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Preliminary Communication

INHIBITION AND ENHANCEMENT OF MICROSOMAL DRUG METABOLISM BY DIETHYL MALEATE

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Diethyl maleate has been shown to deplete hepatic glutathione levels (1,2) and has been widely used for this purpose in toxicological investigations (3-5). The reaction between diethyl maleate and glutathione is catalyzed by a glutathione transferase located in the cytosol fraction of the liver (6).

Since many reactions which serve to generate electrophilic reactive intermediates are catalyzed by hepatic cytochrome P-450 dependent mixed function oxidases, it was of interest to determine the effect of diethyl maleate on this enzyme system. The results of these studies are reported herein.

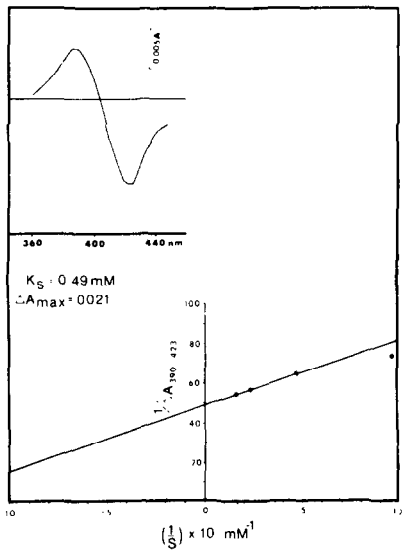
METHODS

Male, Sprague-Dawley rats weighing 240-300 g were used. Hepatic microsomal fractions were isolated as previously described except that the livers were perfused with ice-cold 1.15% potassium chloride solution before homogenization and that homogenates were prepared in potassium chloride solution rather than in sucrose solution (7). Unless otherwise indicated, reaction mixtures contained 50 μmoles of phosphate buffer (pH 7.4), 15 μmoles of magnesium chloride, 10 μmoles of DL-isocitric acid, 1 μmole of $NADP^+$, 1 unit of isocitrate dehydrogenase (Sigma), varying amounts of substrates and diethyl maleate and approximately 3 mg of microsomal protein in a final volume of 3.0 ml. Reaction mixtures were incubated for 15 min at 37°C.

Substrate binding studies were conducted as described by Schenkman *et al.* (8) using a Perkin-Elmer Model 576 spectrophotometer. Formaldehyde was determined according to the method of Nash (9); p-aminophenol and p-hydroxyacetanilide were measured as described by Schenkman *et al.* (8) and Shimazu (10), respectively. Protein was determined by the method of Lowry *et al.* (11). Enzyme kinetic constants were calculated by the method of Wilkinson (12) using BASIC programs written in this laboratory.

RESULTS AND DISCUSSION

Diethyl maleate was found to produce a difference spectrum when mixed with suspensions of oxidized cytochrome P-450; the maximum and minimum absorption occurred at 390 and 423 nm, respectively (Fig. 1). These spectral characteristics are typical of those of a large number of chemicals which are classified as "Type I" compounds (8). The binding spectrum produced by diethyl maleate was dose dependent (Fig. 1). Using diethyl maleate concentrations of 1.04, 2.08, 4.16 and 6.24 mM, the spectral dissociation constant (K_S) and maximal absorbance change ($\Delta A_{max,390-423}$) were found to be 0.60 ± 0.10 mM and 0.021 ± 0.002 A/mg protein (mean ± S.D., n = 3), respectively.



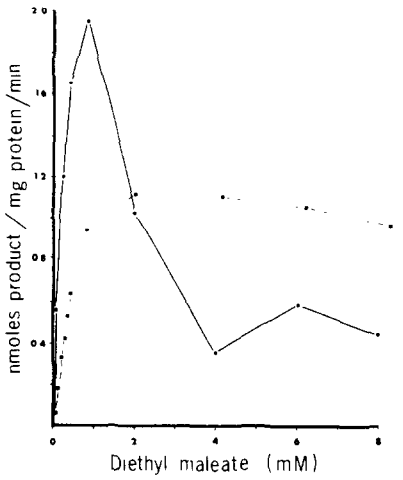


Figure 1 (left). Upper: Difference spectrum produced with diethyl maleate (4.16 mM) and oxidized microsomal suspensions (1 mg protein/ml). Lower: Effect of varying concentrations (1.04, 2.08, 4.16 and 6.24 mM) of diethyl maleate on the difference spectrum produced by diethyl maleate (results of a typical experiment are shown). Diethyl maleate was added as a 10% solution in methanol; an equivalent volume of methanol was added to the reference cuvette.

Figure 2 (right). Effect of varying concentrations of diethyl maleate on aniline (- - - -) and acetanilide (———) hydroxylation. The concentrations of aniline and acetanilide were 0.1 and 1.0 mM and p-aminophenol and p-hydroxyacetanilide were measured as products, respectively.

The effect of diethyl maleate on several pathways of microsomal drug metabolism was also studied. As can be seen from the data in Table 1, diethyl maleate inhibited the N-demethylation of benzphetamine and the O-deethylation of p-ethoxyacetanilide. Analysis of this data as described by Webb (13) showed that diethyl maleate was a mixed inhibitor of benzphetamine N-demethylation and a competitive inhibitor (K_i = 1.55 mM) of p-ethoxyacetanilide O-deethylation. Diethyl maleate (5 mM) did not inhibit the microsomal conversion of dibromomethane to carbon monoxide (data not shown).

In contrast to these inhibitory effects, diethyl maleate enhanced the microsomal hydroxylation of both aniline and acetanilide. In the case of aniline as the substrate, diethyl maleate produced an enhancing effect over a wide range of concentrations; when acetanilide served as the substrate, enhancing and inhibitory effects were observed at low and high diethyl maleate concentrations, respectively (Fig. 2). Furthermore, as can be seen from the data in Table 1, diethyl maleate increased the maximal velocity (V_{max}) for the hydroxylation of both aniline and acetanilide. This enhancing effect of diethyl maleate on microsomal aromatic hydroxylation is similar to that produced by acetone and a number of other compounds (14,15). However, the mechanism by which chemicals produce this enhancing effect is unknown.

These results show that diethyl maleate produces diverse effects on microsomal mixed function oxidations and suggest that experiments employing diethyl maleate to deplete

TABLE 1

EFFECT OF DIETHYL MALEATE ON MICROSOMAL BENZPHETAMINE N-DEMETHYLATION, p-ETHOXYACETANILIDE O-DEETHYLATION AND ANILINE AND ACETANILIDE HYDROXYLATION

Substrate	K_m (mM)	V_{max} (nmoles product/min/mg protein)
Benzphetamine[a]	0.18 ± 0.08[b]	16.06 ± 4.52[b]
+ 5 mM diethyl maleate	0.32 ± 0.07	12.37 ± 3.38
+ 10 mM diethyl maleate	0.63 ± 0.14	10.22 ± 2.69
Aniline[c]	0.054± 0.023	0.97 ± 0.54
+ 0.12 mM diethyl maleate	0.065± 0.024	1.28 ± 0.66
+ 0.28 mM diethyl maleate	0.077± 0.027	1.76 ± 0.88
Acetanilide[d]	0.66 ± 0.04	2.27 ± 0.37
+ 0.14 mM diethyl maleate	1.36 ± 0.28	6.04 ± 1.15
+ 0.34 mM diethyl maleate	0.94 ± 0.05	5.72 ± 1.67
p-Ethoxyacetanilide[d]	0.21 ± 0.02	5.52 ± 1.27
+ 2.5 mM diethyl maleate	0.63 ± 0.10	5.81 ± 0.41
+ 5.0 mM diethyl maleate	0.78 ± 0.17	4.81 ± 0.98

[a] Substrate concentrations used were 0.1, 0.2, 0.5 and 1.0 mM.
[b] Values are shown as the mean ± S.D., n = 3.
[c] Substrate concentrations used were 0.01, 0.02, 0.05 and 0.1 mM.
[d] Substrate concentrations used were 0.1, 0.2, 0.3, 0.5 and 1.0 mM.

hepatic glutathione should be interpreted with care. For example, it has been reported that the administration of diethyl maleate to rats protected against carbon tetrachloride-induced hepatic injury (16). It is possible to speculate that the observed protective effect of diethyl maleate may be due to inhibition of the carbon tetrachloride bioactivation since it is well known that carbon tetrachloride must be metabolized to a toxic species.

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