Noncovalent Interactions of Nucleic Acid Bases (Uracil, Thymine, and Adenine) with Alkali Metal Ions. Threshold Collision-Induced Dissociation and Theoretical Studies

M. T. Rodgers*,† and P. B. Armentrout‡

Contribution from the Department of Chemistry, Wayne State University, Detroit, Michigan 48202, and the Department of Chemistry, University of Utah, Salt Lake City, Utah 84112

Received May 12, 2000

Abstract: Threshold collision-induced dissociation of M^+L ($M^+ = Li^+$, Na^+, and K^+; L = uracil, thymine, and adenine) with xenon is studied using guided ion beam mass spectrometry. In all cases, the primary product formed corresponds to endothermic loss of the intact neutral molecule. The only other product observed is the result of ligand exchange processes to form MXe^+. Cross-section thresholds are interpreted to yield 0 and 298 K bond dissociation energies for M^+-L after accounting for the effects of multiple ion−molecule collisions, internal energy of the reactant ions, and dissociation lifetimes. Ab initio calculations at the MP2(full)/6-311+G-(2d,2p)//MP2(full)/6-31G* level of theory are used to determine the structures and relative energetics of several conformers of these complexes and to provide molecular constants necessary for the thermodynamic analysis of the experimental data. We find that all of the complexes are very nearly planar. Calculated M^+-L bond dissociation energies compare favorably to the experimentally determined bond energies for Na^+ and K^+ binding to uracil and thymine, while theoretical values for Li^+ to all three bases and adenine with all three metal ions are systematically low (by 16 ± 8 kJ/mol). Comparisons with previous values determined by the kinetic method are reasonable, except in the case of Na^+(adenine). A key observation in this work is that the metal ions bind most strongly to adenine at the N7 site coupled with chelation to the amino group. The magnitude of the interaction with the amino group is estimated to be sufficient to disrupt hydrogen bonding in A:T (A:U) nucleic acid base pairs for Li^+, Na^+, and probably transition metal ions and multiply charged ions.

Introduction

The participation of metal ions in biological processes is well-known.[1] Metal ions may play a direct role, as in oxidation−reduction reactions, or may serve an indirect function by inducing conformational changes. For example, metal ions are crucial in determining which of numerous structures nucleic acids can assume, as well as influencing the way that nucleic acids are packed together. The presence of metal ions may constitute one of a number of factors that stabilize a conformer that does not require metals. For example, the transition from the B form of DNA can be prevented by the presence of metal ions that stabilize the B form, including the alkali metal ions, Li^+, K^+, and Cs^+.[2] Although metal ions are essential for many processes, the presence of the wrong metals, or even the essential metals in the wrong concentration, can lead to undesired results.[3] For instance, during protein synthesis, misincorporation of an amino acid could result from mispairing of the bases that can occur at high metal ion concentrations. Further, different metal ions exhibit different structural effects, so that the nature of the metal contact in the nucleus can conceivably influence the course of genetic information transfer. Base binding metal ions can cause more profound effects on the DNA conformation[4] than metal ions that bind to the phosphate backbone.

Alkali metal ions, and other "hard" metal ions, have a low tendency to form covalent bonds and are therefore relatively nonspecific binders. Their primary influence is to neutralize the negative charges on the backbone phosphate groups, thereby stabilizing the double helix. When metal ions bind to the bases instead of the phosphate groups, they also neutralize negative charges on the phosphate in a zwitterion effect. In either event, such stabilization is accompanied by an increase in the "melting temperature of DNA".[5−8] Because each metal ion bound to DNA neutralizes some of the charge on the DNA, additional binding of metal ions becomes increasingly weaker, i.e., the binding is anticooperative.[9] If enough metal ions have been added to neutralize the negative charges in the backbone of the nucleotide, additional metal ions destabilize the DNA double helix.[10] This can occur because the metal ions can bind to the bases in such a way as to interfere with the hydrogen-bonding and stacking interactions that hold the strands together.

† Wayne State University.
‡ University of Utah.

(1) Eichhorn, G. L. *Adv. Inorg. Biochem.* **1981**, *3*, 1.
(2) Ivanov, V. I.; Minchenkova, L. E.; Minyat, E. E.; Frank-Kamenetskii, M. D.; Schyolkina, A. K. *J. Mol. Biol.* **1974**, *87*, 817.
(3) Eichhorn, G. L. In *Inorganic Biochemistry*; Eichhorn, G. L., Ed.; Elsevier: New York, 1973; p 1210.
(4) Shin, Y. A.; Eichhorn, G. L. *Biopolymers* **1977**, *16*, 225.
(5) Shack, J.; Jenkins, R. J.; Thompsett, J. M. *J. Biol. Chem.* **1953**, *203*, 373.
(6) Dove, W. F.; Davidson, N. *J. Mol. Biol.* **1962**, *5*, 467.
(7) Eichhorn, G. L. *Nature* **1962**, *194*, 474.
(8) Eichhorn, G. L.; Shin, Y. A. *J. Am. Chem. Soc.* **1968**, *90*, 7323.
(9) Krakauer, H. *Biopolymers* **1971**, *10*, 2459.
(10) Blagoi, Y. P.; Sorokin, V. A.; Valeyev, V. A.; Khomenko, S. A.; Gladchenko, G. O. *Biopolymers* **1978**, *17*, 1103.

10.1021/ja001638d CCC: $19.00
Published on Web 08/18/2000

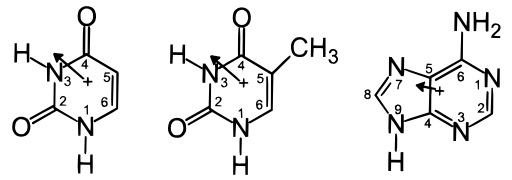

Figure 1. Structures of uracil, thymine, and adenine. Properly scaled dipole moments in Debye are shown as arrows (+ indicates the positive end of the dipole). Values listed are taken from theoretical calculations at the MP2(full)/6-311+G(2d,2p)//MP2(full)/6-31G* level of theory performed here. The measured molecular polarizabilities of thymine and adenine (ref 21) and the estimated molecular polarizability of uracil[22] (in Å^3) are also shown.

In recent work, we have developed methods to allow the application of quantitative threshold collision-induced dissociation (CID) methods to obtain accurate thermodynamic information on increasingly large systems.[11−16] One of the driving forces behind these developments is our interest in applying such techniques to systems having biological relevance. In addition, we seek to perform accurate thermochemical measurements that provide absolute anchors for metal cation affinity scales over a broadening range of energies and for simple to complex ligands. In the present paper, we realize both ambitions in a study of the interactions of three nucleic acid bases, uracil (U), thymine (T), and adenine (A), with the alkali metal ions, Li^+, Na^+, and K^+.[17] The structures of the neutral molecules are shown in Figure 1 along with calculated[18] and measured[19,20] dipole moments, and measured[21] or estimated[22] polarizabilities. Our study utilizes guided ion beam mass spectrometry to measure the cross sections for CID of these nine complexes. The kinetic energy dependent CID cross sections are analyzed using methods developed previously[13] that explicitly include the effects of the internal and translational energy distributions of the reactants, multiple collisions, and the lifetime for dissociation. We derive bond dissociation energies (BDEs) for all metal cation−nucleic acid base complexes and compare these results to values obtained in kinetic method studies.[23] Further, these results are compared to BDEs derived from ab initio calculations of all complexes in several alternate conformations and to previous theoretical results (at lower levels of theory) in the literature.[18,24−27]

Experimental and Theoretical Section

General Procedures. Cross sections for CID of M^+L, where $M^+ = Li^+$, Na^+, and K^+, and L = uracil, thymine, and adenine, are measured using a guided ion beam mass spectrometer that has been described in detail previously.[28,29] The metal−ligand complexes are generated as described below. The ions are extracted from the source, accelerated, and focused into a magnetic sector momentum analyzer for mass analysis. Mass-selected ions are decelerated to a desired kinetic energy and focused into an octopole ion guide, which traps the ions in the radial direction.[30] The octopole passes through a static gas cell containing xenon, used as the collision gas for reasons described elsewhere.[31−33] Low gas pressures in the cell (typically 0.04 to 0.20 mTorr) are used to ensure that multiple ion−molecule collisions are improbable. Product and unreacted reactant ions drift to the end of the octopole where they are focused into a quadrupole mass filter for mass analysis and subsequently detected with a secondary electron scintillation detector and standard pulse counting techniques.

Ion intensities are converted to absolute cross sections as described previously.[28] Absolute uncertainties in cross section magnitudes are estimated to be ±20%, which are largely the result of errors in the pressure measurement and the length of the interaction region. Relative uncertainties are approximately ±5%. Because the radio frequency used for the octopole does not trap light masses with high efficiency, the cross sections for Li^+ products were more scattered and showed more variations in magnitude than is typical for this apparatus. Therefore, absolute magnitudes of the cross sections for production of Li^+ are probably ±50%.

Ion kinetic energies in the laboratory frame, E_{lab}, are converted to energies in the center-of-mass frame, E_{CM}, using the formula $E_{CM} = E_{lab}m/(m + M)$, where M and m are the masses of the ionic and neutral reactants, respectively. All energies reported below are in the CM frame unless otherwise noted. The absolute zero and distribution of the ion kinetic energies are determined using the octopole ion guide as a retarding potential analyzer, as previously described.[28] The distribution of ion kinetic energies is nearly Gaussian with a fwhm typically between 0.2 and 0.5 eV (lab) for these experiments. The uncertainty in the absolute energy scale is ±0.05 eV (lab).

Even when the pressure of the reactant neutral is low, we have previously demonstrated that the effects of multiple collisions can significantly influence the shape of CID cross sections.[34] Because the presence and magnitude of these pressure effects is difficult to predict, we have performed pressure-dependent studies of all cross sections examined here. In the present systems, we observe small cross sections at low energies that have an obvious dependence upon pressure. We attribute this to multiple energizing collisions that lead to an enhanced probability of dissociation below threshold as a result of the longer residence time of these slower moving ions. Data free from pressure effects are obtained by extrapolating to zero reactant pressure, as described previously.[34,35] Thus, results reported below are due to single bimolecular encounters.

Ion Source. The M^+L complexes are formed in a 1 m long flow tube[29,36] operating at a pressure of 0.5−0.7 Torr with a helium flow

(11) Rodgers, M. T.; Armentrout, P. B. *J. Phys. Chem. A* **1997**, *101*, 1238.
(12) Rodgers, M. T.; Armentrout, P. B. *J. Phys. Chem. A* **1997**, *101*, 2614.
(13) Rodgers, M. T.; Ervin, K. M.; Armentrout, P. B. *J. Chem. Phys.* **1997**, *106*, 4499.
(14) Rodgers, M. T.; Armentrout, P. B. *Int. J. Mass Spectrom.* **1999**, *185/186/187*, 359.
(15) Rodgers, M. T.; Armentrout, P. B. *J. Phys. Chem. A* **1999**, *103*, 4955.
(16) Armentrout, P. B.; Rodgers, M. T. *J. Phys. Chem. A* **2000**, *104*, 2238.
(17) Preliminary results for this work were published: Rodgers, M. T.; Armentrout, P. B. *Proceedings of the 44th ASMS Conference on Mass Spectrometry and Allied Topics*, May 12−16, 1996, Portland, OR, p 88.
(18) Basch, H.; Gramer, D. R.; Jasien, P. G.; Krauss, M.; Stevens, W. J. *Chem. Phys. Lett.* **1989**, *163*, 514.
(19) Kulalowska, I.; Geller, M.; Lesyng, B.; Bolewska, K.; Wierzchowski, K. L. *Biochim. Biophys. Acta* **1970**, *407*, 420.
(20) DeVoe, H.; Tinoco, I., Jr. *J. Mol. Biol.* **1962**, *4*, 500.
(21) As cited in ref 22, however, the reference provided does not actually contain the listed values.
(22) Miller, K. J. *J. Am. Chem. Soc.* **1990**, *112*, 8533.
(23) Cerda, B. A.; Wesdemiotis, C. *J. Am. Chem. Soc.* **1996**, *118*, 11884.
(24) Del Bene, J. E. *J. Phys. Chem.* **1984**, *88*, 5927.
(25) Anwander, E. H. S.; Probst, M. M.; Rode, B. M. *Biopolymers* **1990**, *29*, 757.
(26) Jasien, P. G.; Fitzgerald, G. *J. Chem. Phys.* **1990**, *93*, 2554.
(27) Burda, J. V.; Šponer, J.; Hobza, P. *J. Phys. Chem.* **1996**, *100*, 7250.
(28) Ervin, K. M.; Armentrout, P. B. *J. Chem. Phys.* **1985**, *83*, 166.
(29) Schultz, R. H.; Armentrout, P. B. *Int. J. Mass Spectrom. Ion Processes* **1991**, *107*, 29.
(30) Teloy, E.; Gerlich, D. *Chem. Phys.* **1974**, *4*, 417. Gerlich, D. Diplomarbeit, University of Freiburg, Federal Republic of Germany, 1971. Gerlich, D. In State-Selected and State-to-State Ion−Molecule Reaction Dynamics, Part I, Experiment; Ng, C.-Y., Baer, M., Eds.; *Adv. Chem. Phys.* **1992**, *82*, 1.
(31) Dalleska, N. F.; Honma, K.; Armentrout, P. B. *J. Am. Chem. Soc.* **1993**, *115*, 12125.
(32) Aristov, N.; Armentrout, P. B. *J. Phys. Chem.* **1986**, *90*, 5135.
(33) Hales, D. A.; Armentrout, P. B. *J. Cluster Sci.* **1990**, *1*, 127.
(34) Dalleska, N. F.; Honma, K.; Sunderlin, L. S.; Armentrout, P. B. *J. Am. Chem. Soc.* **1994**, *116*, 3519.
(35) Hales, D. A.; Lian, L.; Armentrout, P. B. *Int. J. Mass Spectrom. Ion Processes* **1990**, *102*, 269.

rate of 4000−7000 sccm. Metal ions are generated in a continuous dc discharge by argon ion sputtering of a cathode, made from tantalum or iron, with a cavity containing the alkali metal. Typical operating conditions of the discharge are 2−3 kV and 15−30 mA in a flow of roughly 10% argon in helium. The M^+L complexes are formed by associative reactions of the alkali metal ion with the neutral molecule, which is introduced into the flow 50 cm downstream from the dc discharge. These molecules were vaporized by gentle heating and flowing helium over the sample. The flow conditions used in this ion source provide in excess of 10^4 collisions between a complex ion and the buffer gas, which should thermalize the ions vibrationally and rotationally. In our analysis of the data, we assume that the ions produced in this source are in their ground electronic states and that the internal energies of the M^+L complexes are well described by a Maxwell−Boltzmann distribution of ro-vibrational states at 300 K. Previous work has shown that these assumptions are generally valid.[31,34,36−39]

Thermochemical Analysis. The threshold regions of the reaction cross sections are modeled using the equation

$$\sigma(E) = \sigma_0 \sum_i g_i (E + E_i - E_0)^n / E \qquad (1)$$

where σ_0 is an energy-independent scaling factor, E is the relative translational energy of the reactants, E_0 is the threshold for reaction of the ground electronic and ro-vibrational state, and n is an adjustable parameter. The summation is over the ro-vibrational states of the reactant ions, i, where E_i is the excitation energy of each state and g_i is the population of those states ($\sum g_i = 1$). The populations of excited ro-vibrational levels are not negligible even at 300 K as a result of the many low-frequency modes present in these ions. The relative reactivities of all ro-vibrational states, as reflected by σ_0 and n, are assumed to be equivalent.

The Beyer−Swinehart algorithm[40] is used to evaluate the density of the ro-vibrational states and the relative populations g_i are calculated by an appropriate Maxwell−Boltzmann distribution at the 300 K temperature appropriate for the reactants. Vibrational frequencies and rotational constants obtained by ab initio calculations, as described in the next section, are listed in Tables 1S and 2S, respectively. The average vibrational energies at 298 K of the bases and metal ion bound bases are also given in Table 1S. We have estimated the sensitivity of our analysis to the deviations from the true frequencies by scaling the calculated frequencies to encompass the range of average valence coordinate scale factors needed to bring calculated frequencies into agreement with experimentally determined frequencies found by Pople et al.[41] Thus, the calculated vibrational frequencies (after scaling, see below) were increased and decreased by 10%. The corresponding change in the average vibrational energy is taken to be an estimate of one standard deviation of the uncertainty in vibrational energy (Table 1S) and is included in the uncertainties listed with the E_0 values.

We also consider the possibility that collisionally activated complex ions do not dissociate on the time scale of our experiment (about 10^{-4} s) by including statistical theories for unimolecular dissociation into eq 1 as described in detail elsewhere.[13,37] This requires sets of ro-vibrational frequencies appropriate for the energized molecules and the transition states (TSs) leading to dissociation. The former are given in Tables 1S and 2S while we assume that the TSs are loose and product-like because the interaction between the metal ion and the ligand is largely electrostatic, a treatment that corresponds to a phase space limit (PSL), as described in detail elsewhere.[13] In the present work, the adiabatic 2-D rotational energy is treated using a statistical distribution with explicit summation over the possible values of the rotational quantum number.[13]

The model represented by eq 1 is expected to be appropriate for translationally driven reactions[42] and has been found to reproduce reaction cross sections well in a number of previous studies of both atom−diatom and polyatomic reactions,[43,44] including CID processes.[11,12,31,34,36,37,45−47] The model is convoluted with the kinetic energy distributions of both reactants, and a nonlinear least-squares analysis of the data is performed to give optimized values for the parameters σ_0, E_0, and n. The error associated with the measurement of E_0 is estimated from the range of threshold values determined for different data sets, variations associated with uncertainties in the vibrational frequencies, and the error in the absolute energy scale, 0.05 eV (lab). For analyses that include the RRKM lifetime effect, the uncertainties in the reported E_0 values also include the effects of increasing and decreasing the time assumed available for dissociation (10^{-4} s) by a factor of 2.

Equation 1 explicitly includes the internal energy of the ion, E_i. All energy available is treated statistically, which should be a reasonable assumption because the internal (rotational and vibrational) energy of the reactants is redistributed throughout the ion upon impact with the collision gas. The threshold for dissociation is by definition the minimum energy required for dissociation and thus corresponds to formation of products with no internal excitation. The assumption that products formed at threshold have an internal temperature of 0 K has been tested for several systems.[11,12,31,34,36,37] The threshold energies for dissociation reactions determined by analysis with eq 1 are converted to 0 K bond energies by assuming that E_0 represents the energy difference between reactants and products at 0 K.[48] This requires that there are no activation barriers in excess of the endothermicity of dissociation. This is generally true for ion−molecule reactions[43] and should be valid for the simple heterolytic bond fission reactions examined here.[49]

Ab Initio Calculations. To obtain model structures, vibrational frequencies, and energetics for the neutral and metalated bases, ab initio calculations were performed using Gaussian 98.[50] Geometry optimizations were performed at the MP2(full)/6-31G* level. This level of theory was recently determined by Hoyau et al.[51] and us[16] to be adequate for a good description of sodium cation complexes. This conclusion is reinvestigated here and also tested for lithium and potassium cation complexes. Vibrational analyses of the geometry-optimized structures were performed to determine the vibrational frequencies and rotational

(36) Schultz, R. H.; Crellin, K. C.; Armentrout, P. B. *J. Am. Chem. Soc.* **1991**, *113*, 8590.
(37) Khan, F. A.; Clemmer, D. C.; Schultz, R. H.; Armentrout, P. B. *J. Phys. Chem.* **1993**, *97*, 7978.
(38) Schultz, R. H.; Armentrout, P. B. *J. Chem. Phys.* **1992**, *96*, 1046.
(39) Fisher, E. R.; Kickel, B. L.; Armentrout, P. B. *J. Phys. Chem.* **1993**, *97*, 10204.
(40) Beyer, T. S.; Swinehart, D. F. *Comm. Assoc. Comput. Machines* **1973**, *16*, 379. Stein, S. E.; Rabinovitch, B. S. *J. Chem. Phys.* **1973**, *58*, 2438. Stein, S. E.; Rabinovitch, B. S. *Chem. Phys. Lett.* **1977**, *49*, 1883.
(41) Pople, J. A.; Schlegel, H. B.; Raghavachari, K.; DeFrees, D. J.; Binkley, J. F.; Frisch, M. J.; Whitesides, R. F.; Hout, R. F.; Hehre, W. J. *Int. J. Quantum Chem. Symp.* **1981**, *15*, 269. DeFrees, D. J.; McLean, A. D. *J. Chem. Phys.* **1985**, *82*, 333.
(42) Chesnavich, W. J.; Bowers, M. T. *J. Phys. Chem.* **1979**, *83*, 900.
(43) Armentrout, P. B. In *Advances in Gas-Phase Ion Chemistry*; Adams, N. G., Babcock, L. M., Eds.; JAI: Greenwich, 1992; Vol. 1, pp 83−119.
(44) See, for example: Sunderlin, L. S.; Armentrout, P. B. *Int. J. Mass Spectrom. Ion Processes* **1989**, *94*, 149.
(45) More, M. B.; Glendening, E. D.; Ray, D.; Feller, D.; Armentrout, P. B. *J. Phys. Chem.* **1996**, *100*, 1605.
(46) Ray, D.; Feller, D.; More, M. B.; Glendening, E. D.; Armentrout, P. B. *J. Phys. Chem.* **1996**, *100*, 16116.
(47) Meyer, F.; Khan, F. A.; Armentrout, P. B. *J. Am. Chem. Soc.* **1995**, *117*, 9740.
(48) See, for example, Figure 1 in ref 31.
(49) Armentrout, P. B.; J. Simons, J. *J. Am. Chem. Soc.* **1992**, *114*, 8627.
(50) Gaussian 98, Revision A.7, Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Zakrzewski, V. G.; Montgomery, J. A., Jr.; Stratmann, R. E.; Burant, J. C.; Dapprich, S.; Millam, J. M.; Daniels, A. D.; Kudin, K. N.; Strain, M. C.; Farkas, O.; Tomasi, J.; Barone, V.; Cossi, M.; Cammi, R.; Mennucci, B.; Pomelli, C.; Adamo, C.; Clifford, S.; Ochterski, J.; Petersson, G. A.; Ayala, P. Y.; Cui, Q.; Morokuma, K.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Cioslowski, J.; Ortiz, J. V.; Baboul, A. G.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Gonzalez, C.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Andres, J. L.; Gonzalez, C.; Head-Gordon, M.; Replogle, E. S.; Pople, J. A.; Gaussian, Inc., Pittsburgh, PA, 1998.
(51) Hoyau, S.; Norrman, K.; McMahon, T. B.; Ohanessian, G. *J. Am. Chem. Soc.* **1999**, *121*, 8864.

constants of the molecules. When used to model the data or to calculate thermal energy corrections, the MP2(full)/6-31G* vibrational frequencies are scaled by a factor of 0.9646[52] and listed in Table 1S. Table 2S lists the rotational constants for the ground state conformations. Single point energy calculations were performed at the MP2(full)/6-311+G(2d,2p) level using the MP2(full)/6-31G* geometries. To obtain accurate bond dissociation energies, zero point energy (ZPE) corrections were applied and basis set superposition errors (BSSE) were subtracted from the computed dissociation energies in the full counterpoise approximation,[53,54] as in several other recent papers on Na^+ complexes.[55,56] The ZPE corrections range from 2.1 to 5.1 kJ/mol for the K^+ complexes and increase with the charge density of the cation to 29.5−35.7 kJ/mol for the H^+ complexes. Similarly, the BSSE corrections range from 4 to 5.4 kJ/mol for K^+ complexes to 9.1−9.8 kJ/mol for the H^+ complexes.

We carefully considered various possible binding sites on the three nucleic acids: O2 and O4 on both uracil and thymine, and N1, N3, and N7 on adenine. Stable minima were found for all three metal ions at all of these positions. Complexes in which the metal ion binds out of the plane of the molecule to the π-electrons were also investigated for Li^+ and found to be much less stable than the in-plane complexes. Thus, calculations on these π-complexes were not pursued for Na^+ and K^+.

In a few of the systems, one imaginary frequency was obtained for the optimized structures. In free adenine, this was the NH_2 umbrella motion, i.e., motion of the two amino hydrogens out of the plane of the molecule. This vibration is particularly low because the amino group is planar so that π-electron delocalization can occur. Imaginary frequencies were also obtained for Na^+ and K^+ complexes with adenine bound at the N1 and N7 sites. In these cases, the motion in which the amino group rotates about the C−N bond is found to be imaginary. No imaginary frequencies occur in the corresponding Li^+ complexes as a result of the much stronger binding interaction in these systems. Likewise, no imaginary frequencies occur when the metal ion binds at the N3 site, which is remote from the amino group. This is somewhat surprising because the NH_2 group is again planar, as in neutral adenine. Apparently, metal ion binding at N3 induces sufficient charge transfer to enhance the double bond character of the C−NH_2 bond, see below. In all of the cases that do have imaginary frequencies, we reassigned them as 1-D rotors with a rotational constant equivalent to the appropriate NH_2 rotation. (In assigning these constants, we found that explicit consideration of the rest of the adenine molecule produced insignificant changes.) The appropriate rotational constant, listed in Table 2S, is different in the adenine and the complex systems.

Results

Cross Sections for Collision-Induced Dissociation. Experimental cross sections were obtained for the interaction of Xe with nine M^+L complexes, where $M^+ = Li^+$, Na^+, and K^+, and L = uracil, thymine, and adenine. Figure 2 shows representative data for the Na^+(uracil) complex. (Complete results for all complexes are shown in Figure 1S in the Supporting Information.) As discussed above, the non-zero cross sections observed in these data at the lowest energies are a consequence of multiple collisions and disappear when the data are extrapolated to zero pressure of the Xe reactant, as shown in Figure 2. The complexes with thymine and adenine show similar relative behavior. The dominant process for all complexes is the loss of the intact neutral ligand in the CID reactions 2.

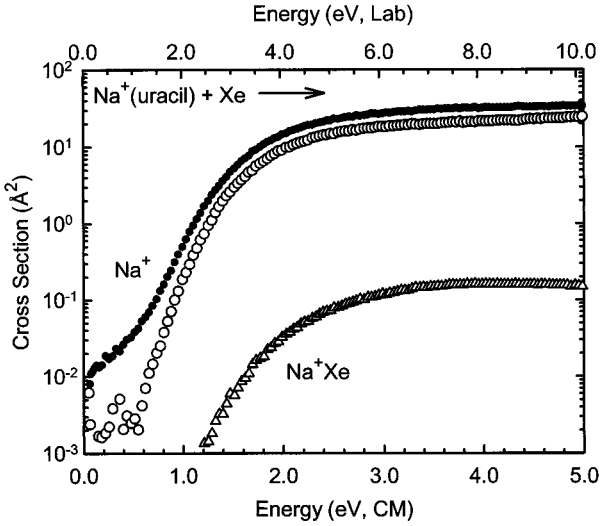

Figure 2. Cross section for collision-induced dissociation of the Na^+(uracil) complex with Xe as a function of kinetic energy in the center-of-mass frame (lower *x*-axis) and the laboratory frame (upper *x*-axis). Data are shown for a xenon pressure of ~0.20 mTorr (●) and extrapolated to zero (○). The cross section for the ligand exchange process to form Na^+Xe is also shown (△).

$$M^+L + Xe \rightarrow M^+ + L + Xe \qquad (2)$$

The magnitudes of the cross sections generally increase in size from $M^+ = Li^+$ to Na^+ to K^+. This is largely because the thresholds decrease in this same order. The only other products that are observed in these reactions are the result of ligand exchange processes to form MXe^+. The cross sections for these products are more than 2 orders of magnitude smaller than those for the primary M^+ product, and the thresholds are slightly lower (by the M^+−Xe binding energy), although this is not apparent given the relative magnitudes of the two cross sections. As little systematic information can be gleaned from these products, they will not be discussed further. Although it is conceivable that this ligand exchange process might cause a competitive shift in the observed thresholds, we do not believe such competition is likely to affect our threshold measurements in any of these systems within the quoted experimental errors. Several reasons for this conclusion have been detailed elsewhere.[47]

Threshold Analysis. The model of eq 1 was used to analyze the thresholds for reactions 2 in nine M^+L systems. The results of these analyses are provided in Table 1 and representative results are shown in Figure 3 for the Na^+(uracil) complex. (Complete results for all complexes are shown in Figure 2S in the Supporting Information.) In all cases, the experimental cross sections for reactions 2 are accurately reproduced using a loose phase space limit (PSL) TS model.[13] Previous work has shown that this model provides the most accurate assessment of the kinetic shifts for CID processes for electrostatic ion−molecule complexes.[11−13,45,46] Good reproduction of the data is obtained over energy ranges exceeding 2 eV and cross section magnitudes of at least a factor of 100. Table 1 also includes values of E_0 obtained without including the RRKM lifetime analysis. Comparison of these values with the E_0(PSL) values shows that the kinetic shifts are minor in the K^+ complexes, ≤0.07 eV. In the more strongly bound systems, the kinetic shift for the Li^+ complexes varied between 0.36 and 0.58 eV, while it only varied between 0.10 and 0.20 eV for the Na^+ complexes. The total number of vibrations changes for these bases (30 for uracil, and

(52) Foresman, J. B.; Frisch, Æ. *Exploring Chemistry with Electronic Structures Methods*, 2nd ed.; Gaussian: Pittsburgh, 1996.
(53) Boys, S. F.; Bernardi, R. *Mol. Phys.* **1979**, *19*, 553.
(54) Van Duijneveldt, F. B.; van Duijneveldt-van de Rijdt, J. G. C. M.; van Lenthe, J. H. *Chem. Rev.* **1994**, *94*, 1873.
(55) Hill, S. E.; Glendening, E. D.; Feller, D. *J. Phys. Chem. A* **1997**, *101*, 6125. Hill, S. E.; Feller, D.; Glendening, E. D. *J. Phys. Chem. A* **1998**, *102*, 3813.
(56) Nicholas, J. B.; Hay, B. P.; Dixon, D. A. *J. Phys. Chem.* **1999**, *103*, 1394.

Table 1. Threshold Dissociation Energies at 0 K and Entropies of Activation at 1000 K of M^+L[a]

M^+L	σ_0[b]	n[b]	E_0[c] (eV)	E_0(PSL) (eV)	kinetic shift[d] (eV)	$\Delta S^\dagger$(PSL) (J mol^{-1} K^{-1})
Li^+(uracil)	3.2 (0.2)	1.4 (0.1)	2.55 (0.04)	2.19 (0.06)	0.36	27 (2)
Na^+(uracil)	29.9 (0.8)	1.3 (0.1)	1.50 (0.09)	1.40 (0.04)	0.10	23 (2)
K^+(uracil)	38.5 (1.4)	1.2 (0.1)	1.11 (0.04)	1.08 (0.03)	0.03	19 (2)
Li^+(thymine)	3.0 (0.2)	1.2 (0.1)	2.76 (0.08)	2.18 (0.07)	0.58	28 (2)
Na^+(thymine)	10.7 (0.5)	1.1 (0.1)	1.60 (0.06)	1.40 (0.04)	0.20	25 (2)
K^+(thymine)	50.8 (4.3)	1.1 (0.1)	1.15 (0.05)	1.08 (0.04)	0.07	21 (2)
Li^+(adenine)	2.4 (0.1)	1.5 (0.1)	2.80 (0.04)	2.34 (0.06)	0.48	55 (3)
Na^+(adenine)	15.7 (0.8)	1.1 (0.1)	1.55 (0.05)	1.45 (0.04)	0.10	62 (2)
K^+(adenine)	43.8 (2.9)	0.9 (0.1)	1.00 (0.04)	0.99 (0.03)	0.01	57 (2)

[a] Uncertainties are listed in parentheses. [b] Average values for loose PSL transition state. [c] No RRKM analysis. [d] Difference between E_0 and E_0(PSL).

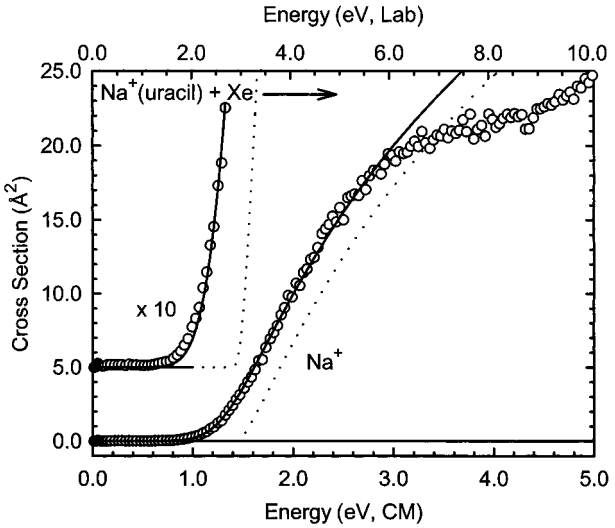

Figure 3. Zero-pressure extrapolated cross sections for collision-induced dissociation of the Na^+(uracil) complex with Xe in the threshold region as a function of kinetic energy in the center-of-mass frame (lower *x*-axis) and the laboratory frame (upper *x*-axis). The solid line shows the best fit to the data using the model of eq 1 convoluted over the neutral and ion kinetic and internal energy distributions. The dashed line shows the model cross sections in the absence of experimental kinetic energy broadening for reactants with an internal energy of 0 K.

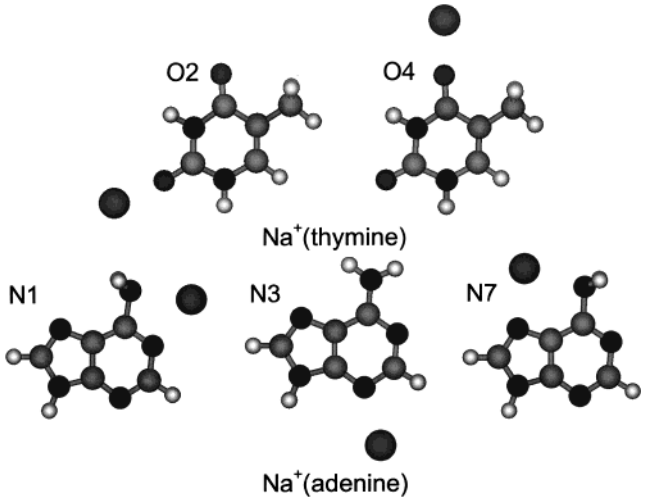

Figure 4. Stable geometries of Na^+(thymine) complexes bound at the O2 and O4 sites, and Na^+(adenine) complexes bound at the N1, N3, and N7 sites, optimized at the MP2(full)/6-31G* level of theory.

39 for thymine and adenine), which explains why uracil has the smallest kinetic shifts. However, the largest kinetic shifts occur in the thymine systems which actually have one fewer heavy atom than adenine, but have lower frequencies (Table 1S) resulting in a higher density of states at a given energy and thus a lower dissociation rate constant.

The entropy of activation, $\Delta S^\dagger$, is a measure of the looseness of the TS and also a reflection of the complexity of the system. It is largely determined by the molecular parameters used to model the energized molecule and the TS, but also depends on the threshold energy. Listed in Table 1, $\Delta S^\dagger$(PSL) values at 1000 K decrease from the adenine to the thymine which is only slightly larger than that for the uracil systems. These entropies of activation can be favorably compared to $\Delta S^\dagger_{1000}$ values in the range of 29−46 J mol^{-1} K^{-1} collected by Lifshitz for several simple bond cleavage dissociations of ions.[57] One of the reasons for the larger entropy change in the adenine systems is related to the frequencies associated with the amino group. In free adenine, there are low-frequency motions associated with the umbrella motion of the NH_2 group and the C−NH_2 rotor, while these motions are constrained in the complexes because of metal ion chelation, discussed in detail below.

The threshold values given in Table 1 differ somewhat from preliminary values previously published.[17] Data for most systems were reacquired to ensure the reliability of the results presented here. In addition, a number of improvements to our data analysis procedure for such large systems have evolved since this preliminary work. One distinction between the two sets of numbers is our previous use of AM1 vibrational frequencies scaled by 0.90. Compared with the frequencies used here, this overestimates the internal energy of the reacting complex and hence yields a threshold that is slightly too high. Despite the changes in the data analysis and the additional data available, the preliminary numbers and those obtained here do not disagree by more than the combined experimental errors, except for the Li^+(thymine) system. Here, our preliminary bond energy appeared anomalously high compared to the uracil systems. On this basis and the theoretical results discussed next, the present value is certainly correct.

Theoretical Results. Theoretical structures for the neutral bases and for the complexes of these molecules with H^+, Li^+, Na^+, and K^+ were calculated as described above. Details of the final geometries for each of these species are provided in the Supporting Information, Table 3S. Results for the most stable conformations of the sodium ion−base complexes are shown in Figure 4 for thymine and adenine.[58] Structures for uracil complexes are very similar to those for thymine. The 0 K calculated proton and metal binding energies, performed at the MP2(full)/6-311+G(2d,2p)//MP2(full)/6-31G* level including

(57) Lifshitz, C. *Adv. Mass Spectrom.* **1989**, *11*, 113.

(58) Figures were generated using the output of Gaussian98 geometry optimizations in Hyperchem Computational Chemistry Software Package, Version 5.0, Hypercube Inc., 1997.

Table 2. Calculated Enthalpies of Proton and Alkali Metal Ion Binding to Nucleic Acid Bases at 0 K in kJ/mol

	experiment			theory (MP2)			
		literature					
complex	TCID[a]	b	adjusted[c]	binding site	D_e[d]	D_0[e]	$D_{0,BSSE}$[f]
H^+(uracil)		866.6		O4	879.5	846.7	837.5
					867.6	835.5	826.4
				O2	845.2	815.1	805.8
					840.2	810.4	801.0
Li^+(uracil)	211.5 (6.1)	209 (4)	197 (20)	O4	207.5	201.2	194.9
				O2	192.0	186.7	180.5
				π	68.3	65.0	56.5
Na^+(uracil)	134.6 (3.4)	140 (4)	129 (25)	O4	146.2	142.5	134.5
				O2	132.6	129.7	122.8
K^+(uracil)	104.3 (2.8)	100 (4)	96 (12)	O4	110.8	107.8	103.8
				O2	98.4	96.1	92.2
H^+(thymine)		874.8		O4	886.8	854.4	844.9
					875.5	843.6	834.5
				O2	860.0	830.2	820.9
					855.5	825.9	816.6
Li^+(thymine)	210.1 (7.0)	213 (4)	200 (20)	O4	208.3	202.2	195.8
				O2	201.0	195.8	189.6
				π	79.0	75.6	66.6
Na^+(thymine)	135.3 (3.8)	143 (4)	136 (25)	O4	146.0	142.4	135.2
				O2	140.0	137.2	130.2
K^+(thymine)	104.0 (3.8)	101 (4)	97 (12)	O4	110.4	107.6	103.4
				O2	104.7	102.6	98.6
H^+(adenine)		936.8		N1	961.6	926.1	916.3
				N3	953.4	917.7	908.0
				N7	924.0	888.7	879.3
Li^+(adenine)	226.1 (6.1)	222 (4)	210 (20)	N7	218.6	208.3	199.8
				N1	209.4	199.8	191.8
				N3	184.1	177.5	171.2
				π	135.8	129.6	121.3
Na^+(adenine)	139.6 (4.2)	166 (4)	160 (25)	N7	144.7	138.0	128.6
				N1	139.9	133.6	124.6
				N3	125.0	121.2	114.0
K^+(adenine)	95.1 (3.2)	104 (4)	105 (11)	N7	93.5	88.3	82.9
				N1	92.3	87.5	82.4
				N3	89.1	86.2	82.0
H^+(imidazole)		942.8			967.9	931.9	922.2
Li^+(imidazole)	210.8 (9.5)[g]				215.5	208.7	202.7
Na^+(imidazole)	139.7 (5.2)[g]				155.1	150.8	144.5
K^+(imidazole)	109.0 (5.6)[g]				115.6	112.3	108.6

[a] Threshold collision-induced dissociation. Present results except as noted. [b] All literature values for protonated systems taken from: Hunter, E. P.; Lias, S. G. Proton Affinity Evaluation. In *NIST Chemistry WebBook*, NIST Standard Reference Database Number 69; Mallard, W. G., Lindstrom, P. J., Eds.; November, 1998, National Institute of Standards and Technology: Gaithersburg MD, 20899 (http://webbook.nist.gov). Values for metalated systems are taken from ref 23 and adjusted to 0 K. [c] Literature values from ref 23 adjusted as described in the text. Uncertainties are estimated in the text. [d] Calculated at the MP2(full)/6-311+G(2d,2p) level of theory using MP2(full)/6-31G* optimized geometries. [e] Including zero-point energy corrections with frequencies scaled by 0.9646. [f] Also includes basis set superposition error corrections. [g] Reference 14.

ZPE corrections and BSSE corrections,[59−61] are listed in Table 2. Independent ZPE and BSSE corrections are made for all conformers listed.

Uracil. The calculations find that the preferred binding site for the alkali cations to uracil is at O4. The C═O−M^+ bond angle is very nearly linear but shifted slightly away from the adjacent NH group. It is interesting to note that this shift is *away* from the global dipole moment of uracil, Figure 1. Changes to the structure of the uracil molecule upon metal ion complexation are minor (Table 3S). We find that the alternate binding site at O2 is higher in energy by 14.4 kJ/mol for Li^+, 11.7 kJ/mol for Na^+, and 11.6 kJ/mol for K^+.

The possibility that the metal ion might be bound to the π-electrons above the uracil ring was also investigated. For Li^+, we located a stable geometry for such a π-complex but find it is less stable by 138 kJ/mol (Table 2) than the ground-state geometry (O4 binding). In this geometry, the Li^+ ion interacts most strongly with the two nitrogen atoms, which appear to have undergone a change in hybridization from sp^2 toward sp^3. As a consequence, there is a gross distortion of the aromatic ring from planarity that destroys much of the resonance delocalization leading to a high-energy species. Because of the relative instability of this conformation, we did not pursue calculations of π-complexes for Na^+ and K^+.

We also obtained results for protonated uracil, Tables 2 and 3S. Here, the preferred binding site is the same as the metal ions, O4, but there are larger perturbations on the structure. This is seen most clearly by noting that the C−O−H bond angle is 112°, indicating sp^2 hybridization, in direct contrast to the C═O−M^+ bond angles which are nearly linear. This rehybridization results in structural changes throughout the aromatic molecule (Table 3S). It is interesting to note that the preferred binding site places the proton in the plane and directed away from the adjacent NH group. The conformation in which the proton is directed toward the NH group lies 11.1 kJ/mol higher in energy.

(59) Møller, C.; Plesset, M. S. *Phys. Rev.* **1934**, *46*, 618.
(60) Bartlett, R. J. *Annu. Rev. Phys. Chem.* **1981**, *32*, 359.
(61) Hehre, W. J.; Radom, L.; Schleyer, P. v. R.; Pople, J. A. *Ab Initio Molecular Orbital Theory*; Wiley: New York, 1986.

The proton affinity of the O2 site of uracil is calculated to be lower than the O4 site by 31.7 kJ/mol (Table 2).

Thymine. Thymine is 5-methyluracil and, as such, it is not surprising that the calculations find geometries for the alkali metal ion−thymine complexes that are very similar to those for uracil. The preferred binding site is again at O4 with slightly shorter metal−oxygen bond lengths than the uracil complexes. Changes to the structure of the thymine molecule upon metal ion complexation are again minor. The alternate conformation where M^+ is attached to O2 is higher in energy by 6.2 kJ/mol for Li^+, 5.0 kJ/mol for Na^+, and 4.8 kJ/mol for K^+, about half the differences calculated for the uracil complexes. A π-complex could again be located for this base bound to Li^+, but it is 129 kJ/mol less stable than the ground-state geometry (Table 2). As for the planar structures, the π-complex for thymine is structurally similar to that found for uracil.

As for uracil, thymine prefers protonation at the O4 site, with the proton lying in the plane directed away from the NH group. In this case, it is interesting to note that the steric interaction with the nearby methyl group is insufficient to change the geometry from that observed for uracil. As with uracil, protonation results in large perturbations to the structure of the thymine molecule, again a consequence of the rehybridization at the O4 center.

Adenine. Calculations find that the metal ions prefer to be bound to the imidazole nitrogen atom, N7, and are further stabilized by interaction with the amino group of the six-membered ring. In neutral adenine, the NH_2 group is planar and sp^2-hybridized to allow delocalization of the lone pair electrons with the aromatic π-electrons of the purine rings. (The C6−NH_2 bond order is generally regarded as 1.41−1.47.)[62] Upon metal complexation, chelation with the amino group requires that it rotate out of the plane of the molecule and rehybridize from sp^2 to sp^3. This rehybridization is indicated by the H−N−H bond angle, which changes from 120.6° in neutral adenine to 103−104° in the metal complexes, and by the C6−NH_2 bond length, which increases by almost 0.1 Å upon metal complexation (Table 3S). Other than these changes, the distortion of the adenine molecule that occurs upon complexation to a metal cation is minor.

We also investigated conformations in which the metal ion binds to the N1 and N3 sites of adenine. For the N1 site, complexes with Li^+, Na^+, and K^+ lie 8.0, 4.0, and 0.5 kJ/mol, respectively, above the complexes bound at the N7 site. Binding at the N1 site is similar to the ground state conformation in that chelation with the amino group is again observed; however, this forms a four-membered rather than a five-membered ring. Presumably, the sterics of this smaller ring help explain why the N1 binding site is slightly less favorable for alkali ions than the N7 site. Binding at the N3 site is much less preferred; complexes with Li^+, Na^+, and K^+ lie higher than the ground state by 28.6, 14.6, and 0.9 kJ/mol, respectively. This preference is apparently a result of the chelation with the amino group available at the N7 and N1 sites, even though rotation of the amino group costs π-resonance delocalization energy (as discussed in detail below). We note that in contrast to the N1 and N7 complexes, the C6−NH_2 bond lengths in the N3 complexes decrease slightly, ∼0.02 Å, consistent with an increase in double bond character and with the observation made above that the NH_2 group umbrella vibration no longer has a calculated imaginary value.

In the gas phase, we also need to consider the possibility of alternate tautomers to the structure shown in Figure 1. While such tautomers are not possible in nucleosides, it is conceivable that they are accessible in our gas-phase studies. However, in previous work on the interactions of alkali metal ions with azoles,[14] we demonstrated fairly conclusively that tautomerization of N-heterocycles in the gas phase or upon metal ion complexation did not occur. This was attributed to excessively high potential energy barriers between the possible complexes.[63] Rather, complexation to the most stable gas-phase tautomer of the free ligand was observed exclusively. For adenine, this is the tautomer identified in Figure 1, N9H, which our calculations indicate is more stable than the tautomer where the H atom is located at N7 by 31.0 kJ/mol. This latter tautomer, N7H, is of potential interest because metal ion binding at the N9 or N9/N3 chelation site of such a tautomer is known to be preferred to binding at N7 for purine free bases in solution, where tautomerization occurs readily.[62] Indeed, our calculations show that alkali ion complexes of N7H-adenine do chelate between the N9 and N3 sites, and are more stable by 17.3, 30.3, and 37.9 kJ/mol for Li^+, Na^+, and K^+ compared to the N9H-adenine complexes (excluding ZPE and BSSE corrections). Thus, bond dissociation energies for M^+(N7H-adenine) are 48.3, 61.3, and 68.9 kJ/mol, respectively, stronger than those of the ground-state M^+(N9H-adenine) complexes. This assumes that tautomerization does not occur upon CID, an assumption consistent with the results of our previous studies.[14]

The possibility that the metal ion could be bound to the π-electrons of either the five- or six-membered ring of adenine was investigated for Li^+. We located a π-complex in which Li^+ lies above the six-membered ring shifted away from the center toward the N1−(CH)2−N3 side of the ring. Distortion of the adenine ligand from planarity is small. This complex is 78.5 kJ/mol higher in energy than the ground-state complex, in contrast to the much larger differences in stability for the planar and π-complexes of Li^+ with uracil and thymine. This distinction is easily understood because there is much less loss of resonance stabilization in the adenine system. These results are consistent with those of Amunugama and Rodgers,[64] who have thoroughly investigated π-complexes of pyrimidine (1,3-*c*-$C_4H_4N_2$), the aromatic *N*-heterocycle most closely related to the six-membered ring of adenine. For adenine, we could find no stable minimum with Li^+ located above the five-membered ring, consistent with previous theoretical results in which no π-complexes were found for imidazole, the analogous five-membered *N*-heterocycle.[14]

In contrast to the results for the metal ion affinities, the proton affinity of adenine is highest at the N1 site, which is slightly higher than N3, which is significantly greater than N7. This reordering is clearly a result of the inability of the proton to chelate, and it emphasizes the energetic consequences of such chelation in the metal complexes. Geometries with the proton located at the imidazole NH and amine sites were not pursued because calculations at the AM1 level of theory indicate that these are much less stable (by 40 and 90 kJ/mol, respectively) than that for N7, the weakest of the other three nitrogen sites.

Discussion

Trends in the Binding of Metal Ions to the Nucleic Acid Bases. In all of the M^+L systems, the measured binding strength

(62) Saenger, W. *Principles of Nucleic Acid Structure*; Springer-Verlag: New York, 1984.

(63) Wong, M. W.; Leung-Toung, R.; Wentrup, C. *J. Am. Chem. Soc.* **1993**, *115*, 2465 and references therein.

(64) Amunugama, R.; Rodgers, M. T. *Int. J. Mass Spectrom.* **2000**, *195/196*, 439.

varies with the metal ion such that Li^+ binds ~60% more strongly than Na^+, which in turn binds ~35% more strongly than K^+. Because these complexes are largely electrostatic in nature, this is easily understood on the basis of the size or, equivalently, the charge density on the metal. The smaller the ion, the greater the charge density of the metal, and therefore, the greater the strength of the ion−dipole and ion−induced dipole interactions in these systems. In this simplistic point of view, the strength of the interaction of the metal ions with the nucleic acid bases appears to be driven principally by the ion−induced dipole interaction, which does not change appreciably for the structurally similar uracil and thymine complexes. The structure of adenine is sufficiently distinct from these two bases that direct comparisons are difficult. The relative bond strengths are inversely related to the dipole moments (Table 2 and Figure 1). To some extent, this is because the metal ions are not able to bind in sites that allow alignment with the dipole moment (Figures 1 and 4). Indeed, in complexes with uracil and thymine bound at the O4 position, the metal ions prefer to tilt *away* from the adjacent NH group (by 4−8°) even though this moves the ion into a position almost perpendicular to the dipole of the molecule. This motion is because the steric interactions with the NH group are substantially greater than those with the CH (or CCH_3) group. In contrast, when the metal ions bind to the O2 position, where there is an adjacent NH group on both sides, the metal ion tilts toward the direction leading to a stronger interaction with the dipole of the molecule (by a more uniform 6−7°).

Theoretical examination of the charge retained on the metals in these complexes shows that for the uracil and thymine complexes, Li^+ has a charge of ~0.81e, which is lower than that for Na^+ (~0.86e), which is lower than that for K^+ (~0.92e). The adenine complexes have a different dependence, 0.76e, 0.90e, and 0.98e, respectively. These results confirm the electrostatic nature of the bonding, but also demonstrate that there is some covalency in the metal−ligand interaction, especially in the Li^+ systems. In these systems, the shorter bond distance and greater charge density allow the metal ion to more effectively withdraw electron density from the neutral ligand, thus reducing the charge retained on the metal. The different variation for adenine parallels the metal-dependent changes in BDEs, i.e., Li^+ binds more strongly to adenine than to uracil and thymine, hence the effective charge retained by the metal is less. For K^+, adenine binds less strongly than uracil and thymine, reflecting the lower degree of electron donation to the metal ion. For Na^+, the calculated charge on the metal ion is slightly greater in the adenine system, consistent with a weaker theoretical BDE compared to the uracil and thymine systems. Experimentally, the adenine BDE is slightly stronger, which may suggest more charge transfer than theoretically determined.

The metal dependence of the binding to adenine can be understood more clearly by comparing these results to those for these same metal ions bound to imidazole, measured previously in our laboratory.[14] Imidazole, *c*-$C_3N_2H_4$, has a binding site equivalent to the N7 binding site in adenine, but does not have the nearby amino group. The BDEs of Li^+, Na^+, and K^+ to imidazole, listed in Table 2, are found to be similar to those to uracil and thymine, but compared to adenine BDEs, they are weaker for Li^+, comparable for Na^+, and weaker for K^+. Apparently, the strong metal dependence in the adenine BDEs is a balance between three competing effects, all associated with the interaction of the metal ion with the amino group. Rotation of the amino group out of the plane (i) decreases the stabilization associated with π-resonance delocalization, (ii) decreases repulsion between the metal ion and nearest hydrogen when the amino group is planar, and (iii) enhances binding as a result of chelation. The combination of these effects leads to chelation for all three metal ions as indicated by theory, which finds that the most stable conformations of the M^+(N7-adenine) complexes have the amino group rotated out of the plane.

The first effect, π-resonance delocalization, should have little dependence upon the metal ion. This effect was estimated as the difference in energies of free adenine in its most stable conformation and that of free adenine in the distorted geometries of the M^+(N7-adenine) complexes. These calculations indicate that this distortion costs about 55 kJ/mol, independent of the metal ion inducing the distortion. Nuclear magnetic resonance studies indicate that a similar distortion for cytosine costs 63−75 kJ/mol and less for adenine.[62] We estimate the second effect, H-atom repulsion, by calculating the binding energies of the three metal ions at the N7 site of adenine with its geometry fixed to that of the free base, i.e., planar. These complexes are less stable than the N7-adenine complexes by 62, 44, and 27 kJ/mol for Li^+, Na^+, and K^+, respectively. Compared to the interactions with imidazole, binding to planar adenine is weaker by approximately 59, 55, and 49 kJ/mol, respectively. We take these values to be a rough estimate of the H-atom repulsive energy. Combining the results of these calculations with the measured BDEs to imidazole and N7-adenine (Table 2), we arrive at estimates for the chelation effect, which is strongest for Li^+, ~129 kJ/mol, and decreases for the larger metal ions, ~110 kJ/mol for Na^+ and ~90 kJ/mol for K^+. Thus, Li^+ binds adenine more strongly than imidazole because the chelation energy is larger than the sum of the π-resonance delocalization energy lost and H-atom repulsion energy. For Na^+, these contributions balance, while for K^+, the sum of the latter two effects is larger than the chelation energy, such that binding to adenine is weaker than that to imidazole.

As for the metal ions, protons are bound to uracil and thymine with comparable BDEs (Table 2), consistent with theory finding that O4 is the favored binding site for all of these complexes. The proton affinity (PA) measured for thymine is somewhat greater than that for uracil (by 8.2 kJ/mol or 1%), presumably because of favorable inductive effects of the additional methyl group. A comparable 1% enhancement in the bonding for the metal ions (1−2 kJ/mol) is within the noise of the present experimental determinations. The PA for adenine is considerably higher than that for uracil or thymine; however, theory indicates that the preferred binding site for a proton is N1 rather than the N7 site used by the metal ions. Hence, a direct comparison of the PA of adenine with the metal ion BDEs is not informative.

Comparison between Theory and Experiment. The alkali metal cation affinities of uracil, thymine, and adenine at 0 K measured by guided ion beam mass spectrometry and calculated here are summarized in Table 2. The agreement between theory and experiment is illustrated in Figure 5. Values for imidazole[14] are also included for comparison. It can be seen that the agreement is reasonable over the nearly 130 kJ/mol variation in binding affinities measured here. For all 12 systems, the mean absolute deviation (MAD) between experiment and theory is 7.9 ± 8.4 kJ/mol, comparable to the expected computational accuracy (about 8 kJ/mol) and slightly larger than the average experimental error of 5.1 ± 1.9 kJ/mol. However, more careful inspection of the data makes it clear that principal contributors to the deviations are the Li^+ systems and the adenine complexes. For the four Li^+ systems, the MAD is 16.3 ± 7.6 kJ/mol, while the Na^+ and K^+ systems have a MAD of 3.7 ± 5.1 kJ/mol. The three adenine systems have a MAD of 16.5 ± 8.5 kJ/mol,

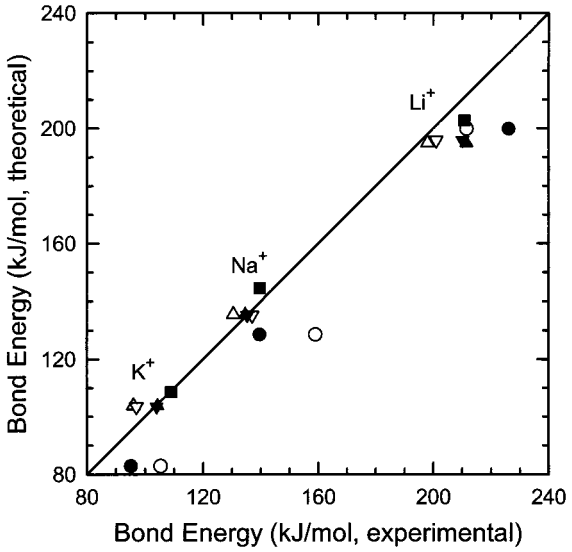

Figure 5. Theoretical versus experimental bond dissociation energies (in kJ/mol) for M^+-L where $M^+ = Li^+$, Na^+, and K^+ and L = uracil (▼, ▽), thymine (▲, △), adenine (●, ○), and imidazole (■). All values are at 0 K and are taken from Table 2. Experimental results include those from the present work and ref 14 as closed symbols, while open symbols show adjusted values from Cerda and Wesdemiotis (ref 23, Table 2). The diagonal line indicates the values for which calculated and measured bond dissociation energies are equal.

while the other nine systems have a MAD of 5.1 ± 6.5 kJ/mol. Theory systematically underestimates the bond energies for the Li^+ complexes, which may be a result of the higher degree of covalency in the metal−ligand bond. This is plausible as the discrepancy between theory at this level and experiment is even higher for the protonated complexes (MAD = 25.0 ± 5.2 kJ/mol), where the bonds are truly covalent. In the case of the adenine complexes, theory is again systematically low. Given the good agreement for the imidazole complexes, this could be a result of difficulties with describing the chelation accurately, especially given the subtle electronic effects associated with disrupting the delocalized π network when the amino group rotates out of the plane. Nevertheless, it is useful to note that theory and experiment agree on the trends in the BDEs for adenine relative to uracil and thymine, namely, Li^+(adenine) > Li^+(uracil/thymine) and K^+(adenine) < K^+(uracil/thymine). The situation is ambiguous for sodium although theory and experiment agree that Na^+(adenine) ≈ Na^+(uracil/thymine).

We also considered whether the strong experimental bond energies to adenine might be explained by the possibility that the metal ion binds to an alternative tautomer of adenine, namely N7H. The bond energies of this tautomer binding at the N9/N3 chelation site are approximately 248, 190, and 152 kJ/mol. (This assumes equivalent ZPE and BSSE corrections as those explicitly calculated for the N7 complex.) These values are well above the experimentally determined BDEs (Table 2). Hence, we conclude that the adenine complexes are formed by three-body associative reactions between the metal ion and the most stable gas-phase tautomer, N9H (Figure 1), in analogy with our previous work.[14]

Conversion from 0 to 298 K. To allow comparison to previous literature values and commonly used experimental conditions, we convert the 0 K bond energies determined here to 298 K bond enthalpies and free energies. The enthalpy and entropy conversions are calculated using standard formulas and the vibrational and rotational constants determined for the MP2-(full)/6-31G* optimized geometries, which are given in Tables 1S and 2S. Table 4S lists 0 and 298 K enthalpy, free energy, and enthalpic and entropic corrections for all systems experimentally determined (from Table 1) along with the corresponding theoretical values (from Table 2). Using these values, we have adjusted enthalpy values taken from the literature[23] (listed at 298 K) to 0 K. These are compared to the present results in Table 2 and Figure 5.

Comparison with Literature Values: **Experiment.** The only previous experimental values for the nucleic acid bases bound to alkali metal ions come from work of Cerda and Wesdemiotis (CW).[23] These authors used the kinetic method[65] to measure the alkali metal ion binding affinities of the bases. Before comparing these values with those obtained here, it is worth understanding the limitations on the absolute accuracy of these numbers. Of course, the kinetic method is not capable of directly measuring absolute metal ion affinities, but instead, relies on reference bases of known absolute affinity. In the study of CW, pyridine, *n*-propylamine, and aniline were used as references for K^+, with affinities taken from work of Davidson and Kebarle.[66] This work used high-pressure mass spectrometry to acquire absolute K^+ affinities with uncertainties of ±4 kJ/mol. This set of references should be adequate, although these three-nitrogen-based molecules are all monodentate. In the context of the kinetic method, where comparisons between similar ligands are highly desirable, these should be reasonable references for the monodentate thymine and uracil bases, but may be questionable for guanine, cytosine, and adenine, which are bidentate. This point was realized by CW, who included an explicit consideration of the entropic effects that these differences would cause.[67] Recently, we have pointed out that the method used in this work to extract entropies and to extrapolate to enthalpies free of such entropic effects is not statistically rigorous.[68] Unfortunately, CW used only two excitation conditions (metastable, MI, and collisional-activated dissociation, CAD, with He), such that the measurements of the metal ion affinity, $\Delta H°_{M^+}$, and entropy difference between the competing dissociation channels, $\Delta(\Delta S°_{M^+})$, are underdetermined, i.e., these two thermodynamic values are obtained from a linear regression analysis of only two experimental points. As such, the statistical uncertainties in these values are infinitely large. In our work,[68] we have shown that similar kinetic method experiments involving at least three excitation conditions yield relative uncertainties of 2−12 kJ/mol (but can be much higher) compared to the <±2 kJ/mol value estimated by CW. These relative uncertainties must then be combined with the absolute uncertainties of the reference to yield the final uncertainties. Thus, a conservative estimate of the relative uncertainties in the K^+ affinities of CW is at least 10 kJ/mol (but technically, the values are underdetermined). This estimate is sensible when it is realized that CW obtained K^+ affinities at 298 K for uracil, thymine, and adenine of 101, 102, and 106 kJ/mol, respectively, from measurements relative to the references. However, they obtained 93, 94, and 107/109 kJ/mol, respectively, when measured relative to the other nucleic acids. Although the "final" numbers reported by CW are the former set, the latter set demonstrates that a relative uncertainty of at least 10 kJ/mol is appropriate. Table 2 lists an average of both sets of numbers from CW (corrected to 0 K) along with the appropriate estimated uncertainties.

(65) Cooks, R. G.; Kruger, T. L. *J. Am. Chem. Soc.* **1977**, *99*, 1279. McLuckey, S. A.; Cameron, D.; Cooks, R. G. *J. Am. Chem. Soc.* **1981**, *103*, 1313. Cooks, R. G.; Koskinen, J. T.; Thomas, P. D. *J. Mass Spectrom.* **1999**, *34*, 85.

(66) Davidson, W. R.; Kebarle, P. *J. Am. Chem. Soc.* **1976**, *98*, 6133.

(67) Cheng, X.; Wu, Z.; Fenselau, C. *J. Am. Chem. Soc.* **1993**, *115*, 4844. Wu, Z.; Fenselau, C. *Rapid Commun. Mass Spectrom.* **1994**, *8*, 777.

(68) Armentrout, P. B. *J. Am. Soc. Mass Spectrom.* **2000**, *11*, 371.

The same difficulties in the kinetic method analysis also plague the values determined for Li^+ and Na^+, but here, there are more serious problems as well. For these two metal ions, the amino acids, glycine, alanine, and valine, were used as references, with values taken from a study by Bojesen et al.,[69] who also used the kinetic method. The amino acids are bidentate ligands (binding at the carbonyl oxygen and amino sites) such that the kinetic comparison with adenine, guanine, and cytosine is favorable, but not with the monodentate uracil and thymine bases. The use of only two excitation methods (MI and CAD/He) restricts the ability of CW to extract both $\Delta H°_{M^+}$ and $\Delta(\Delta S_{M^+})$ in a statistically meaningful way. Again the relative uncertainties in these procedures are probably about 10 kJ/mol.

Oddly, CW state that the uncertainties in their final values are limited by the references, which they claim have errors of ±4 kJ/mol (which is correct for K^+). In contrast, Bojesen et al.[69] explicitly state that the uncertainties in their values are of the order of 12−20 kJ/mol. In our view, this is not a conservative estimate. Bojesen et al. found it difficult to generate complexes containing the desired amino acids with ligands having known metal ion affinities. They settled on using a single reference, dimethyl formamide (DMF), for Li^+ and no reference at all for sodium. Because only a single reference was used for the lithium case, the authors needed to estimate the scaling parameter (generally referred to as the effective temperature) used to obtain quantitative differences in the metal ion affinities. They made this estimate using a value determined from proton affinity determinations made in the same apparatus. No estimate of the error in this procedure was specified, but it is now well-known that the effective temperature is a sensitive function of many parameters (not just the instrument used).[70,71] It seems unlikely that the effective temperature in proton vs lithium bound systems can be equated, hence, the error in this estimate is appreciable. Indeed, Bojesen et al. justify this estimate by pointing out that the ΔH_{Li^+}(alanine) value they obtain, 220.1 kJ/mol, "is in agreement with the calculated value" of 249.8 kJ/mol.[72] They suggest that the theory is likely to be too high by about 13 kJ/mol on the basis of comparison to proton affinities. Even being generous, the authors clearly believe their values could be in error by about 17 kJ/mol. When this is combined with the uncertainty of 10 kJ/mol associated with the extraction of $\Delta H°_{M^+}$ and $\Delta(\Delta S°_{M^+})$, the likely uncertainty in the lithium ion affinities of CW is at least 20 kJ/mol and could be much higher.

In addition, the lithium ion affinity of DMF was taken from work of Taft et al.[73] Bojesen et al. do not specify how they convert the free energy measured by Taft et al. at 373 K, $\Delta G_{373} = 185.4$ kJ/mol, to the lithium ion affinity they used, $\Delta H_{Li^+} = 209.2$ kJ/mol. (Presumably, this value refers to 298 K, although no temperature is actually specified.) Since this paper was published, we have pointed out that the scale of Taft et al. was incorrectly calibrated and should be reduced by 7.1−13.4 kJ/mol, with a shift of 12.1 ± 1 kJ/mol being most likely.[12] This has now been confirmed by Burk et al., who suggest that the lithium ion free energies of Taft et al. (and affinities derived therefrom) be decreased by 10.9 kJ/mol, in good agreement with our suggested shift.[74] Thus, all the lithium ion affinity values of Bojesen et al. and CW should be decreased by 12 kJ/mol. (Note that this adjustment makes the agreement between experiment and theory for ΔH_{Li^+}(alanine) worse.)

In the case of sodium, no adequate references were available to Bojesen et al.[69] Instead, the authors assumed that the sodium ion affinity of alanine was 75% of the lithium ion affinity. This estimate was based on four systems where both metal ion affinities are known (and an average ratio of 73% is obtained) and theory where a ratio of 68% is obtained. Hence, the uncertainty in these values is at least 7%, which propagates to a value of 15 kJ/mol. This uncertainty does not include the uncertainty of ΔH_{Li^+}(alanine), about 17 kJ/mol (or more), or the uncertainty associated with the derivation of $\Delta H°_{M^+}$ and $\Delta(\Delta S°_{M^+})$, about 10 kJ/mol. This means that the uncertainties in the absolute sodium ion affinities are at least ±25 kJ/mol. Further, the shift in the lithium ion affinities should be propagated to the sodium values by decreasing them by 9 kJ/mol (75% of 12 kJ/mol). Adjusted values of the lithium and sodium ion affinities from CW (average values of all determinations) with more properly estimated errors are included in Table 2.

Having evaluated the reliability of the previous experimental determinations of CW, we can now compare these results with those from the present study, Table 2 and Figure 5. This comparison shows that there is rather good agreement between the present results and those measured previously for most of the nine systems. For the uracil and thymine systems, the mean average deviation for the Li^+, Na^+, and K^+ complexes is 7.6 ± 4.9 kJ/mol, respectively, well within the experimental uncertainties. For the adenine systems, the comparisons are less favorable, with a MAD of 15.5 ± 5.5 kJ/mol. Furthermore, the differences are not systematic, with the results of CW being higher than our values for Na^+(adenine) and K^+(adenine), but lower for Li^+(adenine). Compared to theory, the values of CW for the uracil, thymine, and adenine complexes with all three metal ions have a MAD of 9.9 ± 10.4 kJ/mol (vs the MAD between our results and theory of 9.1 ± 9.4 kJ/mol). Similarly, the MAD for CW's results compared to theory for the adenine systems is 21.5 ± 10.7 kJ/mol, compared to our MAD vs theory of 16.5 ± 8.5 kJ/mol. Given the good agreement between our results and theory and our comprehensive investigation of alternate conformers, it appears that the kinetic method result is clearly too high in the Na^+(adenine) case, although this result is probably still accurate within the conservative errors reassigned here. Because the other values are in reasonable agreement with those in the present study, the source of this particularly large discrepancy is unclear. It is worth noting that the Na^+ affinities to guanine and cytosine cited by CW appear to be equivalently high.

Comparison with Literature Values: Theory. We can also compare our theoretical results to those obtained previously. There appear to be few previous studies of the interactions of the alkali metal cations with nucleic acid bases.[24,25,27] Early calculations by Del Bene[24] and Anwander et al.[25] were at low levels and used constrained geometries, leading to results not directly comparable to the results obtained here. However, it is interesting to note that Anwander et al. conclude that metal ion complexation enhances the hydrogen bonding in both A:T and G:C base pairs, a direct consequence of charge relocalization induced by the positively charged metal ions.

(69) Bojesen, G.; Breindahl, T.; Andersen, U. N. *Org. Mass Spectrom.* **1993**, *28*, 1448.
(70) Ervin, K. M. *Int. J. Mass Spectrom.* **2000**, *195*/*196*, 271.
(71) Drahos, L.; Vékey, K. *J. Mass Spectrom.* **1999**, *34*, 79.
(72) Jensen, F. *J. Am. Chem. Soc.* **1992**, *114*, 9533.
(73) Taft, R. W.; Anvia, F.; Gal, J.-F.; Walsh, S.; Capon, M.; Holmes, M. C.; Hosn, K.; Oloumi, G.; Vasanwala, R.; Yazdani, S. *Pure Appl. Chem.* **1990**, *62*, 17.
(74) Burk, P.; Koppel. I. A.; Koppel. I.; Kurg, R.; Gal, J.-F.; Maria, P.-C.; Herreros, M.; Notario, R.; Abboud, J.-L. M.; Anvia, F.; Taft, R. W. *J. Phys. Chem. A* **2000**, *104*, 2824.

A more recent calculation[27] considered several mono- and divalent cations interacting with adenine and guanine. These calculations were performed at the MP2 level (both all electron and pseudopotential methods, although no appreciable differences between these two were observed) with a 6-31G** basis set for the ligands and the DZ basis set of Schäfer et al.[75] for the metal ions. In this work, Burda et al. considered only binding at the N7 position of adenine because this is the position known to be active in various biological systems.[25] The geometry of adenine was constrained to be planar such that no chelation with the amino group was possible analogous to calculations performed here to estimate the H-atom repulsion energy. Compared to our results, the bond lengths calculated by Burda et al. are slightly shorter for Li^+ and Na^+ but longer for K^+. Because chelation interactions are not considered, the bond energies calculated in this work (which include BSSE corrections but not ZPE) are much weaker than those determined here for M^+(N7-adenine), by 41, 28, and 27 kJ/mol for Li^+, Na^+, and K^+, respectively (where the comparison is to our numbers excluding the ZPE corrections). This differs somewhat with our results, which find the planar complexes to be less stable by 62, 44 and 27 kJ/mol compared to M^+(N7-adenine), respectively.

This comparison raises an interesting point. In the gas phase, the interaction of the metal ions with isolated adenine is such that the amino group is free to rotate, although some π-resonance energy is lost. In double-stranded nucleic acids, however, adenine is base-paired with thymine (or uracil) such that the hydrogen bonds hinder rotation of the amino group. However, the interaction energy of the hydrogen bonding in an A:T pair is about 54 kJ/mol,[76] as measured for isolated bases methylated at the glycosidic bond. Thus, rotation of the amino group breaks a single hydrogen bond worth about 27 kJ/mol, and disrupts the π-delocalization worth about 55 kJ/mol. As estimated above, chelation increases the metal ion binding by 90−129 kJ/mol, depending on the metal ion. According to these estimates, binding at the N7 chelation site of adenine is clearly favorable for Li^+ (by 47 kJ/mol) and Na^+ (by 28 kJ/mol) and nearly thermoneutral for K^+ (by 8 kJ/mol). Thus, chelation could explain why metal ion complexation to the bases disrupts the stability of double-stranded nucleic acids. This conclusion differs from that of Anwander et al.,[25] who constrained their calculations such that chelation could not be considered. However, an alternative site for metal ion binding in an A:T (A:U) base pair is the O2 site of uracil or thymine, in which case no hydrogen bonds need be broken. Thus, in an A:T (or A:U) base pair, the O2 site is likely to be the thermodynamically preferred binding site.

(75) Schäfer, A.; Horn, H.; Ahlrichs, R. *J. Chem. Phys*. **1992**, *97*, 2571.
(76) Yanson, I.; Teplitsky, A.; Sukhodub, L. *Biopolymers* **1979**, *18*, 1149.

Conclusions

The kinetic energy dependence of the CID of M^+L, where $M^+ = Li^+$, Na^+, and K^+ and L = uracil, thymine, and adenine, with Xe is examined in a guided ion beam mass spectrometer. The dominant dissociation process is loss of the intact neutral ligand. Thresholds for these processes are determined after consideration of the effects of reactant internal energy, multiple collisions with Xe, and lifetime effects (using methodology described in detail elsewhere).[13] Insight into the structures and binding of the metal ions to the nucleic acid bases is provided by ab initio calculations of these complexes performed at the MP2(full)/6-311+G(2d,2p)//MP2(full)/6-31G* level of theory. Reasonable agreement between the experimentally determined alkali metal ion affinities and ab initio calculations is obtained, although the calculated values are systematically low for the Li^+ complexes and the three adenine systems (16 ± 8 kJ/mol). These discrepancies, which are not that severe, are not completely understood but appear to be a result of several subtle electronic effects that may require higher levels of correlation to predict accurately the BDEs in these systems. We note that the excellent agreement between our results, theory, and previous experimental results for the three metal ions bound to uracil and thymine led us to conclude that these ligands can act as reliable anchors for alkali metal cation affinity scales and can broaden the range of ligands available as absolute thermochemical anchors. Further, the combined experimental and theoretical results provide an understanding of the magnitude of the chelation effect on metal ion bonding. Such chelation appears to be sufficiently strong for Li^+ and Na^+ when bound to adenine that it can disrupt the hydrogen bonding involved in A:T (A:U) base pairs. Although not considered explicitly, it seems likely that other metal ions with high charge densities, e.g., biologically relevant divalent ions such as Mg^{2+}, Ca^{2+} and any of the monovalent or divalent transition metal ions, will behave similarly.[27]

Acknowledgment. This work is supported by the National Science Foundation, Grant CHE-9877162, and in part by an ASMS Research Award from Micromass (M.T.R.). Early parts of this work were also supported by the Petroleum Research Fund, administered by the American Chemical Society.

Supporting Information Available: Tables of vibrational frequencies and average vibrational energies at 298 K, rotational constants, MP2(full)/6-31G* geometry optimized structures, and enthalpies and free energies; figures giving cross sections for collision-induced dissociation (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

JA001638D