Mechanism of Nucleophilic Aromatic Substitution of 1-Chloro-2,4-dinitrobenzene by Glutathione in the Gas Phase and in Solution. Implications for the Mode of Action of Glutathione *S*-Transferases

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Abstract: The reaction mechanism of nucleophilic aromatic substitution of 1-chloro-2,4-dinitrobenzene by glutathione (as modeled by a thiomethoxide ion) in the gas phase and in solution was elucidated using *ab initio* molecular orbital theory in combination with a continuum solvent model at the HF/6-31G*, HF/6-31+G**, and MP2/6-31+G** levels of theory. Two ion−molecule complexes were located in the gas phase at the HF level, but only one exits at the MP2/6-31+G** level, while neither exits in aqueous solution. In aqueous solution, there is a large free energy barrier and C−S bond formation is the rate-determining step, which is in agreement with experimental observation. The calculated free energy barrier (30.2 kcal/mol) at the HF/6-31+G** level of theory seems to be in good agreement with experiment (23.8 kcal/mol), while the MP2/6-31+G** barrier is too low, indicating that the MP2/6-31+G** level of theory probably overestimates the stability of the transition state for C−S bond formation. Implications for the mode of action of glutathione *S*-transferases (GSTs) and a related enzyme are discussed in light of the results of the current study.

Introduction

Glutathione *S*-transferases (GSTs) (EC 2.5.1.18) catalyze the addition of the tripeptide glutathione (GSH or HSG) to a wide variety of compounds that have electrophilic groups.[1−6] This generic reaction is involved in detoxification of potentially toxic alkylating agents and active metabolic intermediates. Glutathione *S*-transferases have also been implicated in the development of resistance toward xenobiotics such as carcinogens, therapeutic agents, pesticides, and insecticides by cells and organisms.[7] So far, five subclasses of glutathione *S*-transferases have been identified; they are either homodimers or heterodimers with a molecular weight of about 50 kD. X-ray crystal structures have been solved for several glutathione *S*-transferases with substrates, products, and inhibitors.[10−18] From these crystal structures, an active site tyrosine residue has been implicated in catalysis. The primary function of this tyrosine is to stabilize the thiolate.

The reaction between glutathione and 1-chloro-2,4-dinitrobenzene (CDNB) is catalyzed by glutathione *S*-transferases; CDNB has been used widely to assay glutathione *S*-transferases. The nucleophilic conjugation of glutathione to CDNB is believed to follow a two-step S_NAr addition/elimination (or attachment/detachment) mechanism. Reactions of the S_NAr type usually proceed in solution via a σ-complex intermediate or Meisenheimer complex, named after its discover (Scheme 1).[8−9]

An early study with a series of 4-substituted 1-chloro-2-nitrobenzenes demonstrated that electron-withdrawing *para* substituents accelerate both the uncatalyzed and enzymatic reactions, confirming the nucleophilic nature of the reactions.[19]

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(1) Armstrong, R. N. *Adv. Enzymol. Relat. Areas Mol. Biol.* **1994**, *69*, 1.

(2) Dirr, H.; Reinemer, P.; Huber, R. *Eur. J. Biochem.* **1994**, *220*, 645.

(3) Wilce, M. C. J.; Parker, M. W. *Biochim. Biophys. Acta* **1994**, *1205*, 1.

(4) Rushmore, T. H.; Pickett, C. B. *J. Biol. Chem.* **1993**, *268*, 11475.

(5) Jakoby, W. B.; Ziegler, D. M. *J. Biol. Chem.* **1990**, *265*, 20715.

(6) Mannervik, B.; Danielson, U. H. *CRC Crit. Rev. Biochem.* **1988**, *23*, 283.

(7) Hayes, J. D.; Wolf, C. R. In *Glutathione Conjugation: Mechanisms and Biological Significance*; Sies, H., Ketterer, B., Eds; Academic Press: London, 1988; pp 316−356. Tsuchida, S.; Sata, K. *CRC Crit. Rev. Biochem. Mol. Biol*. **1992**, *27*, 337.

(8) (a) Bunnett, J. F.; Zahler, R. E. *Chem. Rev.* **1951**, *49*, 340. Bunnett, J. F.; Davis, G. T. *J. Am. Chem. Soc*. **1954**, *76*, 3011. Bunnett, J. F.; Snipes, R. F. *J. Am. Chem. Soc*. **1955**, *77*, 5422. Bunnett, J. F.; Garbisch, E. W., Jr.; Pruitt, K. M. *J. Am. Chem. Soc*. **1957**, *79*,385. Bunnett, J. F.; Merritt, W. D., Jr. *J. Am. Chem. Soc*. **1957**, *79*, 5967. (b) Miller, J. *Aromatic Nucleophilic Substitution*, Elsevier: Amsterdam, 1968.

(9) (a) Terrier, F. *Nucleophilic Aromatic Displacement: The Influence of the Nitro Group* (*Organic Nitro Chemistry Series)*; Feuer, H., Ed.; VCH Publishers: New York, 1991. (b) Chupakhin, O. N.; Charushin, V. N.; Plas, H. C. van der. *Nucleophilic Aromatic Substitution of Hydrogen*, Academic Press: San Diego, CA 1994.

(10) Reinemer, P.; Dirr, H. W.; Ladenstein, R.; Schaffer, J.; Gallay, O.; Huber, R. *EMBO J*. **1991**, *10*, 1997.

(11) Ji, X.; Zhang, P.; Armstrong, R. N.; Gilliland, G. L. *Biochemistry* **1992**, *31*, 10169. Xiao, G.; Liu, S.; Ji, X.; Johnson, W. W.; Chen, J.; Parsons, J. F.; Stevens, W. J.; Gilliland, G. L.; Armstrong, R. N. *Biochemistry* **1996**, *35*, 4753.

(12) Ji, X.; Armstrong, R. N.; Gilliland, G. L. *Biochemistry* **1993,** *32*, 12949.

(13) Sinning, I.; Gilliland, G. L.; Armstrong, R. N.; Ji, X.; Board, P. G.; Olin, B.; Mannervik, B.; Jones, T. A. *J. Mol. Biol*. **1993**, *232*, 192.

(14) Ji, X.; von Rosenvinge, E. C.; Johnson, W. W.; Tomarey, S. I.; Piatigorsky, J.; Armstrong, R. N.; Gilliland, G. L. *Biochemistry* **1995**, *34*, 5317. Lim, K.; Ho, J. X.; Keeling, K.; Gilliland, G. L.; Ji, X.; Ruker, F.; Carter, D. C. *Protein Science* **1994**, *3*, 2233. McTigue, M.; Williams, D. R.; Tainer, J. A. *J. Mol. Biol*. **1995**, *246*, 21. Wilce, M. C. J.; Board, P. G.; Feil, S. C.; Parker, M. W. *EMBO J*. **1995**, *14*, 2133.

(15) Garcia-Saez, I.; Parraga, A.; Phillips, M. F.; Mantle, T. J.; Coll, M. *J. Mol. Biol*. **1994**, *237*, 298.

(16) Dirr, H. W.; Reinemer, P.; Huber, R. *J. Mol. Biol*. **1994**, *243*, 72.

(17) Raghunathan, S.; Chandross, R. J.; Kretsinger, R. H.; Allison, T. J.; Penington, C. J.; Rule, G. S. *J. Mol. Biol*. **1994**, *238*, 815.

(18) Reinemer, P.; Prade, L.; Hof, P.; Neuefeind, T.; Huber, R.; Zettl, R.; Palme, K.; Schell, J.; Koelln, I.; Bartunik, H. D.; Bieseler, B. *J. Mol. Biol*. **1996**, *255*, 289.

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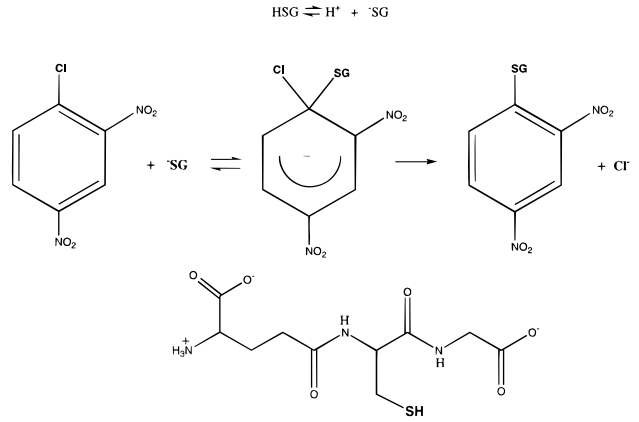
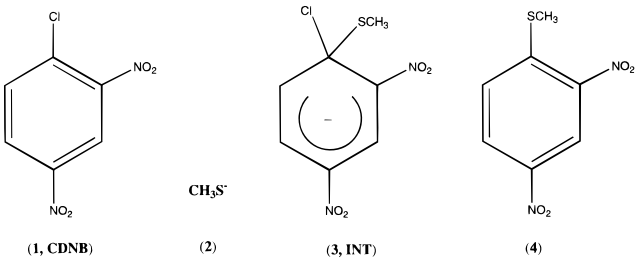
Scheme 1



Scheme 2



Subsequent analysis of substituent effects on reaction rates measured with substituted chlorobenzenes and leaving group effects are consistent with C−S bond formation being the rate-limiting step for k_{cat}.[20] The dead-end Meisenheimer complex formed between 1,3,5-trinitrobenzene and an enzyme-bound glutathione has been analyzed crystallographically.[21] This dead-end complex is very stable owing to the lack of a good leaving group. However, for a normal GST-catalyzed nucleophilic conjugation of GSH to a substituted haloaromatic compound,[20] it is not clear whether the Meisenheimer complex is a true intermediate or a transition state. Furthermore, even if the Meisenheimer complex is a true intermediate and can be isolated, these studies may not be able to reveal exactly how GSTs reduce the reaction barrier for nucleophilic aromatic substitution reactions. Before the mechanism of GST catalyzed reactions can be fully understood, it is necessary to better understand the uncatalyzed reaction. Here we report a detailed investigation of the reaction between glutathione and 1-chloro-2,4-dinitrobenzene in the gas phase and in solution.

Theoretical Procedure

In the present study, deprotonated glutathione is modeled using thiomethoxide (CH_3S^-, see Scheme 2). All gas-phase *ab initio* calculations were carried out using either GAUSSIAN 92[22] or GAUSSIAN 94[23] programs. Geometry optimizations were carried out at the HF/6-31G* and the HF/6-31+G** levels of theory. Energies were calculated at the MP2/6-31+G** level of theory using the HF/6-31+G** geometry. The inclusion of diffuse functions has been shown to be important for anionic species.[24] Stationary points on the potential energy surface were characterized by calculating force constants. Thermodynamic properties were calculated at 298 K using the calculated vibrational frequencies at the HF/6-31G* level of theory. Entropies were calculated using standard techniques.

The solvation free energy of each species involved in the reaction pathway was calculated (based on gas-phase geometry) using the PS-GVB program[25] at the HF/6-31G* level. The solvation free energy calculations involve *ab initio* quantum mechanical calculations coupled to a continuum solvent, which takes advantage of accurate charge distributions from quantum mechanical calculations and the success of numerical Poisson−Boltzmann methods.[26] We did not include corrections for basis set superposition error (BSSE) in the current study for several reasons. The normal counterpoise correction procedure can only provide an approximate estimate on the error.[27a] However, even for the water dimer there is still some debate regarding this issue.[27b] Sometimes, unrealistic results can occur after correction. For example, the alanine dimer gives an interaction energy of −19.7 kcal/mol, while after the counterpoise correction it becomes +14.4 kcal/mol.[27c]

Results and Discussion

Gas Phase. Advances in mass spectrometry and ion cyclotron resonance have allowed ion−molecule reactions to be investigated in detail in the gas-phase, which has made a great impact on studying gas-phase nucleophilic substitution reactions, e.g., S_N2 reactions.[28,29] Although there are numerous studies on S_N2 reactions,[28] few studies have considered nucleophilic aromatic substitution reactions.[29] The situation is similar for theoretical studies of gas-phase nucleophilic substitution reactions.[28,30] Several semiempirical (AM1 and MNDO) molecular orbital studies were reported for reactions between halobenzenes and nucleophiles such as methoxide ion (CH_3O^-) and fluoride.[30]

(19) Keen, J. H.; Habig, W. H.; Jakoby, W. B. *J. Biol. Chem.* **1976**, *251*, 6183.
(20) (a) Chen, W. J.; Graminski, G. F.; Armstrong, R. N. *Biochemistry* **1988**, *27*, 647. (b) Graminski, G. F.; Kubo, Y.; Armstrong, R. N. *Biochemistry* **1989**, *28*, 3562. (c) Graminski, G. F.; Zhang, P.; Sesay, M. A.; Ammon, H. L.; Armstrong, R. N. *Biochemistry* **1989**, *28*, 6252. (d) Huskey, S. -E. W.; Huskey, W. P.; Lu, A. Y. H. *J. Am. Chem. Soc.* **1991**, *113*, 2283.
(21) Ji, X.; Armstrong, R. N.; Gilliland, G. L. *Biochemistry* **1993**, *32*, 12949.
(22) *GAUSSIAN 92*: Frisch; M. J.; Trucks, G. W.; Head-Gordon, M.; Gill, P. M. W.; Wong, M. W.; Foresman, J. B.; Johnson, B. G.; Schlegel, H. B.; Robb, M. A.; Replogle, E. S.; Gomperts, R. ; Andres, J. L.; Raghavachari, K. ; Binkley, J. S. ; Gonzalez, C. ; Martin, R. L.; Fox, D. J. ; Defrees, D. J.; Baker, J.; Steward, J. J. P.; Pople, J. A. Gaussian, Inc.: Pittsburgh, PA, 1993.
(23) *GAUSSIAN 94*, Revision B.2: Frisch, M. J.; Trucks, G. W.; Schlegel, H. B. Gill, ; P. M. W. ; Johnson, B. G.; Robb, M. A.; Cheeseman, J. R.; Keith, T.; Petersson, G. A.; Montgomery, J. A.; Raghavachari, K.; Al-Laham, M. A.; Zakrzewski, V. G.; Ortiz, J. V.; Foresman, J. B.; Cioslowski, J. ; Stefanov, B. B.; Nanayakkara, A.; Challacombe, M.; Peng, C. Y.; Ayala, P. Y.; Chen, W.; Wong, M. W.; Andres, J. L.; Replogle, E. S.; Gomperts, R.; Martin, R. L. Fox, ; D. J.; Binkley, J. S.; Defrees, D. J.; Baker, J.; Stewart, J. P.; Head-Gordon, M.; Gonzalez, C.; Pople, J. A. Gaussian, Inc.: Pittsburgh, PA, 1995.
(24) Chandrasekhar, J.; Andrade, J. G.; Schleyer, P. v. R. *J. Am. Chem. Soc.* **1981**, *103*, 5609, 5612.
(25) *PS-GVB*, Version 2.3: Ringnalda, M. N.; Langlois, J.-M.; Murphy, R. B.; Greeley, B. H.; Cortis, C.; Russo, T. V.; Marten, B.; Donnelly, R. E., Jr.; Pollard, W. T.; Cao, Y.; Muller, R. P.; Mainz, D. T.; Wright, J. R.; Miller, G. H.; Goddard, W. A., III; Friesner, R. A. Schrödinger, Inc., 1996.
(26) Tannor, D. J.; Marten, B.; Murphy, R.; Friesner, R. A.; Stikoff, D.; Nicholls, A.; Ringalda, M.; Goddard, W. A., III; Honig, B. *J. Am. Chem. Soc.* **1994**, *116*, 11875.
(27) (a) Frisch, M. J.; Del Bene, J. E.; Binkly, J. S.; Schaefer, H. F., III. *J. Chem. Phys.* **1986**, *84*, 2279. Schwenke, D. W.; Truhlar, D. G. *J. Chem. Phys.* **1985**, *82*, 2418. (b) P.-O. Astrand, A.; Wallqvist, A.; Karlstrom, G. *J. Phys. Chem.* **1991**, *95*, 6395. Dannenberg, J. J.; Mezei, M. *J. Phys. Chem.* **1991**, *95*, 6396. (c) Cook, D. B.; Sordo, T. L.; Sordo, J. A. *J. Chem. Soc., Chem. Commun.* **1990**, 185.
(28) (a) Olmstead, W. N.; Brauman, J. I. *J. Am. Chem. Soc.* **1979**, *101*, 3715. Brauman, J. L.; Olmstead, W. N.; Lieder, C. A. *J. Am. Chem. Soc.* **1974**, *96*, 4030. Olmstead, W. N.; Brauman, J. I. *J. Am. Chem. Soc.* **1977**, *99*, 4219. (b) Shaik, S. S.; Schlegel, H. B.; Wolfe, S. *Theoretical Aspects of Physical Organic Chemistry: The S_N2 Mechanism*; Wiley: New York, 1992. (c) Minkin, V. I.; Simkin, B. Y.; Minyaev, R. M. *Quantum Chemistry of Organic Compounds: Mechanisms of Reactions*; Springer-Verlag: Berlin, 1990.
(29) Ingemann, S.; Nibbering, N. M. M. *J. Org. Chem.* **1983**, *48*, 183.
(30) (a) Simkin, B. Y.; Gluz, E. B.; Glukhovtsev, M. N.; Minkin, V. I. *J. Mol. Struct. (THEOCHEM)* **1993**, *284*, 123. (b) Dotterer, S. K.; Harris, R. L. *J. Org. Chem.* **1988**, *53*, 777. (c) Bacaloglu, R.; Bunton, C. A.; Ortega, F. *J. Am. Chem. Soc.* **1989**, *111*, 1041.

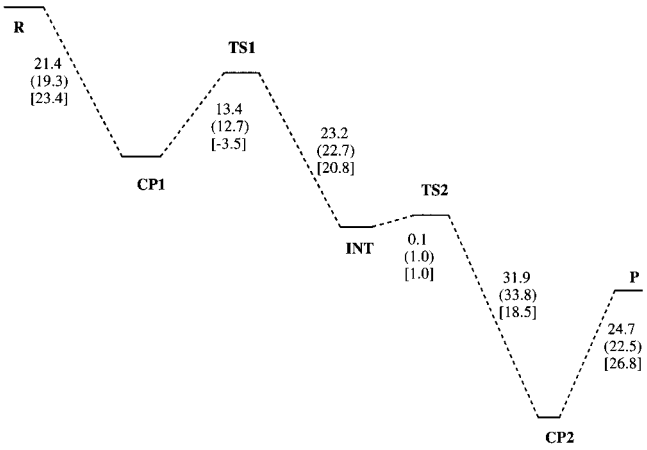


Figure 1. The calculated potential energy surface at HF/6-31G*, HF/6-31+G** (in parentheses), and MP2/6-31+G** (in square brackets) levels.

Table 1. Calculated Total Electronic Energies (au)

compound	HF/6-31G*	HF/6-31+G**	MP2/6-31+G**
CH_3S^-	−437.114 863 5	−437.131 656 3	−437.409 528 1
CDNB	−1096.521 297 1	−1096.544 794 1	−1098.545 583 5
CP1	−1533.670 320 6	−1533.707 197 6	−1535.992 406 6
TS1	−1533.648 947 7	−1533.686 963 8	−1535.998 008 5
INT	−1533.685 878 2	−1533.723 187 9	−1536.031 221 8
TS2	−1533.685 761 7	−1533.721 556 1	−1536.029 637 5
CP2	−1533.736 642 2	−1533.775 423 8	−1536.059 157 8
thioether (**4**)	−1074.171 255 0	−1074.199 833 9	−1076.345 314 3
Cl^-	−459.525 996 9	−459.539 660 9	−459.671 145 4

According to these studies, in some reactions, a Meisenheimer complex is found to be a real intermediate, while in others it is a transition state. Since there is no reliable experimental data to compare, it is difficult to assess the quality of these calculations. The study of gas-phase nucleophilic aromatic substitution reactions is usually complicated by competing reactions. Previous study of the reaction between fluorobenzene and methoxide ion indicated that there are competing proton transfer and S_N2 reactions; in some cases, the nucleophilic aromatic substitution reaction is not even the dominant reaction.[29] Here we examine the gas-phase nucleophilic aromatic substitution reaction between 1-chloro-2,4-dinitrobenzene and thiolate (CH_3S^-) using *ab initio* molecular orbital theory. Table 1 lists the calculated total electronic energy of each species involved in this study at different levels of theory.

First, we searched the potential energy hypersurface at the HF/6-31G* level of theory. We found that when CDNB and thiolate approach each other, an ion−molecule complex is formed. Addition of thiolate at the C1 position of CDNB results in a Meisenheimer complex via a transition state (TS1), while breaking the C−Cl bond in the Meisenheimer complex gives rise to another ion−molecule complex. The calculated potential energy hypersurface is shown in Figure 1. Since anions are involved in this reaction, we also repeated the above calculations at the HF/6-31+G** level. The calculated potential energy surface is very similar to that at the HF/6-31G* level (see Figure 1); Figure 2 shows the calculated structures.

At both HF/6-31G* and HF/6-31+G** levels of theory, there are no barriers for the overall reaction; both transition states are lower in energy than the sum of isolated CDNB and thiomethoxide. Both levels of theory predict a significant barrier from the initial ion−molecule complex, with the barrier being 13.4 and 12.7 kcal/mol at the HF/6-31G* and HF/6-31+G** levels, respectively. The present calculations also demonstrate that the barrier for formation of the Meisenheimer complex is higher than the breakdown of the Meisenheimer complex, which is in agreement with previous experimental observations in solution.[20] Of course, the presence of water molecules in solution could change the reaction profile (see below). Another interesting question is about the stability of the Meisenheimer complex. Is it stable enough to be qualified as a true intermediate in a typical nucleophilic aromatic substitution reaction? In cases where a good leaving group is not present, Meisenheimer complexes have been isolated and characterized. It is not clear whether it could be isolated in reactions between chlorobenzene and methoxide (or thiomethoxide).

The presence of ion−molecule complexes in gas-phase S_N2 reactions is well established. The present calculations have indicated the presence of ion−molecule complexes in nucleophilic aromatic substitution reactions. The presence of an ion−molecule complex in the gas-phase reaction between fluorobenzene and methoxide was not directly demonstrated by mass spectrometry, instead it was only assumed. However, the formation of $F^-(C_6H_6)$, $Cl^-(C_6H_6)$, and $F^-(C_6F_6)$ has been demonstrated and the stabilities of these complexes were determined using a pulsed electron beam mass spectrometer.[31] The measured formation enthalpies for these complexes are −15.3, −9.4, and −27.5 kcal/mol, respectively. In the case of F^- (C_6H_6) and Cl^- (C_6H_6), the complexes are loosely bound ion−molecule complexes, while in the case of $F^-(C_6F_6)$, it is believed to be a σ-complex (Meisenheimer complex).

Not surprisingly, according to our calculations, in both ion−molecule complexes, the anion lies in the plane of the aromatic ring, which is consistent with the charge-dipole interaction. Previous MNDO and AM1 studies also located charge-transfer complexes, in which the nucleophile lies above the aromatic ring.[30b,c] However, when a chloride ion is placed above the aromatic ring of the thioether (**4**), energy minimization at the HF/6-31G* level leads again to a normal ion−molecule complex, probably suggesting that the charge-transfer complex may not exist as a minimum on the potential energy hypersurface. No further attempt was made to search for such complexes. Inclusion of electron correlation effects at the MP2/6-31+G** level has a dramatic effect on the potential energy surface. The most noticeable change is the relative energy between the initial ion−molecule complex and the first transition state. At the MP2/6-31+G** level, the barrier separating the first ion−molecule complex and the Meisenheimer intermediate disappears, indicating that formation of the Meisenheimer complex from CDNB and thiolate (CH_3S^-) is spontaneous. However, the second ion−molecule complex still exists at the MP2/6-31+G** level. Since this reaction is very exothermic and the reverse barrier from the second ion−molecule complex is high, it would be very easy to study this ion−molecule complex (starting with chloride ion and the thioether) using mass spectrometry. It is possible that the MP2/6-31+G** level of theory may overestimate the stability of TS1 relative to CP1, causing a large change in relative energy between the transition state and the ion−molecule complex. It would be desirable to repeat the above calculations at a higher level of theory and relax the geometries at the same level of theory to test the reliability of the MP2/6-31+G**//HF/6-31+G** results. Unfortunately, we cannot presently perform these calculations owing to the large size of the system.

In the ion−molecule complex formed between thiomethoxide and CDNB, the negatively charged sulfur atom of thiomethoxide forms two S^----H-C hydrogen bonds. The S---H distances are about 2.7−3.0 Å. In the complex formed between chloride and the thioether product, the chloride ion interacts with the thioether

(31) Hiraoka, K.; Mizuse, S.; Yamabe, S. *J. Chem. Phys.* **1987**, *86*, 4102.

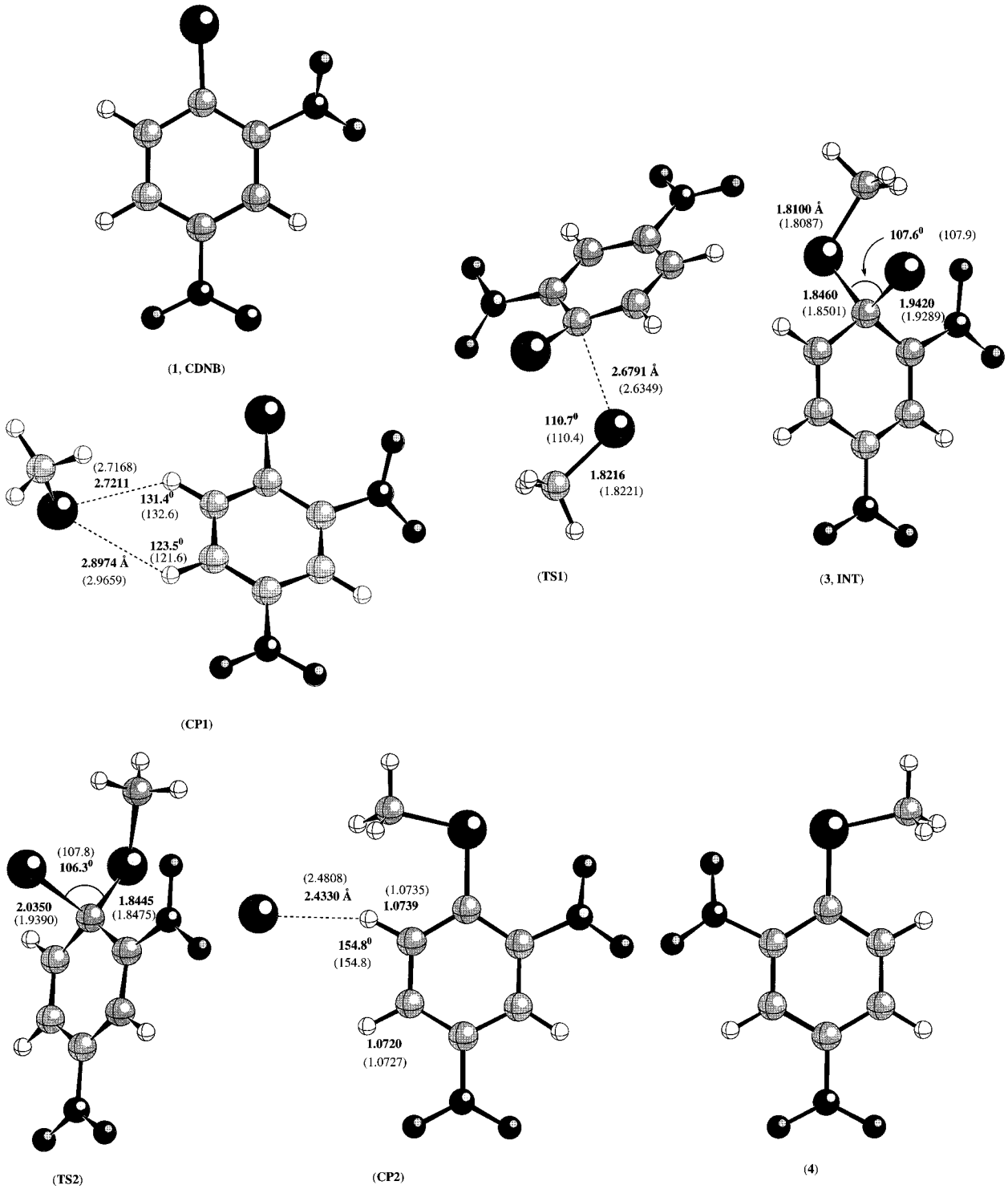


Figure 2. Calculated geometries for compounds involved in this study at the HF/6-31G* and HF/6-31+G** (in parentheses) levels of theory.

through one of the aromatic hydrogen atoms; the Cl---H distance and Cl---H-C angle are about 2.4−2.5 Å and 155°, respectively. It is interesting to note that in the Meisenheimer complex the six-membered ring is still planar, indicating the presence of strong hyperconjugation between the π-orbital and the σ^*-antibonding orbitals of the C−Cl and C−S bonds. The presence of strong hyperconjugation is also evident in the unusually long C−Cl and C−S bonds as shown in Figure 2.

We also calculated the free energy surface in the gas-phase at the HF/6-31+G** and MP2/6-31+G** levels. Thermal corrections and entropies were calculated using the harmonic vibrational frequencies calculated at the HF/6-31G* level (see Table 3). Figure 3 shows the free energy surface at the HF/6-31+G** and MP2/6-31+G** levels. Again, there is a significant difference between these two levels of theory concerning the relative stability between complex **1** (CP1) and TS1. Since formation of ion−molecule complexes is entropically unfavored, the minima at the ion−molecule complexes are shallower on the free energy surfaces. The calculated enthalpy and free energy of formation of CP2 from an isolated chloride ion and **4** are −22.2 (−26.5) and −16.1 (−20.4) kcal/mol, respectively, at the HF/6-31+G** (MP2/6-31+G**) level. At the HF level, the calculated overall free energy barrier is about 4 kcal/mol, while there is no barrier at the MP2 level.

Aqueous Solution. Like all nucleophilic substitution reactions that involve charged species, it is expected that solvation

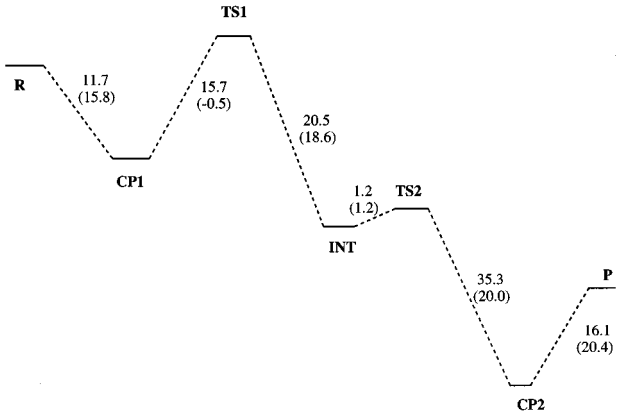


Figure 3. The calculated free energy surface in the gas-phase at the HF/6-31+G** level (MP2/6-31+G** level, in parentheses).

Table 2. Calculated and Experimental Solvation Free Energies

compound	solvation free energy (calcd, kcal/mol)	exptl
CH_3S^-	−79.2	
CDNB	−6.3	
CP1	−66.6	
TS1	−59.3	
INT	−48.9	
TS2	−48.5	
CP2	−66.2	
thioether (**4**)	−8.2	
Cl^-	−79.3	−76

Table 3. Calculated Thermal Corrections and Entropy for Each Species at the HF/6-31G* Level under Standard Conditions (298 K and 1 atm)

compound	*E* (thermal, kcal/mol)	*S* (cal/(mol·K))
CH_3S^-	26.247	55.737
CDNB	72.094	101.698
CP1	100.191	136.372
TS1	99.166	123.128
INT	100.016	118.631
TS2	99.438	115.896
CP2	101.092	126.406
thioether (**4**)	99.303	110.209
Cl^-	0.889	36.586

will have a dramatic effect on nucleophilic aromatic substitution reactions.[32] Since the hydration free energies for small anions like Cl^- are much larger than the free energy of formation of these ion−molecule complexes in the gas phase, in aqueous solution these ion−molecule complexes may not exist. To estimate the solvation effect on this nucleophilic aromatic substitution reaction, one needs to calculate the solvation free energy for each species involved. Geometry optimization and searching for a transition state in aqueous solution require an enormous amount of computer resources for the molecules considered in the present study. We, thus, did not attempt to do geometry optimization; instead, we only estimated the solvation free energy based on the gas-phase geometry. These calculations were done using a combined *ab initio* molecular orbital theory with a dielectric continuum solvent model, as implemented in the PS-GVB program at the HF/6-31G* level. Table 2 lists the calculated solvation free energy and available experimental values.

The calculated solvation free energy for a chloride ion is about 3 kcal/mol larger than the experimental value[33] (−79 vs −76 kcal/mol); for CH_3S^-, we could not find an experimental value, however, the solvation free energy for HS^- is known (−76 kcal/mol). The calculated value for CH_3S^- is comparable with the experimental value for HS^-. Given the fact that in PS-GVB solvation calculations only one parameter per atom is used and also the parameters for chloride and the thiolate are not specifically tuned (general parameters for normal neutral organic compounds were used), these results seem excellent. For the neutral species involved in this study, 1-chloro-2,4-dinitrobenzene and the thioether product, we also could not find experimental values; however, experimental solvation free energy data are available for related compounds such as chlorobenzene and nitrobenzene.[32] The calculated solvation free energies using the gas-phase HF/6-31G* geometry are −1.0 and −3.9 kcal/mol, respectively; the corresponding experimental values are −1.1 and −4.1 kcal/mol, respectively. Clearly, the solvation model implemented in PS-GVB is very good. However, it should be pointed out that this method has not been tested on transition states and molecular complexes. As the reaction proceeds toward the intermediate, the negative charge gets delocalized and the solvation free energy becomes smaller. In the second step of the reaction, the situation reverses and solvation becomes larger again as the reaction proceeds toward product. The overall free energy profile for this nucleophilic aromatic substitution reaction in aqueous solution is constructed by adding the solvation free energy to the gas-phase free energy profile; the resulting free energy profile is shown in Figure 4. For comparison, we constructed free energy surfaces at two levels of theory (HF/6-31+G** and MP2/6-31+G**).

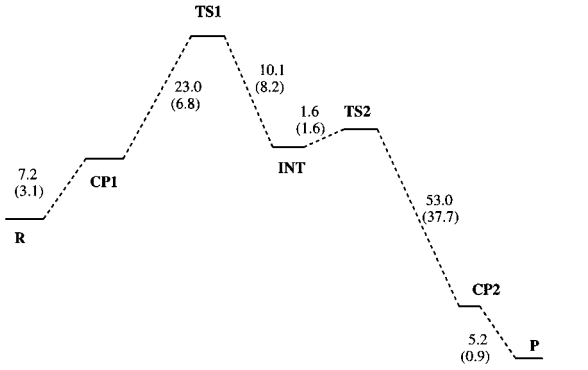


Figure 4. Calculated free energy surface in aqueous solution at the HF/6-31+G** level (MP2/6-31+G** level, in parentheses).

First, as expected, minima for the two ion−molecule complexes vanished in aqueous solution. There is a barrier for this nucleophilic aromatic substitution reaction in aqueous solution. Depending on the level of theory, the barrier varies from about 10 kcal/mol to about 30 kcal/mol. Second, the intermediate still exists and it is still shallow on the free energy surface, which could deepen when geometrical relaxation is allowed. As in the gas-phase, the solution reaction is also exothermic, which is expected since both the chloride ion and thiomethoxide have similar solvation free energy; thus the solvation effect on equilibrium is very small. Most importantly, both methods indicate that the rate-determining step for this reaction is the formation of the C−S bond, and again this is in agreement with experiments.[20] Since the experimental free energy barrier for this reaction is not known, it is difficult to assess the accuracy of the present calculations. However, the barrier for the uncatalyzed reaction between CDNB and glutathione was estimated to be about 23.8 kcal/mol in aqueous solution.[20d] Our calculated barriers bracket this estimated experimental value. The MP2/6-31+G** free energy barrier is too low, while the

(32) Jorgensen, W. L. *Acc. Chem. Res.* **1989**, *22*, 184.

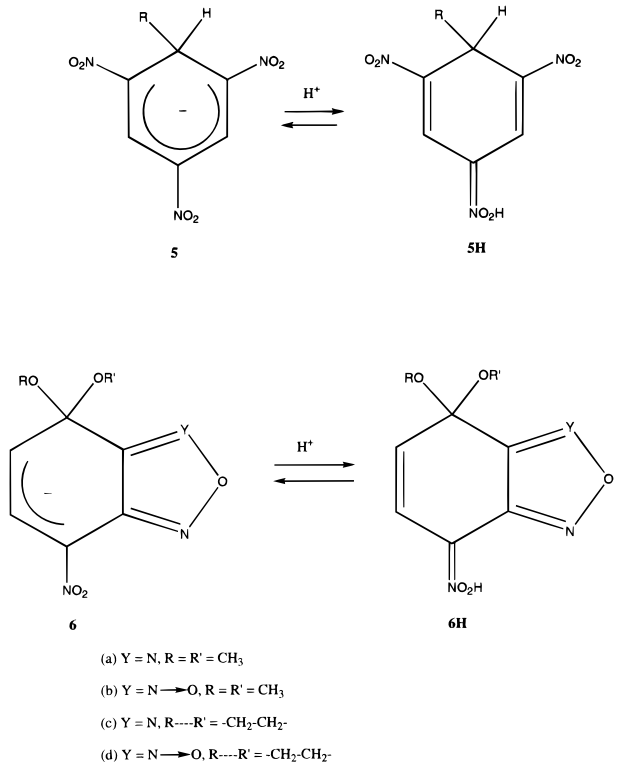
(33) For experimental details see: Carlson, H. A.; Jorgensen, W. L. *J. Phys. Chem*. **1995**, *99*, 10667. Cramer, C. J.; Truhlar, D. G. *J. Computer-Aided Mol. Design* **1993**, *6*, 629.

HF/6-31+G** barrier seems to be in good agreement with the experimentally estimated value of about 23.8 kcal/mol.[20d] This is very encouraging in view of the simplicity of the solvation model and the fact that the van der Waals parameters were not specifically adjusted for reaction pathway calculations.

One obvious source of error is the use of gas-phase geometries in solvation calculations. Although geometrical relaxation for reactants and products may be small, it is expected to be large for ion−molecule complexes, the Meisenheimer intermediate, and transition states. The procedure we employed probably underestimates the solvation free energy for the intermediate; therefore, the stability of the intermediate could be higher than indicated by this procedure. A second potential problem is also associated with geometrical relaxation, namely, the possible shift of the transition state. In the presence of solvent, the location of the transition state on the potential energy surface could change, which will affect the calculated barrier. These errors can be eliminated by relaxing the geometries. However, this is computationally very expensive, especially for locating a transition state in solution. Another factor is that the parameters used in the solvation calculations were not specifically parameterized for this reaction. The calculated free energy of solvation for a chloride ion, for example, is off by about 3 kcal/mol.

Enzymatic Reaction. Our study has shown that (a) in the gas-phase the nucleophilic aromatic substitution reaction of CDNB with the deprotonated glutathione (as modeled by thiomethoxide) does not require much activation energy, and (b) in aqueous solution there is a large barrier for this reaction due to solvation effects. The obvious way for GSTs to reduce the reaction barrier is to provide a nonaqueous environment, where the reaction can occur with little activation energy. The caveat is that the thiolate (deprotonated glutathione) must first be generated. In aqueous solution, the pK_a of the thiol group of glutathione is about 9.0,[1−3] so at neutral pH, the concentration of thiolate ion is very low. Glutathione probably binds to the enzyme not as a thiolate, but as a thiol. Clearly, the enzyme has to provide a mechanism to make the generation of thiolate feasible. This is exactly what GSTs do in general; the thiol group of glutathione when bound to GST has a much lower pK_a (between 6 and 7). X-ray crystallographic studies[1−3] and theoretical calculations[34] suggested that the thiolate in the GST active site is stabilized by hydrogen bonding via the hydroxyl group of tyrosine. It should be pointed out that the presence of this hydrogen bonding interaction stabilizes the thiolate, facilitating the generation of thiolate, but it also slows down the subsequent chemical reaction since the nucleophilicity of thiolate is reduced by virtue of this hydrogen bond. The hydrogen bonding interaction between Tyr-O-H---$^-$SG is expected to become weaker as the reaction proceeds. Such differential "solvation" (or hydrogen bonding) of the reactant and transition state could lead to a deceleration of the reaction relative to the gas-phase. Experimental studies on S_N2 reactions indicate that the presence of even a single solvent molecule to the ionic reactant could significantly increase the activation energy.[35] Whether GSTs stabilize the intermediate still requires further investigation. However, since GSTs normally have such broad substrate specificity and each GST can detoxify many different kinds of compounds, it is unlikely that these enzymes have evolved an effective mechanism to tightly bind each of these different compounds; the fact that GSTs do not have a well-defined hydrophobic binding site is consistent with this notion.

Scheme 3



In the X-ray crystal structure of a class *mu* GST with bound Meisenheimer complex, three active site amino acid residues (Tyr6, Tyr115, and Arg107) and one crystallographic water were found to be close to the six-membered ring of the Meisenheimer complex. The two tyrosine residues (Tyr6 and Tyr115) are hydrogen bonded to the *o*-nitro group in the intermediate, while Tyr6 is hydrogen bonded to the thiolate of glutathione in the reactant. Arg107 is too far away to form a hydrogen bond with the Meisenheimer complex directly, but it could form a water-mediated one. The crystallographic water forms a hydrogen bond with the *p*-nitro group of the six-membered ring of the intermediate. Otherwise, there are no other polar groups nearby to provide any stabilization. The closest α-helix is still too far away to provide significant stabilization. Since a crystal structure of the ternary complex (E·GSH·CDNB) is not available, it is not clear whether the hydrogen bond between Tyr115 and the *o*-nitro group and the hydrogen bond between the *p*-nitro group and the crystallographic water are present in the initial complex or not. If they do and if they contribute equally to the stabilization of reactant and transition state, the impact of these groups on catalysis will be small. Therefore, it is not immediately clear from the X-ray crystal structure of a class *mu* GST with bound Meisenheimer complex how glutathione *S*-transferases accelerate the glutathione conjugation reaction. However, it is known experimentally that the anionic nitro-substituted cyclohexadienylide structures can be protonated to form nitronic acids. Typical examples of these nitronic acids are **5H** and **6H** as shown in Scheme 3 which have been characterized using experimental techniques such as UV−vis and/or NMR spectroscopy.[36] The crystallographic water in the Meisenheimer complex may play a similar role as the proton

(34) Zheng, Y.-J.; Ornstein, R. L. *J. Am. Chem. Soc.* In press. Liu, S.; Ji, X.; Gilliland, G. L.; Stevens, W. J.; Armstrong, R. N. *J. Am. Chem. Soc.* **1993**, *115*, 7910.

(35) O'Hair, R. A. J.; Davico, G. E.; Hacaloglu, J.; Dang, T. T.; DePuy, C. H.; Bierbaum, V. M. *J. Am. Chem. Soc.* **1994**, *116*, 3609. van der Wel, H.; Nibbering, N. M. M.; Sheldon, J. C.; Hayes, R. N. Bowie, J. H. *J. Am. Chem. Soc.* **1987**, *109*, 5823. Henchman, M.; Paulson, J. F.; Hierl, P. M. *J. Am. Chem. Soc.* **1983**, *105*, 5509. Bohme, D. K.; Mackay, G. I. *J. Am. Chem. Soc.* **1981**, *103*, 978.

(36) Moutiers, G.; Fahid, B. E.; Collot, A.-G.; Terrier, F. *J. Chem. Soc., Perkin Trans. 2*, **1996**, 49.

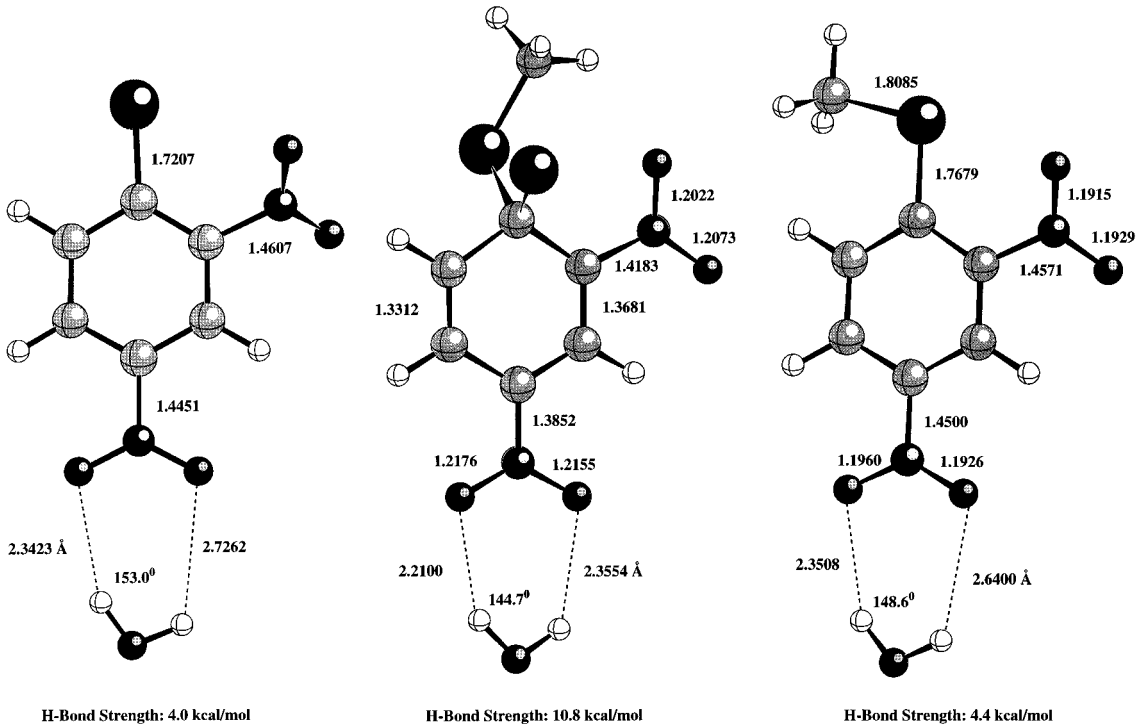


Figure 5. Calculated geometries and interaction energies for the hydrogen bonded complexes formed between CNDB (**1**), INT (**3**), thioether (**4**), and one water molecule at the HF/6-31G* level of theory.

in compounds **5H** and **6H**. If this is the case, this would be a very elegant mechanism by which glutathione *S*-transferases selectively stabilize the intermediate (or transition state) of the glutathione conjugation reaction.

To examine the potential role of such a crystallographic water, we first calculated hydrogen bonding interactions between a water molecule with CDNB (reactant **1**), the Meisenheimer intermediate (**3**), and the thioether (product **4**) (see Scheme 2). Figure 5 shows the calculated geometries and hydrogen bond strengths for the complexes formed between reactant, intermediate, and product with a water molecule at the HF/6-31G* level of theory. It is clear that the hydrogen bonding is the strongest in the intermediate. This differential hydrogen bonding will stabilize the intermediate state by about 6 to 6.4 kcal/mol with respect to the reactant and product, respectively. In the active site of the enzyme, there is an Arg (Arg107) near this important water and the water molecule is hydrogen bonded to Arg107 and Gln165, which could further polarize the water and make the hydrogen bonding between the intermediate and water even stronger. Clearly, this provides a very elegant way to selectively stabilize the intermediate state.

As expected, the presence of a water molecule causes little structural perturbation to CDNB and the thioether product since both of them are neutral aromatic species. The hydrogen bonding between a water molecule and either of them will be relatively weak. However, for the Meisenheimer intermediate, the situation is completely different for two reasons: (a) the intermediate is negatively charged and (b) the aromaticity is destroyed. The interaction between a water and the intermediate is very strong; there is a significant structural change in the cyclohexadienylide structure and the structure is best described as a nitronate. In the presence of a water molecule, the bond between the nitrogen of the *p*-nitro group and the C-4 becomes a double bond. A similar mechanism for selectively stabilizing the intermediate (or transition state) also seems to be employed by 4-chlorobenzoyl CoA dehalogenase.[37] Such differential "solvation" has also been observed experimentally in some gas-phase S_N2 reactions.[38]

In the gas-phase calculations, two ion−molecule complexes were found on the potential energy surface at the HF level of theory. An unanswered question is what is the relevance of these structures to the enzymatic reaction. First, in the active site of the enzyme, the geometrical constraint may not allow the formation of such complexes. Second, because of the presence of polar groups in the active site, both Cl^- and the thiolate anion (of the deprotonated glutathione (GS^-)) can form hydrogen bonds. For instance, it is known that the thiolate (GS^-) is hydrogen bonded to Tyr6 before the reaction. Therefore, it is quite clear that these gas-phase ion−molecule complexes play little role in the enzymatic reaction.

Tetrachloro-*p*-hydroquinone Reductive Dehalogenase (TeCH-RD). Recently, it was proposed that the enzyme involved in the biodegradation pathway of pentachlorophenol, tetrachloro-*p*-hydroquinone reductive dehalogenase (TeCH-RD), is also a glutathione *S*-transferase.[39] This enzyme catalyzes the removal of a chlorine atom from tetrachloro-*p*-hydroquinone. The reaction is shown in Scheme 4. It is worthwhile to examine the reaction mechanism in light of the current study. Naturally, if TeCH-RD is a GST, the mechanism should be similar. A possible mechanism involving an S_NAr mechanism is shown below:

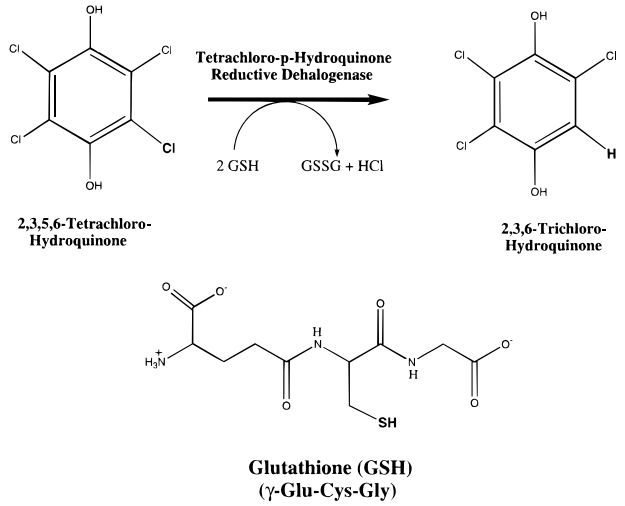
$$\text{Ar-Cl} + {}^-\text{SG} \rightarrow \text{Ar-SG} + \text{Cl}^- \quad (1)$$

$$\text{Ar-SG} + \text{HSG} \rightarrow \text{Ar-H} + \text{GSSG} \quad (2)$$

where Ar−Cl refers to tetrachloro-*p*-hydroquinone. While step 1 is a normal S_NAr reaction, the second step does not seem to be a fast reaction. It is difficult to see how the enzyme catalyzes the second step of this reaction if it is catalyzed by the enzyme at all. Although CDNB is normally a good substrate for GST, it is not a substrate for TeCH-RD. This fact may indicate that

(37) Taylor, K. L.; Liu, R.-Q.; Liang, P.-H.; Price, J.; Dunaway-Mariano, D.; Tonge, P. J.; Clarkson, J.; Carey, P. R. *Biochemistry* **1995**, *34*, 13881.
(38) Craig, S. L.; Brauman, J. I. *J. Am. Chem. Soc.* **1996**, *118*, 6786.
(39) Orser, C. S.; Dutton, J.; Lange, C.; Jablonski, P.; Xun, L.; Hargis, M. *J. Bacteriol.* **1993**, *175*, 2640.

Scheme 4



there are some differences between the mode of action of TeCH-RD and other GSTs.

Although thiolates are very powerful nucleophiles, they are also strong reducing agents. The chances for involvement of free radicals are high in nucleophilic reactions between aromatic compounds and thiolates. The reaction of isomeric chloronitrobenzene with thiolates (RSNa, R= Me, 2-Pr, *t*-Bu, Ph), for example, could produce RS−SR and nitrobenzene, but nitro reduction was also detected for *m*-chloronitrobenzene.[40] Therefore, an alternative mechanism for TeCH-RD which involves radicals is also a possibility:

$$\text{Ar-Cl} + {}^{-}\text{SG} \rightarrow (\text{Ar-Cl})^{-\bullet} + \text{SG}^{\bullet}$$

$$(\text{Ar-Cl})^{-\bullet} \rightarrow \text{Ar}^{\bullet} + \text{Cl}^{-}$$

$$\text{Ar}^{\bullet} + \text{HSG} \rightarrow \text{Ar-H} + \text{SG}^{\bullet}$$

$$\text{SG}^{\bullet} + \text{SG}^{\bullet} \rightarrow \text{GSSG}$$

Here the function of TeCH-RD again is to facilitate the generation of thiolate. Of course, combination of radical $\text{Ar}^{\bullet}$ and $\text{SG}^{\bullet}$ could also generate Ar-SG. ESR techniques could be used to investigate the possible involvement of this alternative pathway. The mechanism of uncatalyzed and enzymatic reactions of tetrachloro-*p*-hydroquinone with glutathione is largely unknown.

Conclusions

Ab initio molecular orbital theory in combination with a continuum solvent model was used to examine the nucleophilic aromatic substitution reaction of 1-chloro-2,4-dinitrobenzene with glutathione in the gas-phase and in solution. First, gas-phase calculations at different HF levels show that, when CDNB and thiomethoxide approach each other, an ion−molecule complex is formed. Attack by thiomethoxide on CDNB at C1 generates a Meisenheimer intermediate via a transition state, and breaking of the C-Cl bond in the intermediate results in the formation of a second ion−molecule complex via a smaller barrier before reaching the isolated chloride ion and thioether product. Inclusion of electron correlation effects changes the reaction profile, with the most significant change being the relative energy between the first ion−molecule complex and the first transition state; whether the MP2 calculation overestimates the stability of the first transition state relative to the first ion−molecule complex is not known and may deserve further investigation. The enthalpy and free energy of formation for the second ion−molecule complex from an isolated chloride ion and the thioether (**4**) are predicted to be −22.2 (−26.5) and −16.1 (−20.4) kcal/mol, respectively, at the HF/6-31+G** (MP2/6-31+G**) level.

The free energy profile for this reaction in aqueous solution has also been calculated at the HF/6-31+G** and MP/6-31+G** levels of theory. The formation of the C-S bond is calculated to be the rate-determining step, which is in agreement with experimental observations.[20] Again, there is an intermediate in this reaction as in the gas-phase. The calculated free energy barriers are 30.2 and 9.9 kcal/mol at HF/6-31+G** and MP2/6-31+G** levels of theory, respectively. The experimentally estimated free energy barrier for the reaction of CDNB with glutathione in solution is about 23.8 kcal/mol, which is higher than the MP2 value, but lower than the HF/6-31+G** value. In view of the possible errors in the calculated solvation free energy of thiomethoxide, the HF/6-31+G** barrier seems to be in excellent agreement with the experimental barrier.

The mode of action of glutathione *S*-transferases in general and TeCH-RD in particular was also discussed in light of the current study in the gas-phase and in solution. It is proposed that the main function of GSTs is to facilitate the generation of thiolate and provide a nonaqueous environment for the reaction. Selective stabilization of the Meisenheimer intermediate (or transition state) is provided by an active site water. As far as TeCH-RD is concerned, although it is proposed that TeCH-RD is a GST,[39] the reaction mechanism seems to be different. Whether TeCH-RD follows a normal S_NAr mechanism or an alternative pathway like a free radical process is not clear and requires detailed chemical and biochemical investigations.

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(40) Montanari, S.; Paradisi, C.; Scorrano, G. *J. Org. Chem.* **1993**, *58*, 5628 and references cited therein.