially purified transferases from insecticide-resistant and -susceptible houseflies (Table 3) showed little strain difference. The inhibition constants for other effective inhibitors were also found to be between 1×10^{-5} to 4×10^{-5}M (MOTOYAMA et al unpublished observations) and thus indicate their potential as effective inhibitors at physiological concentrations.

TABLE 3

Inhibition of Glutathione S-Transferase from Insecticide Resistant (Rutgers) and Susceptible (CSMA) Houseflies[a]

Inhibitor	I_{50} (M)	
	Rutgers	CSMA
1,4-naphthoquinone	7.15×10^{-6}	1.20×10^{-5}
1,4-benzoquinone	2.75×10^{-4}	7.70×10^{-5}

[a]The incubation conditions and assay procedure are described under Materials and Methods except that the preincubation time was 1.0 min in the case of enzyme from Rutgers strain of housefly.

Although quinones can be reduced to dihydroxy compounds non-enzymatically be glutathione, the effect of added tyrosinase and alkaline assay conditions indicate that under these conditions it is the quinones or semiquinone that are probably involved in the inhibition process. Both the oxidation of glutathione and the formation of glutathione-adducts by such compounds are known (WEBB 1966). However, investigations in progress have demonstrated that glutathione has not become binding under the conditions used. Although quinones can oxidize protein SH groups to a disulfide linkage (WEBB 1966), such inhibition is usually reversible in the presence of glutathione (HOFFMAN-OSTENHOF 1963).

The inhibition may be due to quinones reacting at the catalytic site of the transferase, possibly in a manner analogous to a substrate. In this regard, the possible covalent binding of semiquinones to enzyme protein cannot be overlooked. Covalent binding of the semiquinone radical anion of dopa and dopamine to microsomal proteins and bovine serum albumin has been observed in NADPH-supplemented rat liver microsomes (SCHEULEN et al. 1975). Further studies of the inhibition mechanism are in progress.

It is important to note that most of the inhibitors evaluated in this study are naturally occurring compounds, some with function, either in the nervous system or, in insects, in the tanning process. Since contamination of subcellular fractions by them is likely to occur, interpretation of the data from crude enzyme preparations, especially from insects, must be done with great care. Endogenous inhibition of glutathione S-transferases of the housefly abdomen has been demonstrated (MOTOYAMA et al. unpublished results).

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REFERENCES

ASKELOF, P., C. GUTHENBERG, I. JAKOBSON, and B. MAMERVICK: Biochem. J. 147, 513 (1975).
BOOTH, J., E. BOYLAND and P. SIMS: Biochem. J. 79, 516 (1961).
CHASSEAUD, L. F.: Drug Metabol. Rev. 2, 185 (1973).
CLARK, A. G., F. J. DARBY, and J. N. SMITH: Biochem. J. 103, 49 (1967).
HABIG, W. H., M. J. PABST, and W. B. JAKOBY: J. Biol. Chem. 249, 7130 (1974).
HOFFMANN-OSTENHOF, O.: in Metabolic Inhibitors, Vol. 2, p. 145, Academic Press, New York (1963).
MOTOYAMA, N. and W. C. DAUTERMAN: J. Agr. Food Chem. 22, 350 (1974).
MOTOYAMA, N. and W. C. DAUTERMAN: Insect Biochem. in press (1977).
MOTOYAMA, N., A. P. KULKARNI, E. HODGSON and W. C. DAUTERMAN: unpublished observations.
OHL, V. S., and G. LITWACK: Arch. Biochem. Biophys. 180, 186 (1977).
SCHEULEN, M., P. WOLLENBERG, H. M. BOLT, H. KAPPUS, and H. REMMER: Biochem. Biophys. Res. Commun. 66, 1396 (1975).

WEBB, J. L.: in Enzyme and Metabolic Inhibitors, Vol. 3, p. 421, Academic Press, New York (1966).
WILKINSON, C. F. and L. B. BRATTSTEN: Drug Metabol. Rev. 1, 153 (1972).
YANG, R. S. H.: in Insecticide Biochemistry and Physiology (WILKINSON, C. F. ed.) Plenum Press, New York, p. 177 (1976).