Reverse micelles as a catalyst for the nucleophilic aromatic substitution between glutathione and 2,4-dinitrochlorobenzene

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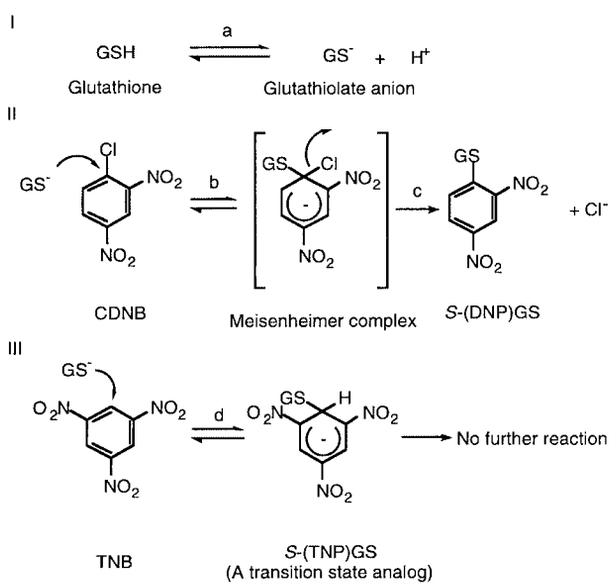
The nucleophilic aromatic substitution (S_NAr) between GSH and 2,4-dinitrochlorobenzene was studied in reverse micellar systems composed of limited amounts of water, a surfactant with a polar head and a nonpolar tail, and the organic solvent 2,2,4-trimethylpentane. When the surfactant was positively charged and contained an aromatic ring in the polar head, the second-order rate constant was increased by approximately two orders of magnitude as compared to that in aqueous solution. The rate enhancement could be attributed to the stabilization of the negatively charged Meisenheimer σ-complex by the positively charged polar head and the weak aromatic ring's electric quadrupole interactions of the surfactant. The reaction rate in reverse micelles composed of neutral polar head groups (Triton X-100) was increased by 3-fold, which may be explained by the interactions of the hydroxy groups of Triton molecules with the π-system of the Meisenheimer complex. An inverse relationship between the molar concentration $[H_2O]$/[surfactant] ratio, which reflects the inclusive volume of the reverse micellar particle, and the rate enhancement was observed for positively charged or hydroxy-containing reverse micelles, but opposite results were obtained with negatively charged reverse micelles. These reverse micellar systems thus mimic the active site of a detoxification enzyme, glutathione transferase, in which stabilization of the Meisenheimer complex by a positively charged arginine residue, on-edge quadrupole interactions of aromatic amino acids, and the hydroxy group of tyrosine or threonine have been proposed in the enzyme-catalysed S_NAr conjugation.

Introduction

Glutathione transferase (GST,† EC 2.5.1.18) catalyses the conjugation of glutathione (GSH) with various xenobiotic or endogenous electrophilic compounds, constituting a major detoxification mechanism of biological systems and is responsible for the development of drug resistance in cancer chemotherapy as well as for pesticide and insecticide resistance in agriculture.[1] The GST-catalysed nucleophilic aromatic substitution (S_NAr) between GSH (a L-γ-Glu-Cys-Gly tripeptide) and 2,4-dinitrochlorobenzene (CDNB) involves a general-base catalysed ionisation of the enzyme-bound GSH in which the cysteinyl -SH group is ionised to yield a better nucleophile glutathiolate anion (GS^-). This anion then attacks the *ipso* carbon bearing the chlorine atom in CDNB, forming a Meisenheimer σ-complex. Leaving of the chloride ion completes the reaction (Scheme 1, reactions I and II).[2] The formation of the *S*-(2,4-dinitrophenyl)glutathione conjugate renders the less water soluble CDNB into a more water soluble product which is then ready for excretion from the cells by a membranous ATP-dependent glutathione-conjugate pump system.[3] The rate-limiting step has been proposed at the σ-bond formation in the Meisenheimer complex.[4] Some of the factors contributing to the transition-state stabilization have been proposed, including the role of conserved positively charged amino acid residues in the active site.[4,5]



Scheme 1 Proposed chemical mechanism for the base-catalysed nucleophilic aromatic substitution of GSH and CDNB or GSH and TNB. (I) Ionisation of glutathione catalysed by a general base (step a). (II) Nucleophilic attack of the glutathiolate anion at the *ipso* carbon of CDNB forming the Meisenheimer complex (step b) and leaving of chloride ion forming the *S*-(DNP)GS product (step c). (III) Nucleophilic attack of the glutathiolate anion on TNB forming the transition state analogue *S*-(TNP)GS (step d).

The non-enzymatic reaction between GSH and CDNB has a substantially measurable rate, which needs to be corrected in the assay of GST-catalysed reaction. On the other hand, this

† Abbreviations used. GST, glutathione transferase; GSH, reduced glutathione; CDNB, 2,4-dinitrochlorobenzene (1-chloro-2,4-dinitrobenzene); TNB, 1,3,5-trinitrobenzene; *S*-(DNP)GS, *S*-(2,4-dinitrophenyl)glutathione; *S*-(TNP)GS, *S*-(2,4,6-trinitrophenyl)glutathione; S_NAr, nucleophilic aromatic substitution; AOT (Aerosol-OT), sodium bis(2-ethylhexyl) sulfosuccinate ($C_{20}H_{37}O_7SNa$); BDAC, benzyldodecylbis(2-hydroxyethyl)ammonium chloride ($C_{23}H_{42}NO_2Cl$); BTAC, benzyldimethyltetradecylammonium chloride ($C_{23}H_{42}NCl$); CBAC, cetylbenzyldimethylammonium chloride ($C_{25}H_{46}NCl$); CPB, cetylpyridinium bromide ($C_{21}H_{38}NBr$); CTAB, cetyltrimethylammonium bromide ($C_{19}H_{42}NBr$); DEAB, dodecylethyldimethylammonium bromide ($C_{16}H_{36}NBr$); DTAB, dodecyltrimethylammonium bromide ($C_{15}H_{34}NBr$); EDAB, ethylhexadecyldimethylammonium bromide ($C_{20}H_{44}NBr$); OTAB, octadecyltrimethylammonium bromide ($C_{21}H_{46}NBr$); TDAB, tetradecyltrimethylammonium bromide ($C_{17}H_{38}NBr$); Triton X-100, *tert*-octylphenoxypolyethoxyethanol ($C_{32-34}H_{58-62}O_{10-11}$).

non-enzymatic reaction provides one of the few reactions suitable for comparison with the enzymatic-catalysed reaction that is extremely useful in characterizing the fundamental role of the enzyme in catalysis and thus is helpful in elucidating the GST mechanism. We have previously isolated and characterized the octopus hepatopancreatic GST.[6] The octopus GST is a sigma-class GST,[7,8] which has been recruited as *S*-crystallin in octopus lens.[9] The detailed kinetic analysis of the reaction catalysed by this enzyme was performed by steady-state kinetics.[10,11] Our results indicate that octopus GST conforms to a steady-state random Bi-Bi kinetic mechanism similar to other classes of GST.

Since CDNB is less soluble in water and was dissolved in organic solvent before mixing with GSH, we have also characterized the kinetic behavior of the enzyme in a plasma membrane mimicking reverse micellar system composed of water–surfactant–2,2,4-trimethylpentane which produced a macro-homogeneous transparent solution.[11] During those studies, we found that the non-enzymatic S_NAr reaction of the GSH–CDNB system was enhanced in reverse micelles composed of positively charged surfactant but not in the negatively charged reverse micelles.[12]

Furthermore, we previously found that GST may have some affinity with the membranous structure.[11] We postulated that association of the cytoplasmic GST with the membranous pumping system is beneficial for the cells to efficiently excrete toxic xenobiotics.[11] These results prompted us to systematically examine the charge and other effects of the polar heads on the S_NAr reaction rate and to critically evaluate the application of using these reverse micelles as a model system in elucidating the reaction mechanism of GST.

In this article, the non-enzymatic conjugation between GSH and CDNB in aqueous solution and in various water–surfactant–2,2,4-trimethylpentane reverse micellar systems is examined. Our results reveal that reverse micelles are ideal systems to access the various factors that contribute to the rate enhancement of the GST-catalysed S_NAr reaction.

Experimental

Materials

GSH and CDNB were obtained from Sigma-Aldrich (St. Louis MO, USA). 2,2,4-Trimethylpentane was obtained from Merck (Darmstadt, Germany). The purity of AOT was previously examined.[13] Other surfactants were from Sigma-Aldrich or Merck and were used without further purification. Distilled water further purified through a MilliQ system (Millipore, Bedford, USA) was used throughout this work.

Enzyme purification

Digestive gland GST from octopus was purified once by GSH-Sepharose 4B affinity chromatography as described previously.[6] The dimeric enzyme was judged to be apparently homogeneous by SDS/PAGE with subunit M_r 24 000. Protein concentration was determined by the protein-dye binding method.[14]

Enzyme assay in aqueous system

Assay for GST activity was carried out at 25 °C. The reaction mixture (1 ml) contained 89 mM potassium phosphate buffer, pH 6.5, 1 mM each of GSH and CDNB, 0.89 mM EDTA and an appropriate amount of the enzyme. The reaction rate was colinear with the GST concentration up to 3.69 μg assay^{-1}.[11] The formation of *S*-(DNP)GS was continuously monitored at 340 nm. One unit of enzyme activity is defined as an initial rate of 1 μmol *S*-(DNP)GS formed per minute under the assay conditions using a molar absorption coefficient of 9.6×10^3 M^{-1} cm^{-1} for the conjugate.

Non-enzymatic reaction between GSH and CDNB in reverse micellar systems

CDNB is the generally used substrate for the enzymatic assay of GST activity. However, GSH reacts with CDNB non-enzymatically at alkaline pH. Under the near neutral assay conditions (pH 6.5) used in the enzymatic assay described above, the minor non-enzymatic conjugation was corrected using a double beam spectrophotometer that allows all reaction components except the enzyme to be placed in the reference cell. Thus, the recorder tracing will represent only the enzyme-catalysed conjugation rate.

In the reverse micellar systems, the non-enzymatic reaction between GSH and CDNB was examined at 25 °C in Bis-Tris–HCl buffer (6 mM, pH 6.5). The final total volume, not the less definable volume entrapped in reverse micelles,[15] was used for measurement of concentrations. The CDNB and other less water-soluble substrates were prepared as dimethyl sulfoxide solutions. The final organic solvent's concentration in the assay mixture was kept below 1% and did not affect the results. An injection method was employed in mixing the two substrate solutions.[15]

The second-order rate constant for the non-enzymatic reaction between GSH and CDNB in reverse micellar systems was estimated by the following equation (eqn. (1)) in which

$$\frac{1}{[A]_o - [S\text{-(DNP)GS}]} = k_2 \times t + 1/[A] \qquad (1)$$

[GSH] = [CDNB],[16] where $[A_o]$ is the initial substrate concentration and k_2 denotes the second-order rate constant. To simplify the calculation further, we used 1 mM concentration for both GSH and CDNB. In this particular case, eqn. (1) can be simplified to eqn. (2).

$$\frac{1}{1 - [S\text{-(DNP)GS}]} = k_2 \times t + 1 \qquad (2)$$

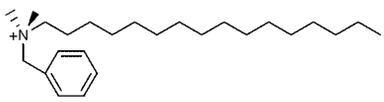
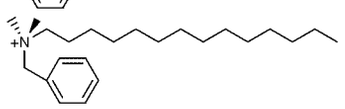
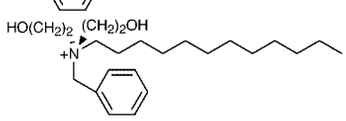
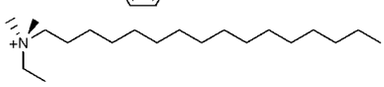
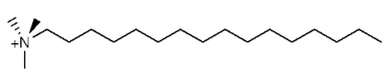
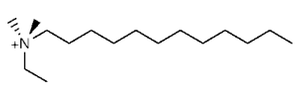
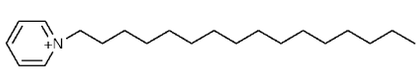
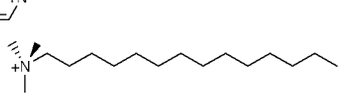
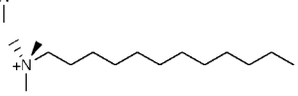
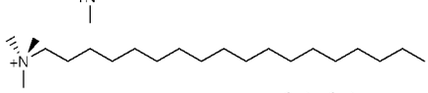
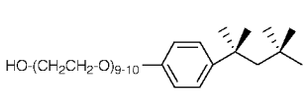
A plot of $1/\{1 - [S\text{-(DNP)GS}]\}$ *versus* time (t) will give a straight line which intercepts the vertical axis at 1.0 and whose slope denotes the second-order rate constant.

Results

Rate enhancement of nucleophilic aromatic substitution in reverse micelles

We have previously suggested that a reverse micellar system composed of positively charged polar head surfactant is an exemplary model system to study the GST-catalysed S_NAr reaction.[12] To further characterize this model system, we have systematically examined the conjugation between GSH and CDNB in reverse micelles composed of various surfactants. The reactions were conveniently monitored with a UV-VIS spectrophotometer by following the formation of *S*-(DNP)GS which absorbs at 340 nm. The reaction rate measurements in all surfactant reverse micelles followed a linear plot with respect to the CDNB concentration and the reaction time. Some typical plots of the data according to eqn. (2) are shown in Fig. 1. Table 1 lists the detergent structures and the second-order rate constants for the S_NAr reaction in those reverse micelles. We have chosen surfactants with positively charged polar heads and nonpolar tails of different chain lengths. However, no obvious difference was observed for the reaction rate of surfactants with different nonpolar tails. The same organic solvent 2,2,4-trimethylpentane was used in all reverse micellar systems. The partitioning of water insoluble CDNB between the aqueous and organic phases and/or the interface does not seem to account for the rate differences. The clear conclusion that can be drawn from the data shown in Table 1 is that the

Table 1 Second-order rate constants of the nucleophilic aromatic substitution reaction between GSH and CDNB in reverse micelles composed of different surfactants. The $[H_2O]$/[surfactant] molar ratio was held constant at 8.3 for all reverse micellar systems in Bis-Tris–HCl buffer (6 mM, pH 6.5). The ratios of second-order rate constants in reverse micelles ($k_{2(rm)}$) to that in aqueous solution ($k_{2(H_2O)}$) are compared in the last column

Surfactant	Chemical structure of surfactant	Second-order rate constant/ M^{-1} s^{-1}	$k_{2(rm)}/k_{2(H_2O)}$
CBAC		0.209	91
BTAC		0.200	87
BDAC		0.124	54
EDAB		0.109	47
CTAB		0.103	45
DEAB		0.101	44
CPB		0.96	42
TDAB		0.095	41
DTAB		0.092	40
OTAB		0.089	39
TRITON X-100		0.007	3
None		0.0023	1

S_NAr reaction rate in positively charged polar head reverse micelles is enhanced up to approximately two orders of magnitude when compared to the reaction rate in aqueous solution.

The CBAC and BTAC have the fastest reaction rates. Besides the positive charge, the weak electric quadrupole of the benzyl group might provide an on-edge interaction with the Meisenheimer complex that accounts for the approximately 2-fold rate enhancement.

The surfactant with a hydroxy polar head (Triton X-100) enhanced the rate by 3-fold. However, BDAC, which possesses positively charged, benzyl and hydroxy groups, did not provide a faster rate than those surfactants with only positive charge and benzyl group (CBAC and BTAC). It is possible that the hydroxy groups of BDAC shield the more important positive charge interactions. The basicities of the amino functions from which the positively charged head groups are derived seems to have no effect on the rate constant. Thus values are similar in reversed micelles derived from CPB and CTAB which contain pyridinium and quaternary ammonium head groups respectively.

Effect of particle size of the reverse micellar system on the rate enhancement of the nucleophilic aromatic substitution

To further access the charge effect, we prepared reverse micelles under various hydration degrees of the system by adjusting the water content of the system, thus producing reverse micelles of different sizes.[17] The degree of freedom of GSH in the reverse micelles is also restricted in small reverse micelles and thus decreases the entropy of the system. Fig. 2 shows the results of four reverse micellar systems. If the reverse micelles are composed of negatively charged polar heads (AOT), the reaction rate decreased with decreasing water content for the smaller reverse micelles (Fig. 2A). On the other hand, the rate in reverse micelles composed of positively charged (CTAB and CBAC) or hydroxy (Triton X-100) polar heads increased for the smaller reverse micelles (Fig. 2B–D). CBAC had a positive charge and an extra benzyl group, and gave the fastest rate enhancement among the surfactants we examined. These results clearly substantiate our hypothesis that the rate enhancement in the positively charged polar head reverse micelles is due to the ionic interactions between the polar heads and the negatively charged Meisenheimer complex.[12] In other words, the reaction is facilitated by stabilizing the charged Meisenheimer complex and these reverse micelles provide an excellent model system for studying the S_NAr reaction catalysed by GST.

Discussion

Analogy between reverse micelles and glutathione transferase

In this article, we provide evidence indicating that the rate enhancement of S_NAr in reverse micelles is due to the stabilization of the negatively charged Meisenheimer σ-complex by the

positively charged polar head and the weak aromatic ring's electric quadrupole interactions of the surfactant. The reaction rate enhancement in reverse micelles composed of a neutral polar head (Triton X-100) may be explained by the interactions of the hydroxy groups of Triton molecules with the π-system of the Meisenheimer complex. We found that this reverse micellar catalysed rate enhancement is highly analogous to the rate acceleration of the same reaction catalysed by the detoxification enzyme GST. In GST, a highly conserved positively charged arginine residue (Arg-13) has been proposed to stabilize the negatively charged Meisenheimer complex.[18–23] The functional importance of the corresponding Arg-14 in pi-GST[22] and Arg-15 in alpha-GST[23] have been demonstrated by site-specific mutagenesis.

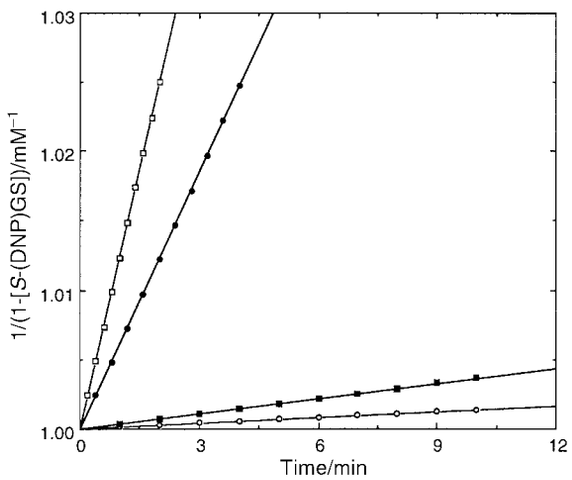


Fig. 1 Effect of surfactants on the rate enhancement of the conjugation between GSH and CDNB. GSH (1 mM) and CDNB (1 mM) in Bis-Tris–HCl buffer (6 mM, pH 6.5) were injected into the reverse micellar system containing 200 mM surfactant: CBAC (□), CTAB (●), Triton X-100 (■), water (no surfactant) (○). The data were fitted to eqn. (2). The slopes of these lines denote the second-order rate constants.

Here we propose that these reverse micellar systems mimic the active site region of a GST molecule and the quaternary amine may play an identical role to Arg-13 in GST. In sigma-GST, a Phe-106, located beside the dinitrophenyl moiety of the *S*-(DNP)GS molecule, may play the role of the weak aromatic ring's electric quadrupole interactions.[24,25] Although the dinitrophenyl moiety of the product seems to have little direct interaction with the enzyme other than a few van der Waals interactions with Phe-106,[8] the nitro groups of *S*-(DNP)GS are nestled against the electropositive edge of the Phe-106 aromatic ring in sigma-GST.[26] We thus conclude that, in mimicking the active site of sigma-GST, CBAC is a better surfactant to choose than the CTAB that we used previously.[12] The benzyl group mimics the Phe-106, whereas Tyr-7 is mimicked by benzyl and hydroxy groups. Scheme 2 shows a schematic model for the CBAC reverse micelles.

The above hypothesis is further supported by the fact that the recruited cephalopods lens protein *S*-crystallin has very little endogenous GST activity and can be regarded as a natural mutant of sigma-GST. Based on homology computer modeling data, we have suggested that the small enzymatic activity of *S*-crystallin is because of its losing the charge stabilization of the Meisenheimer complex.[26] An asparagine residue at position 101 in sigma-GST has been changed, in *S*-crystallin, to an aspartate residue, which is only 3.71 Å apart from Arg-13. A Coulomb charge–charge interaction would diminish the positively charged environment provided by Arg-13 in the active centre. This argument is strengthened by the comparison of surface electrical potential between *S*-crystallin and sigma-GST.[26] A marked feature was noticed in the active centre region, where the overall electric potential is positively charged.

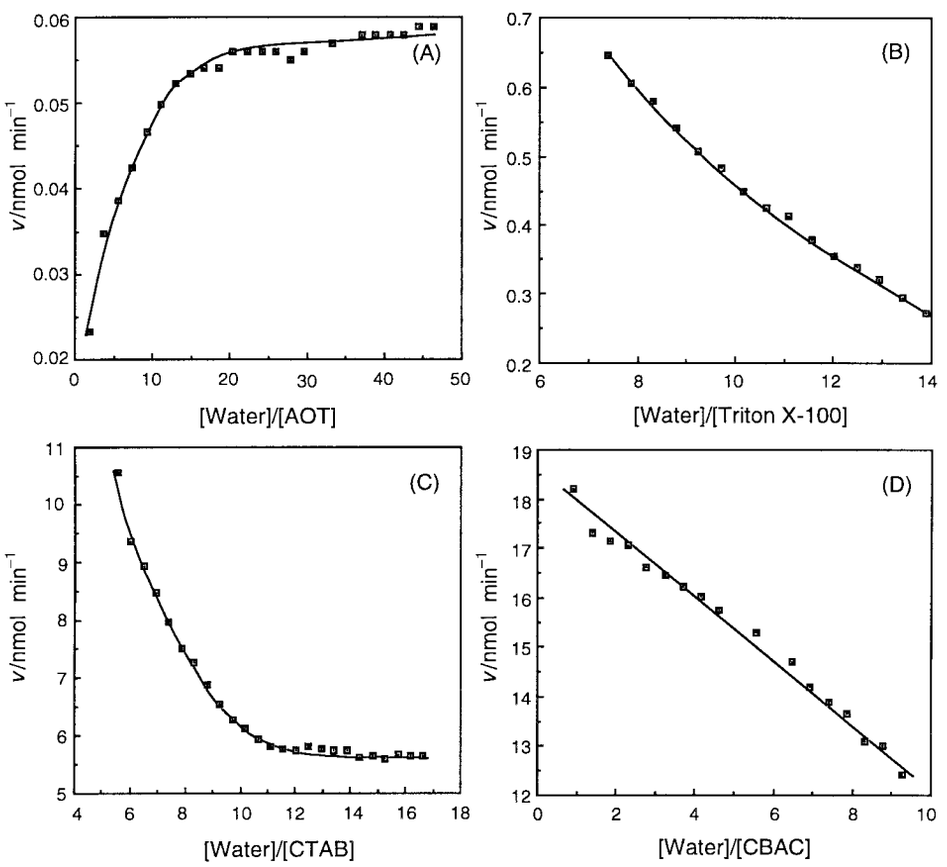
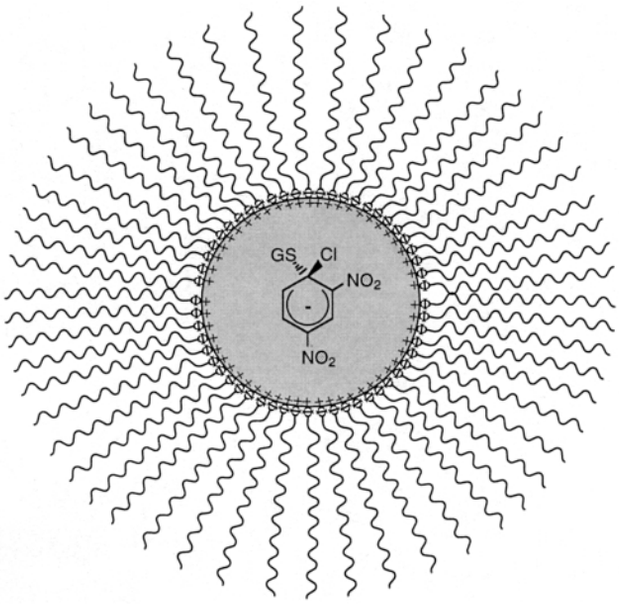


Fig. 2 Effects of [H_2O]/[surfactant] ratio on the conjugation between GSH and CDNB. GSH (1 mM) and CDNB (1 mM) in Bis-Tris–HCl buffer (6 mM, pH 6.5). The surfactant concentration was maintained constant at 200 mM. The [H_2O]/[surfactant] ratio was adjusted by varying the water amount, thus varying the dimensions of the reverse micellar particles. The surfactant used (A) AOT, (B) Triton X-100, (C) CTAB, and (D) CBAC.



Scheme 2 Schematic model showing the Meisenheimer complex entrapped in a CBAC-reverse micelle. The quaternary amine polar heads are represented by positive charges. The benzyl groups are represented by Φ and are drawn adjacent to the water phase.

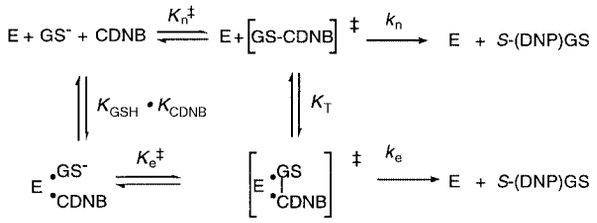
However, quite different surface electrical potentials are observed around Arg-13 between sigma-GST and *S*-crystallin.[26] Arginine-13 has been proposed to be the major residue responsible for the positively charged environment in the active site region.[22,23] Our model system provides further evidence indicating that the positively charged active centre is essential for the S_NAr reaction to proceed in GST.

Contribution of various factors in the rate enhancement of the GST-catalysed S_NAr reaction between GSH and CDNB

The above results provide a basis to quantitatively evaluate the contribution of various factors to the rate enhancement in the GST-catalysed S_NAr reaction. The experimental results from our earlier study indicated that ionisation of GSH does not contribute to the rate-acceleration of the non-enzymatic reaction.[12] A similar conclusion was also drawn for the enzymatic reaction.[27] Furthermore, the detergent does not affect the dissociation of GSH, however, the Meisenheimer complex is stabilized and provides a 91-fold acceleration of the reaction rate.

To estimate the degree to which the tight binding of transition-state contributes to the rate-enhancement of GST-catalysed reaction, *S*-(TNP)GS was generated *in situ* and was assumed to be a transition-state analog (see Scheme 1). This compound was found to be an un-competitive inhibitor for octopus GST with respect to GSH with $K_{I,S\text{-}(TNP)GS}$ value of 1.89 ± 0.17 μM according to the method of Clark and Sinclair.[28] The $K_{m,GSH}$ and $K_{m,CDNB}$ values as determined by initial-velocity studies were 0.344 ± 0.004 mM and 0.769 ± 0.007 mM, respectively.[10,11] According to the thermodynamic relationships of these kinetic parameters[29] (Scheme 3), the rate enhancement due to tight binding of *S*-(TNP)GS with the enzyme can be expressed as $k_e/k_n = K_e^{\ddagger}/K_n^{\ddagger} = K_{m,GSH} \cdot K_{m,CDNB}/K_{i,S\text{-}(TNP)GS}$. An effective concentration of approximately 140 mM was obtained.

If the effects of charge stabilization for the Coulomb and weak aromatic quadrupole electric interactions and the hydroxy group interactions are to be considered, the rate enhancement should be increased at least by another 273-fold (91×3). Assuming that all these effects are multiplicative, *i.e.,* their contributions to the activation free energy are additive, an overall value of approximately 3.8×10^4-fold (140×273) was achieved.



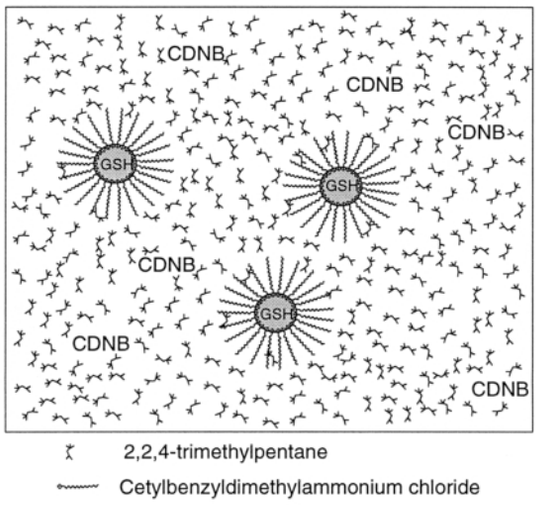
Scheme 3 Thermodynamic cycle for the non-enzymatic and enzymatic reaction of GSH and CDNB. K_{GSH}, K_{CDNB}, and K_T ($K_{i,S\text{-}(TNP)GS}$) represent dissociation constants for GSH, CDNB and the transition-state with the enzyme, respectively. $K_n^{\ddagger}$ and $K_e^{\ddagger}$ represent the dissociation constants of the transition state in non-enzymatic and enzymatic reactions, respectively. k_n denotes the rate for non-enzymatic reaction and k_e denotes the rate for enzymatic reaction.

For the acceleration of an enzymatic reaction rate, when making a comparison with the non-enzymatic reaction rate constant, the second order rate constant (k_{cat}/K_m) of the enzyme reaction should be used.[30] Detoxification enzymes such as GST are characterized as sluggish, with broad substrate specificity, and essentially unidirectional catalysts capable of reacting with a broad spectrum of xenobiotics that cells might encounter.[1] Since the GSH concentration in cells is in the range of 3–10 mM,[1] which is an order magnitude greater than the $K_{m,GSH}$ value, the GST thus can be regarded as saturated with GSH under the *in vivo* conditions. Therefore, the $k_{cat}/K_{m,CDNB}$ is a more appropriate kinetic parameter to be compared with the non-enzymatic second-order rate constants. In the case of octopus GST, the k_{cat} is 213 s^{-1} and a catalytic efficiency of 2.8×10^5 M^{-1} s^{-1} in the $k_{cat}/K_{m,CDNB}$ term is achieved by the binding of substrates.[10,11] As compared with the non-enzymatic second-order rate constant (0.0023 M^{-1} s^{-1}) (Table 1), a rate-acceleration factor (k_e/k_n) for the enzymatic reaction was estimated to be 12×10^7, a value which is still a thousand fold faster than that estimated from the reverse micellar model system (3.8×10^4). Since the reaction rate is increased in smaller reverse micelles containing positive charges or hydroxy groups, and the essential protein groups in the enzyme active centre are more closely in contact with the substrate or transition-state, if the inclusive volume factor is taken into consideration, the non-enzymatic reaction rate enhancement may actually approach the enzymatic reaction rate acceleration.

Proposed reverse micelles as GST model

Our results strongly suggest that electrostatic interaction is the key factor for the GST-catalysed S_NAr reaction rate enhancement. This conclusion conforms to the theoretical derivation for enzyme catalysis by Warshel *et al.*,[31] who predicted that the role of an enzyme molecule in the catalysed reaction is to provide pre-oriented dipoles. These dipoles are polarized to stabilize the transition state charge distribution, thus reducing the activation energy for the chemical reaction. The charge stabilization effect does account for a large part of the enzyme acceleration rate for the S_NAr reaction between GSH and CDNB. The water–CBAC–2,2,4-trimethylpentane reverse micellar system thus mimics the active site of the sigma-GST (or other classes of GST) and provides an excellent model to assess the charge effects in the nucleophilic aromatic substitution (Scheme 4).

Our reverse micellar model may be considered as a refinement of the oil-lake model proposed by Jakoby for GST.[32] In that cartoon model, the lipophilic compounds are accepted by the membranous oily lake. GSH is on an island to accommodate the tripeptide. Whereas in our model (Scheme 4), the reverse micellar particles constitute the active site islands to hold the hydrophilic substrate GSH. This model suggests that GST is an efficient method of detoxification, capable of handling the huge numbers of water insoluble toxic compounds that the cells might encounter, however, it would be inefficient for



Scheme 4 Schematic model for the water–cetylbenzyldimethylammonium chloride–2,2,4-trimethylpentane reverse micelles. The entrapment of the hydrophilic substrate glutathione (GSH) in the water pool (gray area) of the reverse micelles mimics the active centre of the glutathione transferase. The various hydrophobic substrates, *e.g.*, 2,4-dinitrochlorobenzene (CDNB), were dissolved in the organic solvent 2,2,4-trimethylpentane reflecting the broad substrate specificities of the enzyme. This model suggests that GST is not an efficient detoxification enzyme. Proximity and orientation might not be the important factors in the GST-catalysed reaction. Stabilization of the negatively charged Meisenheimer complex in a positively charged environment (Scheme 2) may contribute to the major factor in the rate enhancement.

catalysis. This low catalytic rate is compensated for by the presence of large amounts of the enzyme in cells.[1,32] GST was estimated to constitute as much as 5% of the total soluble protein in cells.[1,32] Our model provides a quantitative evaluation of the previous oil-lake model.

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