presumably will be an underestimate, since the calculation has ignored the entropic contributions of the rotating hydroxyl groups, which may be more constrained in the $^1C_4$ conformer, and the greater frequency of conformational transitions of C4–C5–C6–O6 in the $^4C_1$ conformation has not been included. The number also applies only to the molecule in vacuo, not in aqueous solution. Nonetheless, it is in agreement with the experimental observation that the $^4C_1$ conformer is preferred in nature, and with the number estimated for this and similar surfaces using energy minimization studies.[10,13] Angyal[27] calculated a value of –4.15 kcal/mol for this transition in aqueous solution, and Dunfield and Whittington[28] have estimated the vacuum value to be –0.97 kcal/mol. While the entropic contribution is small compared to the enthalpic term (only 13.5% of the total free energy difference), it is not necessarily negligible, as assumed in ref 13.

This method was also used to compute the configurational entropy difference between the gauche (–60°) and anti orientations for the exocyclic dihedral C4–C5–C6–O6 for the $^4C_1$ conformation. Since only about 38 and 55% as much trajectory time, respectively, is available for averaging for these two conformers, the statistical convergence of the resulting number will be correspondingly poorer. It should also be remembered that since the anti conformer is at a lower potential energy, it should be at a higher temperature in a fixed-energy simulation. In these simulations, the anti conformer was approximately 1.8 K hotter than the gauche (–60°) conformer. Using eq 4, the entropy change in going from gauche to anti was estimated to be –0.43 cal/mol·K, or –0.13 kcal/mol at 300 K. This result, if correct in sign, is somewhat surprising, since an intramolecular hydrogen bond might be expected to restrict internal fluctuations. It is clear that the configurational entropy difference between these two states would be affected by the presence of solvent.

IV. Conclusions

These preliminary trajectory studies demonstrate the utility of molecular dynamics simulations in the study of carbohydrate structure, and indicate the need for similar calculations of such molecules in aqueous solution. The most developed of the proposed potential energy surfaces for glucose was tested and found to adequately describe the molecule's motions in vacuo, with small but possibly significant deviations from the crystal geometry. Far from being rigid, these pyranose rings were found to exhibit considerable flexibility, and even to undergo conformational transitions. The relative probabilities of the three major exocyclic group conformers calculated from these trajectories are not in quantitative agreement with the limited experimental data available, but do seem to support previous models of intramolecular hydrogen bonding opposed by unfavorable "syn-axial" interactions. The results of trajectory simulations were also used to estimate the free energy for conformation transition for glucose in vacuo, and to follow the time course of such transitions. These results can be used to evaluate the conformational effects of solvation when solution calculations, currently beginning, are completed.

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Mechanism of the Photodissociation of *s*-Tetrazine: A Unimolecular Triple Dissociation

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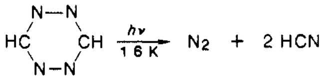
Abstract: The triple dissociation of *s*-tetrazine to HCN + HCN + N_2 has been investigated by ab initio molecular electronic structure theory. The predicted activation energy along the ground-state potential energy hypersurface is 47 kcal, consistent with a one-photon excitation to S_1 (51.8 kcal), followed by radiationless transition to vibrationally excited S_0, and finally by unimolecular triple dissociation.

The symmetric tetraaza-substituted benzene, *s*-tetrazine, has been of interest to chemists for nearly 80 years, with the original studies of this red crystalline heterocycle carried out by Curtius, Darapsky, and Müller[1] in 1907 and Koenigsberger and Vogt[2] in 1913. Since that time much effort has been devoted to the characterization of the ground and excited electronic states of *s*-tetrazine. These studies have led in turn to the experimental examination of the dynamics and dissociative photochemistry of electronically excited *s*-tetrazine.

Perhaps most fascinating among the various photochemical findings for *s*-tetrazine is the appearance of very simple dissociation products, namely, molecular nitrogen and hydrogen cyanide. This was first suggested in the 1975 paper by Hochstrasser and King[3] who observed in mixed crystal systems the reaction



(1)

Three months later, Karl and Innes[4] independently reported the same photochemical reaction in the gas phase. Clearly the mechanism for this decomposition of a medium-sized molecule to three small molecules is of interest, and subsequent studies by Hochstrasser and coworkers pursued this point.[5,6] Other aspects

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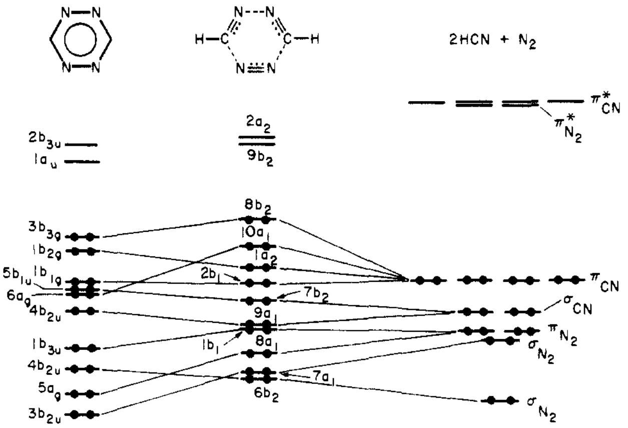
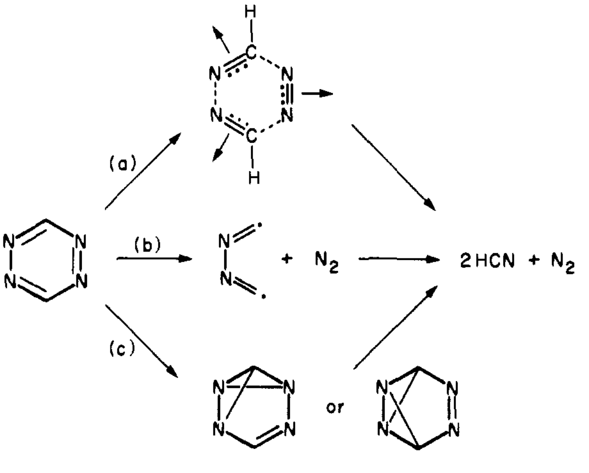


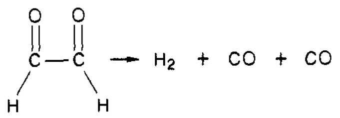
Figure 1. Molecular orbital correlation diagram for the unimolecular triple dissociation (eq 2a) of *s*-tetrazine.

of the *s*-tetrazine triple dissociation have been examined in the laboratory by the groups of Pacansky,[7] Wittig,[8] Schlag,[9] Rentzepis,[10] Wiersma,[11] and Riley.[12]

In 1977 King, Denny, Hochstrasser, and Smith[5] reported experiments which appeared to eliminate all but three mechanisms for the photochemical decomposition of *s*-tetrazine. The three remaining plausible mechanisms are shown in eq 2. Of these the triple dissociation (2a) is perhaps the most intriguing, although Glownia and Riley suggest that it is an unlikely mechanism.[12] Unimolecular reactions ABC → A + B + C involving a single transition state are quite rare, although just such a mechanism has recently been established[13] for the photodissociation of glyoxal (eq 3). The purpose of this communication is to propose from quantitative theoretical studies that the triple dissociation (2a) of *s*-tetrazine is energetically operative under the conditions of the Hochstrasser (and subsequent) experiments.



(2)



$$\mathrm{HC(=O)-C(=O)H} \rightarrow H_2 + CO + CO \quad (3)$$

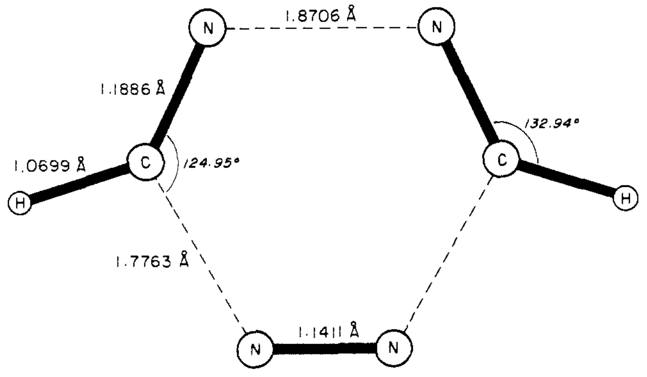


Figure 2. Theoretical transition state for the unimolecular triple dissociation (eq 2a) of *s*-tetrazine at the DZP SCF level of theory.

Table I. Theoretical Predictions (in kcal/mol) of the Classical Barrier Height for the Triple Dissociation of *s*-Tetrazine

	DZ basis set	DZP basis set
self-consistent-field (SCF)	67.4	79.4
configuration interaction (CI)	43.0	67.8
unlinked cluster corrected CI	33.9	60.1
coupled cluster (CCSD)	26.6	52.8
SCF correction for zero-point vibrational energy	−5.5	−5.8
experimental activation energy	≤51.8	

In the photochemical experiments the first step is the electronic excitation $S_0 \rightarrow S_1$. S_1, the first excited singlet state, lies at 18 128 cm^{-1}, or 51.8 kcal/mol.[14] This is followed by a radiationless transition to the higher vibrational manifold of the S_0 ground-state potential energy hypersurface. As noted by Karl and Innes[4] in their 1975 paper, the ensuing ground-state unimolecular reaction must proceed with an activation energy less than or equal to 51.8 kcal/mol. Accordingly, the goal of this research was to ask whether the one-step triple dissociation (2a) does indeed have such a lower energy barrier.

The triple dissociation pathway can in principle maintain C_{2v} symmetry throughout. In light of the massive rearrangement of bonds [two aromatic (bond order 1.5) CN bonds and one aromatic NN bond broken; two aromatic CN bonds and one aromatic NN bond become triple bonds in the reaction products], it seems at first glance unlikely that such a high-symmetry process would be allowed by orbital symmetry. However, as Figure 1 shows, the orbitals of *s*-tetrazine, resolved into C_{2v} symmetry, have precisely the same numbers of a_1, a_2, b_1, and b_2 orbitals as do HCN + HCN + N_2 in the same dissociated C_{2v} arrangement. Thus the triple dissociation is allowed by orbital symmetry in the sense of Woodward and Hoffmann.[15]

Our quantitative theoretical studies were carried out using standard double ζ (DZ) and double ζ plus polarization (DZP) basis sets,[16] designated C,N(9s 5p 1d/4s 2p 1d), H(4s 1p/2s 1p) in the DZP case. Polarization function orbital exponents were $\alpha_d(C) = 0.75$, $\alpha_d(N) = 0.80$, and $\alpha_p(H) = 0.75$. The hydrogen s functions were scaled by a factor of 1.2. Stationary point geometries were precisely located for *s*-tetrazine and the expected C_{2v} transition state at both the DZ self-consistent-field (SCF) and DZP SCF levels of theory using analytic gradient methods.

Harmonic vibrational analyses at both DZ SCF and DZP SCF levels of theory showed that the C_{2v} stationary point seen in Figure 2 is in fact a true transition state. Analytic second derivative

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methods were used for this purpose. The single imaginary vibrational frequency is predicted to be 1166i cm^{-1} (DZ SCF) and 1267i cm^{-1} (DZP SCF), and the corresponding normal mode vibrational eigenvector does indeed connect the transition state to the HCN + HCN + N_2 products.

More reliable estimates of the classical barrier height for the triple dissociation were obtained from configuration interaction (CI) wave functions including all single and double excitations. For the larger DZP basis set the correlated wave functions involved a total of 187 526 configurations. The effects of higher excitations were estimated via Davidson's correction[17] for unlinked clusters. A more reliable treatment of unlinked clusters is provided by the coupled cluster single and double excitation (CCSD) method.[18]

The present energetic predictions are summarized in Table I. At the highest level of theory, CCSD with zero-point vibrational energies appended, the activation energy for reaction 2a is predicted to be (52.8 − 5.8) = 47.0 kcal/mol. This prediction is 4.8 kcal/mol lower than the experimental upper limit. However, it is well established[13b,19] that such levels of theory yield activation energies above available observed E_a values, and an estimate of the expected error might be 3 kcal/mol. Thus we conclude that the unimolecular triple dissociation proceeds with an energy below the $S_0 \rightarrow S_1$ electronic excitation energy of *s*-tetrazine.

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Diffusion-Controlled Association Rate of Cytochrome *c* and Cytochrome *c* Peroxidase in a Simple Electrostatic Model

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Abstract: The diffusion-controlled association of electron transport proteins cytochrome *c* and cytochrome *c* peroxidase is simulated by the Brownian Dynamics method. The proteins are modelled as spheres, each having a central monopole carrying the net charge and two dipolar charges imbedded 1.0 Å inside the protein surface. The magnitudes of the monopole and dipole moments generated by this simplified charge distribution are determined directly from the X-ray crystallographic coordinates of the proteins by assigning partial charges to each non-hydrogen atom. Solvent electrolyte effects are modelled by using the Debye–Hückel screening law modified to include the effect of the large ion-exclusion sizes of macromolecules. Rate constants obtained by the simulation compare moderately well with experimentally determined rate constants for electron transfer at physiological ionic strength. The electrostatic torques generated by inclusion of the dipolar terms in the interaction potential are of crucial importance in overcoming the severe rate-retarding effect of the orientational reactivity constraints. At lower ionic strengths, dissociation rather than diffusion-controlled association is rate limiting for this particular reaction. The computed ionic strength dependence of the rate constant is weaker than that observed in the experiment, presumably because of the failure of the Debye–Hückel screened Coulombic potential to adequately treat solvent mediation effects as ionic strength increases. Simulation results are sensitive to the mode of treatment of the solvent mediation effects and choice of effective protein radii.

I. Introduction

The rates of many important biological processes in solution are influenced or controlled by the diffusional encounter rate of reactants.[1] Important examples include the bimolecular reaction of enzymes and ligands, antibodies and antigens, hormones and receptors, electron transport protein association, and sliding of small molecules on DNA. Molecular recognition in biochemical reactions not only depends on static conformational properties of biomolecules obtainable by X-ray diffraction data, but it may depend on dynamics as well. It has already been argued[2] that molecular specificity may in fact begin in the diffusional encounter stage of the reaction, with long-ranged electrostatic forces and other factors selectively steering particles into favorable orientations for reaction. In this paper we examine the role played by electrostatic structural features in the diffusion-controlled association rate of electron-transport proteins cytochrome *c* and cytochrome *c* peroxidase.

A number of important theoretical studies have addressed the problem of calculating the rate of diffusion-controlled reactions between macromolecules.[3] Smoluchowski and Debye investigated the problem of diffusion-controlled reactions between uniformly reactive spheres in the absence[4] and presence[5] of centrosymmetric Coulombic forces. Since these pioneering works, there has been a proliferation of theoretical studies based on more refined models. In many types of important problems analytical solutions have been obtained for rate constants. Unfortunately, one cannot hope to obtain analytical solutions on the detailed level required to understand molecular recognition in biomolecular interactions in general when a variety of interactions operate simultaneously. For this reason one must resort to numerical methods. A computer simulation approach based on the Brownian Dynamics (BD) trajectory method has been developed[6] to handle the arbitrarily

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