value is not a quantitative measure, its magnitude is similar to those obtained for other pathways[22,23,27] where the interaction of nonpolar residues is the driving force for self-association and suggests this may also be responsible for SCIII dimerization.

The dissociation constant for the binding of calcium to $P^*{}_2Ca$ (K_2) is approximately 300 times smaller than that for calcium binding to the unfolded peptide, P (K_1). One possibility for this observation is that the presence of a single calcium in the dimer, $P^*{}_2Ca$, inhibits further calcium binding via electrostatic repulsion between calcium ions. Complexation studies utilizing model crown ether systems have shown that such a mechanism is possible[49] when the distance between the binding sites is relatively small. However, in the C-terminal domain of troponin-C the calcium ions in calcium-binding sites III and IV are 11.7 Å apart. Since a similar distance is expected in $P^*{}_2Ca_2$ for SCIII it is unlikely that electrostatic repulsion could account for the observed weak binding of the second calcium ion.

The weaker binding of the second calcium ion could also be a result of decreased mobility of the $P^*{}_2Ca$ complex compared to the unfolded peptide, P. Such changes in dynamics have been recently assessed calbindin D_{9k}[50] where the association and dissociation rate constants for calcium binding, k_{on} and k_{off}, respectively, have been measured.[51] A similar analysis for SCIII would be valuable for interpretation of the dissociation constants in SCIII. For example, if calcium binding to $P_2{}^*Ca$ is inhibited because the site is performed one might expect the association rate constant for this step ($k^2{}_{on}$) to be slower than that for initial calcium binding to P ($k^1{}_{on}$). For the binding of the first calcium ion to SCIII no observable line broadening occurs with increasing calcium concentration giving an upper limit of $k^1{}_{off} < 10\ s^{-1}$. However, this estimate actually corresponds to the product of the calcium-binding step (K_1) and the peptide association (k_d), and an estimate of $k^1{}_{on}$ is not easily extracted.

The mechanism and stoichiometry for calcium binding to SCIII appears to be different from previous results with synthetic peptides and fragments from troponin-C. One of the most obvious reasons for our observation of a peptide association pathway is the concentration regime used here as compared to other studies. Previous work with either troponin-C and calmodulin peptide fragments were done with use of concentrations ranging from 5 to 20 μM.[14,15,41] In the present study, the peptide association constant was found to be near this range (K_d = 10 μM). This would imply that if peptide association was occurring in earlier works it may have been incomplete due to the concentrations used. As a result, the calculated dissociation constants for calcium would likely be higher than that found in this study. Indeed in one case a dissociation constant of 30 μM has been found for fragment CB9 (site III of troponin-C),[15] which is about 10 fold higher than K_1 = 3 μM for SCIII.

There have also been a few studies[16,17] conducted with troponin-C peptides at concentrations greater than 0.1 mM, where a high degree of peptide association would be expected to occur. As discussed earlier, these studies assumed 1:1 Ca peptide stoichiometry. However, even if peptide association was suspected, binding of a second calcium ion likely would not have been observed since free calcium ion concentrations were not extended high enough to measure the weak binding of the second ion (K_2 = 1 mM). As a result, the reported dissociation constant should be similar to K_1 (3 μM) in this study. This has been found for at least one case where a dissociation constant of 3.8 mM was found.[16]

Acknowledgment. This work arose in part from studies conducted by Jean Gariepy in 1982 at the University of Alberta and whose NMR titrations of a site III rabbit skeletal troponin-C peptide (residues 90–123) showed 0.5 Ca–peptide stoichiometry. We would like to thank Robert Boyko for his assistance with the curve-fitting programs used, Paul Semchuk for his help with peptide synthesis and purification, and Mike Nattriss for amino acid analysis. We are also grateful to Sue Smith for typing this manuscript and to Wendy Findlay and Carolyn Slupsky for helpful discussions.

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Crossed-Beam Study of the S_N2 Reaction

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Abstract: We have used crossed molecular beams to study the dynamics of chemiionization S_N2 reactions of the type $RX + R'_3N \rightarrow RR'_3N^+ + X^-$, where RX is an alkyl halide and R'_3N is a nucleophile. We have seen reaction between methyl iodide, ethyl iodide, and *n*-propyl iodide with quinuclidine and between methyl iodide and pyridine and tri-*n*-propylamine. The reactive cross sections have a threshold between 4.4 and 6.0 eV depending on the system. They rise rapidly above the threshold and then level off at higher energies showing no dependence on the vibrational energy of the nucleophile.

Introduction

Nucleophilic substitution reactions are sufficiently important that they are generally introduced early in a course in elementary organic chemistry. The S_N2 reaction, with its picturesque umbrella inversion, is the most important of these. It was characterized by Ingold et al.[1] in the 1930's and has been studied in solution ever since. There have been several studies of the general reaction

$$X^- + CH_3Y \rightarrow Y^- + CH_3X \qquad (1)$$

in the gas phase with use of flowing afterglow,[2] ion cyclotron resonance,[3] and tandem mass spectroscopy.[4] Several groups have calculated the potential-energy surface for this type of reaction.[5,6]

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The detailed dynamics has then been studied with use of Monte Carlo simulations.[5]

We report here a beam study of the chemiionization reaction

$$RX + R'_3N \rightarrow RR'_3N^+ + X^- \qquad (2)$$

where RX is an alkyl halide and R'_3N is a nucleophile. Note that the reactants are neutral, but the products are ions. The most significant conclusion of the paper is that the reaction occurs. There are no prior examples of gas-phase S_N2 reactions where the reactants are neutral. In our previous studies[7,8] of gas-phase chemiionization reactions we have found only a few cases where more than one atom is transferred, and then the cross section was very small.

Experimental Section

We have studied several types of organic chemiionization reaction using crossed molecular beams to determine the detailed dynamics of the reaction process.[7,8] The details of the experiment are described in these previous papers so only a summary of the experiment will be given here. Each reactant is prepared with use of a seeded nozzle beam. The two beams intersect at 135° in the middle of a large vacuum chamber where the reaction takes place. The product ions are extracted by an electric field, mass selected, and then detected.

The seeded nozzle beam is prepared by mixing a few hundred millitorr of the reactant gas in ~1 atm of an inert carrier gas. During the expansion in the nozzle, collisions accelerate the reactant up to the velocities of the carrier gas. Because the reactant has a much higher mass, it can reach energies of several electronvolts. The relative translational energy depends on the nozzle temperature, the type of carrier gas, and the beam intersection angle. We use three carrier gases: H_2, He, and a mixture of 60% He and 40% H_2. At a given nozzle temperature H_2 produces a higher kinetic energy than He with the mixture in between. Increasing the nozzle temperature raises the energy. During the nozzle expansion the translational energy distribution is cooled down as the enthalpy due to translation and rotation in the stagnant gas behind the nozzle is converted into bulk translational energy of the beam.[9] The resulting distribution in translational energy is roughly a Maxwell-Boltzmann distribution characterized by a temperature of a few degrees Kelvin added to a large constant velocity. The rotational energy distribution is roughly thermal at a temperature of tens of degrees Kelvin. Vibrational relaxation, however, is a very inefficient process, so an appreciable fraction of the vibrational energy remains in the beam and can participate in the reaction. As the nozzle temperature is raised, the vibrational energy is increased as well as the translational energy. Our experiment consists of measuring the reactive cross section as a function of nozzle temperature for several combinations of carrier gases. If, as is often the case, the vibrational energy has little effect on the cross section, all the data will fall on one common curve if plotted as a function of translational energy. If vibrational energy is important, the curves will be different. To get a given translational energy with He as the carrier gas requires a hotter nozzle than for H_2 and therefore gives more vibrational energy.

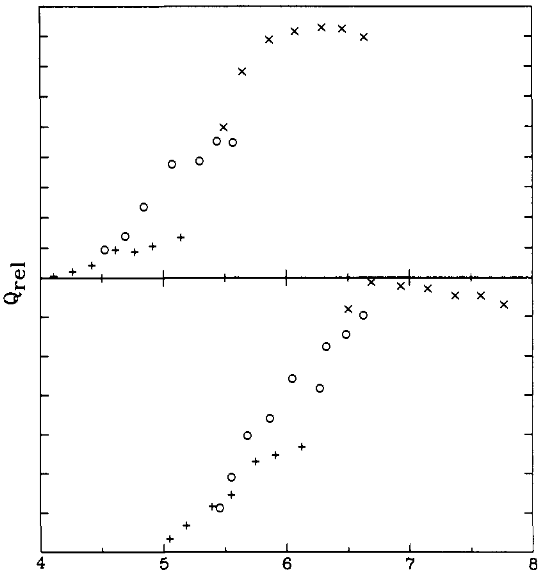


Figure 1. The cross section for the reaction of quinuclidine and methyl iodide vs relative translational energy. The energy is varied by changing the temperature of the nozzle for quinuclidine. Three carrier gases are used: (+) He, (○) a mixture of 60% He and 40% H_2, and (×) H_2. The data in the top panel were taken with the mixture as the carrier gas in the CH_3I beam while those in the bottom panel were taken with H_2. The scale in the ordinate is arbitrary. The data for the different carrier gases are normalized to each other, but the data in the two panels are not.

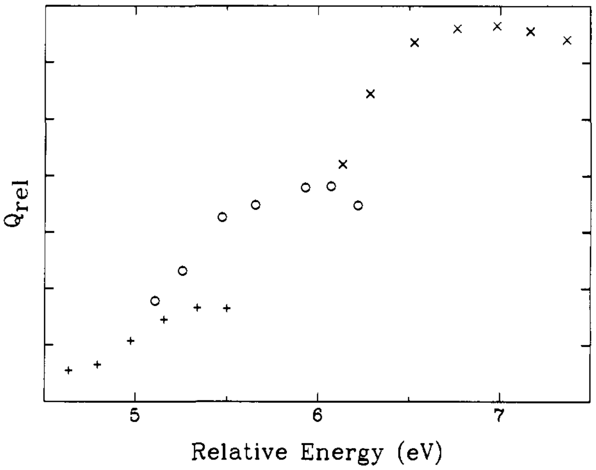


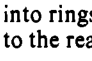
Figure 2. The cross section for the reaction of ethyl iodide with quinuclidine. See the caption of Figure 1 for details. The carrier gas for ethyl iodide was H_2.

Results and Discussion

Figure 1 shows the cross section for the reaction of quinuclidine (Qn) and methyl iodide

$$CH_3I + Qn \rightarrow CH_3Qn^+ + I^- \qquad (3)$$

Quinuclidine (**1**) is a tertiary amine in which all three substituents



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on the nitrogen are bound into rings so that the lone pair on the nitrogen is always exposed to the reaction. Only the two product ions in eq 3 are found. There are no fragment ions at the energies of our experiment. As discussed above, the translational energy is varied by changing the nozzle temperature of the quinuclidine. Three carrier gases are used: He (+), the mixture (○) and H_2 (×). The data in the top panel of Figure 1 were taken with the mixture in the CH_3I beam and the data in the bottom panel were

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taken with H_2. To within experimental error, the effects of carrier gas in each case are negligible. The small differences are due to errors in correcting for the different beam intensity for the two cases and in reaching complete thermal equilibrium in the nozzle. We can obtain a threshold energy by extrapolating the cross section to zero signal. The comparison between the two panels shows that the threshold in the top panel is 4.4 eV, ~0.5 eV lower than that for the bottom panel. This is just beyond our experimental uncertainty of 0.4 eV. We have seen this effect before as we change the intersection angle between the two beams. It may be due to the importance of vibrational energy near the threshold since the vibrational temperatures in the top panel are higher than those in the bottom panel. To get a given translational energy with the mixture as a carrier gas, the CH_3I nozzle must be hotter than in the case of H_2. A more likely explanation is that the cross section is highly nonlinear near the threshold so that a linear extrapolation to the threshold is in error. In the latter case, all we can get from the data is an upper bound to the true threshold.

The results for the different carrier gases are identical within experimental error. This means that vibrational energy in the quinuclidine is not specifically required for the reaction. If it were, we would expect an exponential, Arrhenius-like, dependence on the vibrational energy, and this is clearly not seen in the data. Vibrational energy may be more or less interchangeable with translational energy. Because we have much more translational energy in the reactants, we cannot detect this effect. Quinuclidine has 57 vibrational modes, all thermally populated in our experiments. It is quite likely that only a few of them participate in the reaction, and the amount of energy in these few modes is very small.

Figure 2 shows similar data for the reaction of ethyl iodide with quinuclidine. The threshold is roughly the same. We have no way of normalizing the cross sections for one reactant to those of another because we cannot measure the absolute intensities of the beams. However, the signal intensities for ethyl iodide are very much lower than those for methyl iodide, so that the cross section is probably less by roughly a factor of ~5. This is not surprising since the reaction site on ethyl iodide is shielded by the methyl group in the case of ethyl iodide. We have also seen reaction with *n*-propyl iodide, but the cross section is still smaller.

Figure 3 shows the cross sections for the reaction of methyl iodide with pyridine. They are similar to those with quinuclidine except that a smaller energy range is available because of the lower mass of pyridine. We have also seen reaction of methyl iodide with tri-*n*-propyl amine. Here, the threshold is about 6 eV, higher than the others. We did not see reaction of methyl iodide with tri-*n*-butylamine. A likely explanation is that conformations where the butyl groups are folded out of the way so that the nitrogen lone pair is readily accessible have a low probability.

We have previously seen an elimination reaction between butyl iodide and the strong base tetrakis(dimethylamino)ethylene (TDMAE)[8]

$$\text{BuI} + \text{TDMAE} \rightarrow \text{TDMAE–H}^+ + \text{I}^- + C_4H_8 \qquad (4)$$

All four butyl iodide isomers reacted. All four had similar thresholds of 7.9 ± 1 eV, but the cross sections were roughly in the ratio of the numbers of β-hydrogens on the butyl iodide isomer. We did not see the S_N2 reaction here. In the present study, we looked at isopropyl iodide and quinuclidine. The signal levels were very low, but we clearly saw two cation products, one due to the S_N2 reaction (isopropylquinuclidine ion) and the other due to the elimination reaction (protonated quinuclidine ion). The signal levels were too low to get an accurate plot of cross section vs energy.

The major features of the relevant potential energy surfaces can be surmised from the reaction. Taking reaction 3 as an example, we see that, if the reactants are dissociated, we obtain CH_3, I, and Qn—all neutral. If the products are dissociated, we get CH_3, I^-, and Qn^+. Therefore the products must lie on a different potential energy surface than the reactants. On the reactant side, the covalent surface is lower, but it is relatively flat until the reactants meet. It then becomes repulsive since the neutral products I + CH_3Qn are not stable. On the other hand, the ionic surface is strongly attractive due to the Coulomb attraction between the ions. The two surfaces cross each other, probably at short distances. When the system reaches the crossing, an electron jumps from Qn to CH_3I, and the reaction continues on the ionic surface.

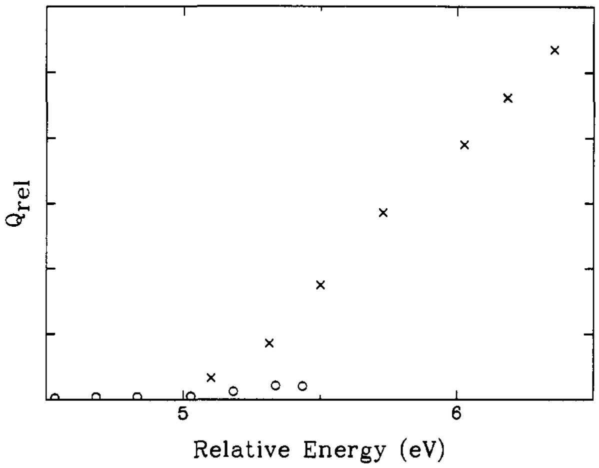

Figure 3. The cross section for the reaction of methyl iodide with pyridine. The carrier gas for methyl iodide was H_2.

We have modeled the reaction

$$\text{HI} + \text{B} \rightarrow \text{I}^- + \text{HB}^+ \qquad (5)$$

using an ionic and a covalent surface. Trajectories were then run on the pair surfaces. The most significant result of the calculations is that the cross section for the formation of I^- and HB^+ is approximately exponential for about 1 eV above the threshold. It then levels out at higher energies. This exponential dependence on energy explains why the apparent threshold varies with beam conditions. A linear extrapolation to get the threshold will give very different results depending on how much of the exponential function is used to do the extrapolation. The details of the computer modeling will be published later.[10] Neither of the surfaces used has a barrier in it. The reaction is exothermic from the covalent reactants to the ion pair at the minimum in the ionic surface but is endothermic to the pair of separated product ions. Similar conclusions probably hold in the S_N2 case.

Conclusion

Nucleopholic substitution reactions are an important class of organic reactions. We have found that many of them can be seen as chemiionization reactions in the gas phase if enough translational energy is supplied to the reactants. The most effective alkyl halide appears to be methyl iodide. Other halides may be reactive, but their smaller mass means that we cannot use seeded nozzle beams to accelerate them. The best bases are quinuclidine and pyridine where the lone pair on the nitrogen is not sterically hindered. In all cases, the cross sections depend strongly on translational energy but not on the vibrational temperature of the base. The cross section is zero below a threshold which ranges from 4.4 to 6.0 eV depending on the reactants. The cross section rises rapidly above the threshold and then levels off at higher energies. Presumably, it then decreases at still higher energies, but we cannot get to these energies in our experiments.

Acknowledgment is made to the donors of the Petroleum Research Fund, administered by the American Chemical Society, for partial support of this research. We are also indebted to the National Science Foundation for the remaining support under Grant No. CHE-8901577. We also thank Ms. Cindy X. H. Wang for her help in some of the experiments and in working on the literature survey.

Registry No. Qn, 100-76-5; CH_3I, 74-88-4; ethyl iodide, 75-03-6; *n*-propyl iodide, 107-08-4; pyridine, 110-86-1; tri-*n*-propylamine, 102-69-2.

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(10) Yen, Y. F.; Cross, R. J. Submitted for publication.