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INHIBITION OF GLUTATHIONE *S*-TRANSFERASES FROM RAT LIVER BY BASIC TRIPHENYLMETHANE DYES

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Abstract—(1) Basic triphenylmethane dyes related to pararosanilin inhibit class α glutathione *S*-transferases (GSTs) from rat liver. The inhibitory potency of each dye correlates with its octanol–water partition coefficient. Values of K_i determined at pH 6.5 ranged from about 1×10^{-7} M for Ethyl violet to 7×10^{-5} M for Methyl green. GST 3-3, a class μ isoenzyme, was an order of magnitude less sensitive to inhibition by Ethyl violet. (2) All of the dyes tested were bleached to varying degrees by glutathione. The bleaching appears to result from the formation of an adduct between the dye and glutathione. At pH 6.5, adduct formation is significant only for Malachite green and Methyl green. There is kinetic evidence that for these dyes the adduct contributes significantly to the overall inhibition. It is probable that at physiological pH, all of the dyes would exist to a significant extent in the adduct form. (3) The dyes are excreted extensively in the bile, at least partly as the glutathione adduct. The free dye is regenerated on standing, it is assumed as a result of removal of glutathione by oxidation.

The glutathione *S*-transferases (GSTs†) are enzymes which are important in the metabolism of many chemicals possessing an electrophilic centre [1]. Such reactions become of toxicological interest when they give rise to resistance to the action of a drug. Failure of alkylating drugs to control tumour development may be caused by elevation of the activity of the GSTs conjugating such drugs [2, 3]. Similarly, failure of pesticides to control pests in the field may in some instances be attributed to enhanced activity of GSTs degrading the control agent in the target organism [4, 5]. An approach to countering the development of this type of resistance is to inhibit the enzyme(s) responsible. In the present work we continue earlier studies on the basic triphenylmethane dye, Malachite green, which has been shown to be a potent inhibitor of some GSTs [6], and investigate further to what extent dyes of its type might be suited to a role as synergists.

MATERIALS AND METHODS

Chemicals. Pharmalytes (pH 8–10.5) and Polybuffer exchanger 11–8 were purchased from Pharmacia Fine Chemicals, AB (Uppsala, Sweden). The triphenylmethane dyes, Brilliant green, Crystal violet, Ethyl violet, Malachite green, Methyl green and pararosanilin were obtained from the Sigma Chemical Co. (St Louis, MO, U.S.A.). These dyes were used as supplied. Chloro-2,4-dinitrobenzene (CDNB), sulphobromophthalein (BSP), glutathione, Coomassie Brilliant blue R250 and buffer constituents were obtained from Sigma. The glutathione conjugate of BSP, immobilized on epichlorohydrin-activated Sepharose [7], was prepared as described previously [8].

Enzyme preparation. Sprague–Dawley rats of both sexes of body weight 250 ± 30 g were used. Rat liver GSTs 1-1, 1-2, 2-2 and 3-3 were prepared by affinity chromatography on immobilized BSP–GSH conjugate, chromatofocusing and chromatography on carboxymethyl-cellulose as described previously [9]. They were homogeneous as judged by SDS electrophoresis by the method of Laemmli [10].

Enzyme assays. GST activity using CDNB as substrate was assayed at 30°. In most experiments the pH was maintained at 6.5 with a sodium phosphate buffer (0.1 M). When CDNB was the varied substrate, the concentration of GSH was held constant at 1.0 mM and the CDNB concentration varied between 0.2 and 1 mM. When, as was more commonly the case, GSH was the varied substrate, CDNB was maintained at a constant 1 mM and GSH concentration was varied between 0.1 and 1 mM. CDNB was dissolved directly into the assay buffer, to avoid the consequences of adding it dissolved in organic solvents [11]. The inhibitory dyes were dissolved immediately before use to a concentration of 6 mM in distilled water. They were added to give final concentrations of between 4 and 45 μM. During the course of kinetic experiments substrate and inhibitor concentrations were varied randomly to avoid systematic errors due, for instance, to progressive loss of enzyme activity with time. Glutathione stock solutions (60 mM) were stored at −20° in small aliquots. These were thawed only when required, when in use were stored on ice to minimize oxidation and were used for no longer than 30 min. Rate determinations were made at least in triplicate.

Analysis of data. Rate data were fitted to the rate equations under consideration by a non-linear least squares procedure (Kinecom2) written for IBM-compatible microcomputers. Data were weighted

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† Abbreviations: GST, glutathione *S*-transferase; GSH, reduced glutathione; BSP, sulphobromophthalein; CDNB, 1-chloro-2,4-dinitrobenzene.

with the square on the reciprocal velocity. In testing alternate model equations, the following criteria were taken into consideration: convergence of the fitting procedure; minimal standard errors on regression parameters: random distribution of residuals and minimal values of the sum of squared residuals [12].

Determination of relative partition coefficients of triphenylmethane dyes. To a known concentration of dye in distilled water was added an equal volume of *n*-octanol. The mixture was shaken thoroughly for 5 min and the concentration of dye left in the aqueous layer was determined spectrophotometrically using molecular extinction coefficients from the literature. The ratio of the dye's concentration in the organic phase to that in the aqueous phase was then calculated. All measurements were made in triplicate.

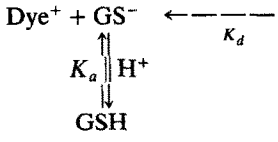
Determination of dissociation constants of dye–GSH adducts. The reduction in concentration of the dye in the presence of glutathione, due to the formation of a GSH–dye adduct, was measured spectrophotometrically at the wavelength of maximum absorption of the dye. For most dyes, determinations of the stability constants were carried out at two pH values, 6.5 and 7.4. Three dye concentrations, between 6 and 20 μM, were employed at the former pH and four at the latter. Glutathione concentrations were varied between 0.2 and 1.2 mM at pH 6.5 and between 0.1 and 0.5 mM at pH 7.4. Equilibrium concentrations of GSH, free dye and adduct were calculated and the best-fit value of the dissociation constant was determined by fitting to Eqn (2) (see Results), using the fitting programme discussed above. For Malachite green, values of the dissociation constant were determined at several pH values.

Biliary excretion. Biliary excretion of Malachite green was followed in male rats of body weight 300–400 g. They were anaesthetized with pentobarbital (60 mg/kg) given by intraperitoneal injection. The bile duct was cannulated with polyethylene tubing and bile was collected in pre-weighed, ice-cold vials. Bile was collected for 20 min prior to the injection of dye. This was injected into the femoral vein at a dose level in the range 2–7 mg/kg. Bile was collected for 5 min intervals over a period of 1 hr. The visible absorption spectrum of the bile fractions was measured using a short path-length, low volume cell. The thiol content of the bile was measured using the method of Ellmann [13]. In some experiments, the liver was excised and homogenized, and the thiol content and total GST activity of the liver were measured, using CDNB as substrate.

RESULTS

Adduct formation

During preliminary experiments with Malachite green it was observed that in the presence of glutathione, the dye solution lost intensity of colour and, in the presence of a sufficiently great excess of glutathione, could be almost completely bleached [6]. The stoicheiometry of the reaction was examined as was its dependence on pH. It was found that these were defined by simple equilibria as shown in the scheme below in which the dye–GSH adduct is assumed to be colourless.



It was found that the apparent dissociation constant of the adduct resulting from the reaction of Malachite green with GSH was linearly related to the hydrogen ion concentration, as in Eqn 1 (A. G. Clark and P. A. Alley, unpublished). The pH-independent value of the dissociation constant (K^{-}) obtained from these experiments was $1.2 \pm 0.2 \times 10^{-5}$ M and the value of the macroscopic pK_a governing the reaction was 9.21 ± 0.12, which corresponds well with values reported in the literature for the sulphydryl group of GSH [14, 15].

$$K_{app} = K^{-} . (1 + [H^+]/K_a). \quad (1)$$

Values for the apparent dissociation constants of the adducts formed from the various dyes at pH 6.5 and 7.4 were determined by measuring the concentration of free dye spectrophotometrically and the concentration of adduct from the reduction in the extinction of dye solutions upon the addition of GSH, and by fitting these quantities into Eqn 2:

$$[\text{Adduct}] = 0.5 \times (([\text{GSH}] + [\text{DYE}] + K_d) - (([\text{GSH}] + [\text{DYE}] + K_d)^2 - 4 \times [\text{DYE}] \times [\text{GSH}])^{0.5}). \quad (2)$$

These values are listed in Table 1.

Table 1. Dissociation constants for the adducts formed from basic triphenylmethane dyes and glutathione

Dye	$K_d \times 10^5$ (M) pH 6.5	pH 7.4
Ethyl violet	2270 ± 10	1480 ± 10
Crystal violet	4110 ± 0.4	1280 ± 6
Brilliant green	ND	174 ± 0.7
Malachite green	381 ± 10	50 ± 0.3
Pararosanilin	855 ± 10	160 ± 1.76
Methyl green	35.7 ± 0.4	5.2 ± 0.35

Values are means ± SD.
ND, not determined.

At pH 6.5 the order of ease of formation of the GSH adducts was Methyl green > Malachite green > pararosanilin > Ethyl violet > Crystal violet. At pH 7.4, the order was very similar: Methyl green > Malachite green > pararosanilin > Brilliant green > Crystal violet > Ethyl violet. The apparent dissociation constants were lower for all dyes at pH 7.4 than at pH 6.5, as would be expected from the results for Malachite green, above.

The dyes examined partitioned to varying degrees between *n*-octanol and water. The experimentally

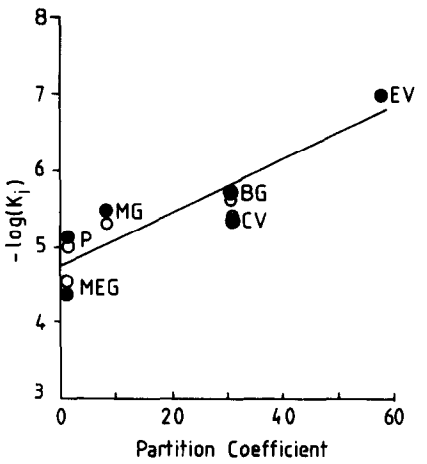
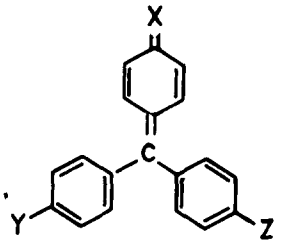


Fig. 1. Inhibition of rat liver GST 1-1 and 1-2 by basic triphenylmethane dyes: correlation of inhibitory potency with octanol–water partition coefficient. The logarithm of the K_i is plotted against the experimental value of the octanol–water partition coefficient. Filled symbols represent data obtained for GST 1-1 and open symbols, data obtained form GST 1-2.



Dye	X	Y	Z
Pararosanilin	$-NH_3^+$	$-NH_2$	$-NH_2$
Malachite green	$-N^+(CH_3)_2$	$-N(CH_3)_2$	$-H$
Crystal violet	$-N^+(CH_3)_2$	$-N(CH_3)_2$	$-N(CH_3)_2$
Methyl green	$-N^+(CH_3)_2$	$-N(CH_3)_2$	$-N^+(C_2H_5)_3$
Brilliant green	$-N^+(C_2H_5)_2$	$-N(C_2H_5)_2$	$-H$
Ethyl violet	$-N^+(C_2H_5)_2$	$-N(C_2H_5)_2$	$-N(C_2H_5)_2$

Fig. 2. Substituents of basic triphenylmethane dyes.

determined partition coefficients are used in Fig. 1. The magnitude of the partition coefficient increased with increasing hydrophobicity of the *para*-substituents of the dyes. All except Methyl green showed a high solubility in the organic phase than in the aqueous phase. The low hydrophobicity of Methyl green is expected because of the highly hydrophilic quaternary ammonium substituent (see Fig. 2).

Kinetic experiments were carried out in which the efficacy of these dyes as inhibitors was tested. It soon became apparent that the dyes varied greatly in their inhibitory powers and, further, that the mechanism of inhibition was not a simple one. Most experiments were concerned with the effect of variation of glutathione concentration on the inhibition since we wished to determine whether the dye–GSH adduct had any inhibitory properties. For both GSTs 1-1 and 1-2, it was found that the inhibition for almost all of the dyes was best represented as involving partially non-competitive inhibition with respect to GSH by both of the dye species present, the adduct making no discernable independent contribution to the overall inhibition. For these cases rate data were fitted to Eqn 3:

$$v = V_{max}\frac{(1 + \beta.[I]/K_i)}{(1 + [I]/K_i)}(1 + K_m/[GSH]) \quad (3)$$

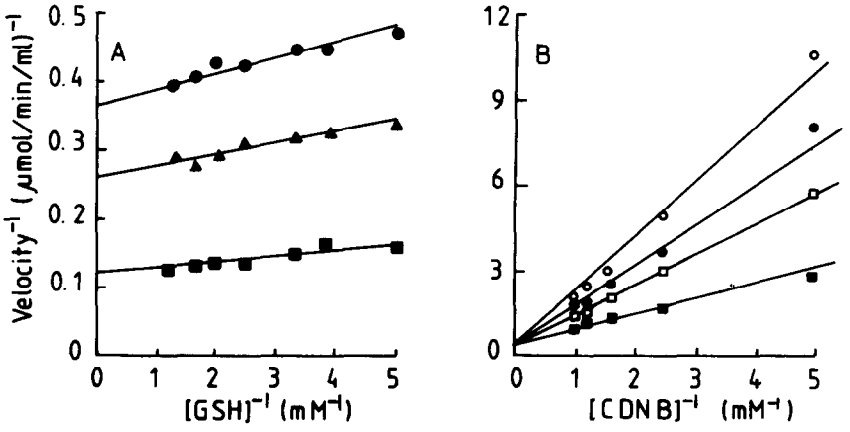


Fig. 3. Inhibition of rat liver GST 1-1 by Malachite green at pH 6.5. (A) Varied substrate GSH. The concentration of CDNB was held constant at 1.0 mM. MG concentrations were (■) 0 mM, (▲) 0.18 mM and (●) 0.74 mM. Solid lines were generated by a least squares fit to a partial non-competitive model (see Eqn 3). Weighting was according to $1/v^2$. (B) Varied substrate CDNB, GSH concentration was held constant at 1.0 mM. MG concentrations were (■) 0 mM; (□) 0.0067 mM; (●) 0.01 mM; (○) 0.02 mM. The solid lines were generated by a fit to a partial competitive mechanism. Weighting was according to $1/v^2$. Rate determinations were made in triplicate.

Table 2. Inhibition of rat liver GST by basic triphenylmethane dyes

Dye	K_m (mM)	K_i (μM)	β
Transferase 1-1			
Ethyl violet	0.10 ± 0.01	0.099 ± 0.02	0.08 ± 0.003
Crystal violet	0.14 ± 0.01	3.4 ± 0.3	0.22 ± 0.013
Brilliant green	0.13 ± 0.026	6.3 ± 0.7	0.077 ± 0.049
Malachite green	0.062 ± 0.006	5.8 ± 0.4	0.114 ± 0.017
Pararosanilin	0.13 ± 0.01	8.0 ± 2.0	0.57 ± 0.0009
Methyl green	0.12 ± 0.01	67 ± 21	0.18 ± 0.15
Transferase 1-2			
Ethyl violet	0.10 ± 0.01	0.1 ± 0.02	0.08 ± 0.003
Crystal violet	0.08 ± 0.01	4.0 ± 0.5	0.21 ± 0.02
Brilliant green	0.06 ± 0.02	1.8 ± 0.9	0.26 ± 0.08
Malachite green	0.09 ± 0.016	3.1 ± 0.4	0.17 ± 0.01
Pararosanilin	0.16 ± 0.015	9.7 ± 1.7	0.41 ± 0.025
Methyl green	0.12 ± 0.024	41 ± 11	0.001 ± 0.002

Data were analysed according to a model in which both the dye and its glutathione adduct were identically inhibitory and the mechanism of inhibition was partial non-competitive (see Eqn 4). Each experiment involved the determination of 21 reaction rates in triplicate.

Values are means ± SD.

where V_{max}, K_m and K_i have their usual meanings but represent only apparent kinetic constants (since they are functions of CDNB concentration), and β measures the residual, fractional catalytic activity of the enzyme–inhibitor complex. Typical results are shown in Fig. 3 and values for these parameters are shown in Table 2. It will be seen that the constant β varies in value quite markedly, but has a value significantly different from zero in all cases but that of the inhibition of GST 1-2 by Methyl green. The values of the inhibition constants correlated well with the measured partition coefficient ($r = 0.911$ $P < 0.01$) (see Fig. 1).

In the case of Malachite green a more detailed kinetic analysis suggested that there was a significant contribution to the inhibition by the adduct. If it were proposed that both Malachite green and the adduct of the dye and GSH inhibited in a partially non-competitive fashion (see Eqn 4) then a detectable improvement in the agreement between kinetic data and predicted values was obtained in the case of GST 1-1 and a substantial improvement in fit for GST 1-2.

$$v = V_{max} \frac{(1 + \beta([\text{Free dye}]K_{i1} + [\text{Adduct}]/K_{i2}))}{(1 + [\text{Free dye}]/K_{i1} + [\text{Adduct}]/K_{i2})} \Big/ (1 + K_m/[\text{GSH}]). \quad (4)$$

K_{i1} and K_{i2} are inhibition constants for the free dye and the adduct respectively.

In the first case the value of ES (the mean sum of squared deviations from the theoretical values) decreased marginally from a value of 0.0072 to one of 0.0068. On the other hand, when GST 1-2 was studied, the values of ES decreased from 0.001 to 0.00019, indicating a very substantial improvement in fit. When GST 1-1 was the enzyme under consideration, the adduct appeared to be twice as effective an inhibitor as the free dye. When GST 1-2 was examined, the adduct appeared to be an order of magnitude more effective. In both instances, the kinetic constants obtained by regression analysis were well defined with coefficients of variation being generally less than 0.2 (see Table 3).

The inhibition of GST 1-1 by Malachite green, at fixed concentrations of GSH and CDNB, was examined at pH values between 6.5 and 7.5 (see Fig. 4). It was observed that the percentage inhibition of the enzyme increased with pH and appeared to be tending towards a limiting level beyond pH 7.5.

In one experiment, the inhibition of GST 1-1 by Malachite green was examined when the varied substrate was CDNB. In this case the mode of inhibition was found to be partially competitive. The K_m for CDNB corresponding to this model was 1.34 ± 0.43 mM, and the apparent K_i value was 8 ± 2 μM.

The inhibition of GST 2-2 by Crystal violet and Malachite green was examined. It was found that the mode of inhibition was again best described as partial non-competitive. The inhibition constants were 2.2 ± 0.2 μM (mean of six experiments) and 5.6 ± 0.1 μM (mean of four experiments) for Crystal violet and Malachite green, respectively. These values are very similar to those obtained for GST 1-1, as shown in Table 2.

A single experiment was carried out in which the effect of Ethyl violet on the μ-class GST 3-3 was studied. Analysis of the rate data suggested that the inhibition mechanism was non-competitive, rather than partially non-competitive, with respect to glutathione. The inhibition constant had a value of 5.8 ± 0.2 μM. Whether class μ GSTs are generally less sensitive to inhibition by this class of compound than the class α isoenzymes, it is premature to say. It is so in this case.

Malachite green appeared to be excreted rapidly

Table 3. Proposed inhibition of rat liver GST 1-1 and 1-2 by Malachite green and its glutathione adduct

Enzyme	K_m	K_{i1}	K_{i2}	β
1-1	0.07 ± 0.005	6 ± 0.5	3.4 ± 0.9	0.12 ± 0.02
1-2	0.20 ± 0.01	12.6 ± 0.5	0.4 ± 0.08	0.17 ± 0.008

Data were analysed according to a model in which both the free Malachite green and its adduct with glutathione inhibited the enzymes by partial non-competitive inhibition (see Eqn 4). K_{i1} and K_{i2} are the inhibition constants for free Malachite green and its GSH adduct, respectively. Each experiment involved the determination of 21 reaction rates in triplicate.

Values are means ± SD.

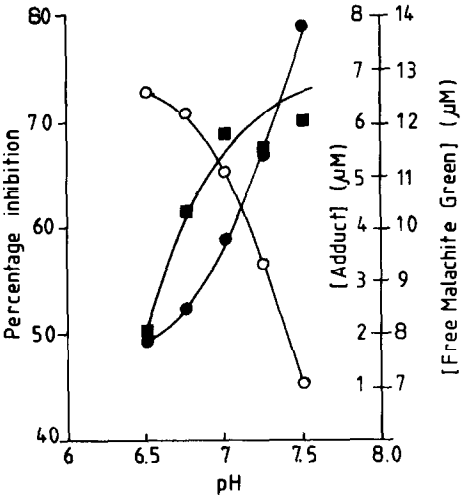


Fig. 4. Inhibition of rat liver GST 1-1 by Malachite green: variation of inhibition with pH. GST activity was measured at fixed concentrations of GSH and CDNB of 1.0 and 0.0144 mM, respectively. (○) Concentration of free Malachite green; (●) concentration of GSH–MG adduct; (■) % inhibition.

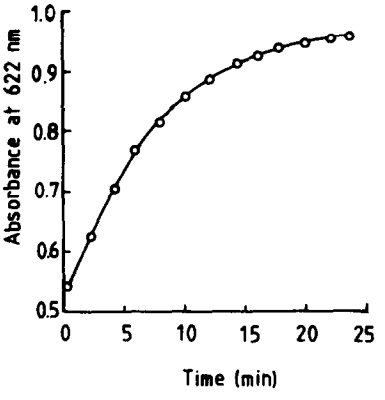


Fig. 5. Change in extinction at 620 nm of bile collected from rats injected with Malachite green. Bile from a rat injected with Malachite green (2 mg/kg) was collected in a 1 mm pathlength spectrophotometer cell and the extinction at 620 nm was measured at intervals of 2 min, starting from the time the cell was filled.

and extensively in the bile after intravenous injection. The bile did not have an unusual appearance initially, but on standing exposed to the air, it rapidly became an intense green. Repeated scans of bile samples obtained from injected animals showed the appearance of a substantial aborption peak at 625 nm which was significant at 2 min after collection and reached a maximum value at about 20 min (Fig. 5). Tests using the method of Ellman [13] suggested that the excretion of Malachite green in the bile was accompanied by a substantial increase in its thiol content. However, since Malachite green has a substantial absorption peak at the wavelength at which the Ellman test is carried out, it was not possible to quantitate these results. In sum, we interpret the above results to indicate that Malachite green is released into the bile largely as a GSH adduct, in equilibrium with both free GSH and free Malachite green. It is assumed that on standing the free GSH in the bile is oxidized by atmospheric oxygen to GSSG. This then causes the equilibrium between dye and adduct to change in favour of the release of free dye.

DISCUSSION

The inhibitory nature of Malachite green with respect to glutathione-dependent enzymes was first recognised when Balabaskaran *et al.* [16] tested it as a substrate analogue for housefly DDT-dehydrochlorinase. It was found to be a potent inhibitor. It was also observed that the dye could inhibit insect GST and that glutathione bleached the dye to a significant extent [6]. The bleaching was attributed to reduction of the dye to its leuco form.

In the present work we have set out to examine inhibition of GSTs of mammalian origin by Malachite green and related compounds, the end in view being to screen for compounds that will provide effective inhibition of the GSTs *in vivo*. Balabaskaran and Smith [6] reported on the difficulty of interpreting inhibition patterns yielded by Malachite green due to bleaching of the dye by GSH. Consequently, our first priority was to obtain a quantitative expression

of the relationship between the dye and its colourless form. The stoicheiometry of the reaction does not support the view that bleaching of the dye is due to a reduction to leuco Malachite green. This would require the participation of two GSH molecules for the reduction of each dye molecule. Rather, it appears probable that the reaction involves nucleophilic attack of a single GS^- anion, probably on the central carbon atom, to give a tetrahedral adduct in which the resonance of the chromophore is disrupted. The observed stoicheiometry of the equilibrium and the variation of the equilibrium constant with pH support this view. The magnitude of the constants is such that at pH 6.5, the proportion of the dye conjugated with GSH at 0.5 mM GSH would be, for Methyl green, pararosanilin, Malachite green, Crystal violet and Ethyl violet: 58, 5.5, 11.6, 1.2 and 2.1%, respectively. The corresponding percentages at pH 7.4 at 0.5 mM GSH concentration would be 90.6, 23.9, 50, 3.7 and 3.3%. Under the latter conditions Brilliant green would be found to be 22% in the form of the adduct. Towards the upper end of the range of physiologically relevant concentrations of GSH (5 mM) [17], the percentage adduct formation for Methyl green, pararosanilin, Malachite green, Crystal violet, Brilliant green and Ethyl violet would be 99, 76, 91, 74, 28 and 25%, respectively. Thus for instance in the liver, compounds such as Methyl green and Malachite green would be almost totally in the adduct form. This is consistent with our observation of the biliary excretion of an initially colourless form of Malachite green. We assume that the dye is excreted as the adduct. These figures also indicate that for all dyes tested in the present work, on injection into an animal, there will be a significant fraction present as a glutathione adduct in the tissues.

We confirm the earlier reports of Malachite green's efficacy as an inhibitor of GST [6] and have shown that structurally related dyes, such as Ethyl violet, may be even more potent inhibitors. Our evidence suggests that the dyes themselves are strong inhibitors of the enzymes and, further, that the adduct between the dye and glutathione may, in some instances, be an even more potent inhibitor. Support for the inhibitory nature of the adduct comes from a detailed analysis of the kinetic behaviour of the enzyme in the presence of Malachite green (see Tables 2 and 3) and also from observation of increased inhibition of the enzyme under conditions in which the formation of the adduct is favoured, i.e. when the pH of the assay system is increased (Fig. 4). Kinetic analysis of the inhibition by Methyl green also gives support to the inhibitory nature of its adduct, though the inhibition by neither dye nor adduct is strong. Under the assay conditions chosen, there would have been insufficient adduct formation from other dyes for any inhibitory effect to have been identified by kinetic analysis. It appears possible that, at physiological pH, inhibition by the adduct may be a significant contributory factor to the overall inhibition for all the dyes tested. This possibility is currently under examination.

There are two aspects of the mechanism of inhibition that merit discussion. In the first case, the instances of inhibition by the adduct that we have been able to characterize so far suggest that this type of compound inhibits about two to 10 times more strongly than the parent dye. Given that the K_m for GSH is about 10^{-4} M and that the K_i of the parent dye about 10^{-6} M, for a bifunctional adduct which bound to both portions of the active site one might expect a K_i value of the order of 10^{-10} M, this value being the product of the two dissociation constants. The experimentally determined values are much more modest. It appears likely that this may be explained by the formation of a GSH adduct through attack on the central carbon of the triphenylmethane dye, this generating a tetrahedral molecule very different in its geometry from the planar parent compound. It is likely that such a molecule would have greater difficulty in fitting into a hydrophobic cleft on the enzyme than the more compact dye. The second point concerns the apparently partial non-competitive nature of the inhibition we report here. We have observed partial modes of inhibition with a variety of different classes of compound, including phthalein dyes [18] and inorganic anions [19], in GSTs from a variety of sources. Others have reported similar behaviour for other types of compound including pesticides [20] and other classes of dye [21]. We have in the past proposed as an explanation of such behaviour a "classical" model in which the inhibitor may partially occlude the active site producing an enzyme form of reduced affinity for the substrate or of reduced catalytic activity. Given that the same type of behaviour is observed with such widely differing classes of inhibitor and given the wide variation in values of β obtained for closely related inhibitors, we believe that such an explanation may be an oversimplification. There is growing evidence that, particularly at the low pH levels at which most kinetic studies on these enzymes are carried out, GST may exist in a number of kinetically stable conformers [22–24]. It appears likely that the complexities of inhibition discussed here may be generated by stabilization of one or more of such conformers by the inhibitor.

It is now well established that, in connection with both chemotherapy [2, 3] and chemical pest control [4, 5], elevation of GSTs may be linked with the development of toxicologically significant resistance to the chemical agent being used. The ability to inhibit these enzymes *in situ* is therefore of considerable interest. Toxicity of the inhibitors used is a factor to be taken into account. The data with respect to the basic triphenyl methane dyes are somewhat contradictory with regard to their effects, *in vivo* and *in vitro*. Crystal violet at a concentration of about 30 μM is reported to impair the function of a number of human cell types *in vitro* [25, 26] and is also reported to inhibit the replication of DNA [27] and its repair [28]. On the other hand, dyes such as Crystal violet and Malachite green have been used as topical sterilants for many years with no adverse effects reported. Furthermore, Crystal violet has been administered intravenously in the treatment of blastomycosis and septicaemias [29] with no untoward effects reported. This dye is also currently used in the control of Chaga's disease. It is added to stored whole blood to prevent the transmission of *Trypanosoma cruzi*, the causative organism, by

blood transfusion. No ill effects have been noted even in the course of transfusions involving large doses (up to 300 mg/day). These results suggest that dyes such as these may be worth investigating further as inhibitors which may bring about the selective inhibition of GST *in vivo*.

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