An anomalous dissociation of protonated cluster ions of DNA guanine-cytosine base-pair

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Received August 29, 2011, Revised September 1, 2011, Accepted September 1, 2011
First published on the web September 15, 2011; http://dx.doi.org/10.5478/MSL.2011.2.3.073

Abstract: In the collisionally-activated dissociation of the proton-bound cluster ions of DNA base guanine (G) and cytosine (C), G••H^+••C, the abundance of [CH^+] ions was found to be higher than that of [GH^+] despite the fact that G has a higher proton affinity than C. This unexpected observation seems to demonstrate another example that the simple kinetic method scheme does not work. We suggest that a kinetic factor or detailed dynamics governing the proton transfer and dissociation should be carefully considered in the applications of the kinetic method to the proton affinity measurements.

Key words: Pronton affinity, Collisional dissociation, Proton transfer, Guanine, Cytosine

Introduction

The kinetic method has been widely used for the thermochemical determination of gas-phase basicities, gas-phase acidities, proton affinities, electron affinities, ionization potentials, metal ion affinities, and so forrth.[1-4] In particular, the Cooks' kinetic method is based on the measurements of relative rates of competitive dissociations of a cluster ion comprised of the compound of interest and a reference compound.[5] For proton affinity (PA) measurements, the proton-bound cluster ions A••H^+••B are dissociated into the constituent ions of AH^+ or BH^+ by applying collision energy to A••H^+••B. By measuring the relative abundance of [AH^+] and [BH^+] peaks, the PA of the compound of interest is determined using the following equation.[1,5]

$$[AHB]^- \xrightarrow{k_1} AH^- + B \qquad [AHB]^- \xrightarrow{k_2} BH + A \tag{1}$$

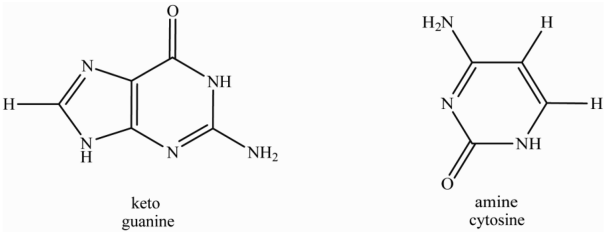
$$ln\left(\frac{[AH^+]}{[BH^+]}\right) = ln\left(\frac{k_1}{k_2}\right) \approx ln\left(\frac{Q_1^*}{Q_2^*}\right) + \frac{\Delta\varepsilon_0}{RT_{eff}} \approx \frac{\Delta(\Delta G)}{RT_{eff}} \tag{2}$$

$$\approx \frac{\Delta(\Delta H)}{RT_{eff}} \approx \frac{1}{RT_{eff}}(PA(A) - PA(B))$$

where Q_1^* and Q_2^* refer to the partition functions of the transition states of the two dissociation channels of A••H^+••B and T_{eff} is the effective temperature that is the characteristic temperature of an activated complex under chosen experimental conditions.

However, the deviation of the simple Cooks' kinetic method regime is observed sometimes and it was suggested that the entropy effect should be considered.[6,7] The consideration of the entropy effect widened the areas of kinetic method applications even to the case where the analyte and reference species are not structurally similar. In addition, the effective temperature was also a subject of extensive concern.[8,9] More interestingly, the failure of the kinetic method was also observed in the determination of proton affinities of bridgehead alkenes, where structural rearrangement of tricycle[3.3.3.0]undec-2(7)-ene occurring upon the addition of H^+ was shown to be responsible for the apparent discrepancy between its experimental proton affinity and the *ab initio* (MP4SDQ/6-311G*) predictions.[10]

In this study, we present another example of an anomalous dissociation of proton-bound complex of DNA guanine (G)-cytosine (C) base-pair (see Scheme 1 for their molecular



Scheme 1. Molecular structures of guanine and cytosine in the canonical keto- and amine-tautomeric forms, respectively.

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structures). This result demonstrates that the Cooks' kinetic method should be used with some discretion when the reaction scheme bears complex nature.

Experimental

Experiments were performed on a quadrupole ion-trap mass spectrometer (LCQ-Fleet, Thermo, CA, USA). The cluster ions of protonated G-C base-pair, *i.e.*, G••H^+••C, were generated in positive-ion mode by electrospraying 50 μM G and C in 49 : 49 : 2 (v/v/v) methanol : water : acetic-acid solution with the interface potential of +5.0 kV. The sample solution was directly infused using a built-in syringe pump at the flow rate of 3 μl/min. The G••H^+••C cluster ions at *m/z* 263.1 were isolated with a 1 Da width window and subjected to collisional activation dissociation (CAD). Each mass spectrum was obtained by averaging twenty scans. Guanine, cytosine, methanol (HPLC grade), and water (HPLC grade) were purchased from Sigma (Seoul, Korea).

Results and Discussion

Figure 1 shows the CAD mass spectra of G••H^+••C cluster ions obtained at three different collision energies. As clearly shown in Figure 1, the abundance of the protonated cytosine, [CH^+], was consistently larger than that of the guanine, [GH^+], irrespective of the collision energies. This result is quite unexpected considering that guanine has a little higher PA than cytosine; 229.3 *vs*. 227.0 kcal/mol.[11] According to the Cooks' kinetic method regime, the compound of the higher PA value should give rise to a higher abundance of its fragmenting ion in the competitive dissociation of the proton-bound complex under the assumption of negligible reverse barriers and entropy effect.[1,5] However, the observed results were quite different from this expectation.

To understand this unexpected results, it is necessary to carefully examine the energetic involved in the dissociation of the G••H^+••C cluster ions. Figure 2 shows the energy diagram of the important cluster and individual ion species that are involved in the dissociation of the G••H^+••C cluster ions. The energy values given in this diagram are from ref. 12, which were obtained by density functional theory (DFT) calculations (B3LYP/6-31+G(d,p)).[12] For consistency, all energy values in Figure 2 are taken only from the theoretical results even though experimental values for proton affinities of G and C are available. Proton affinities of G and C were predicted to be 228.15 and 226.61 kcal/mol, respectively, which is in good agreement of the experimental data.[11] The dissociation energies (D_o) of G••H^+C and GH^+••C were 39.70 and 37.18 kcal/mol, respectively, by calculations. Overall, the G••H^+C cluster ion was more stable than GH^+••C by 0.88 kcal/mol. Although the guanine monomer has a higher PA than the cytosine monomer, the G••H^+C cluster ions were found to be more stable than GH^+••C. In our recent preliminary DFT calculations at the level of B3LYP/6-31+G(d,p), it is also confirmed that the G••H^+C cluster has a higher stability in comparison with GH^+••C.[13]

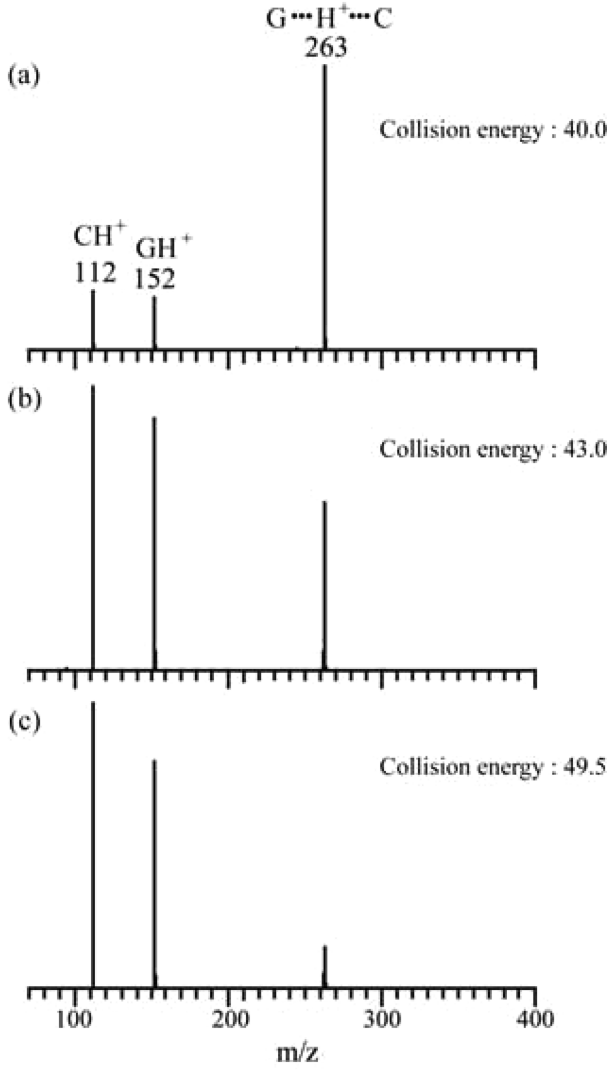

Figure 1. CAD mass spectra of G••H^+••C cluster ions at three different collision energies. The applied normalized collsion energies are (a) 40.0, (b)43.0, and (c) 49.5 in arbitrary unit.

From the perspective of the dissociation energies necessary to overcome to fragment into its corresponding protonated monomer and a neutral, *i.e.*, [GH^+] + C or [CH^+] + G, the dissociation channel into [CH^+] + G requires higher energy than the channel into [GH^+] + C; 39.70 *vs*. 37.18 kcal/mol, under the assumption of negligible reverse barriers. If any, the reverse barrier into the [GH^+] + C channel is not likely to be larger than that into the [CH^+] + G channel. Furthermore, the shallow (or negligible) proton transfer barrier between G and C would allow the proton of G••H^+C to be transferred into GH^+••C when the sufficient collision energy is provided to the cluster ion as it was in the present study. At the configuration of GH^+••C, it is likely that the reaction would follow a low energy dissociation pathway to give rise to [GH^+] + C. In this

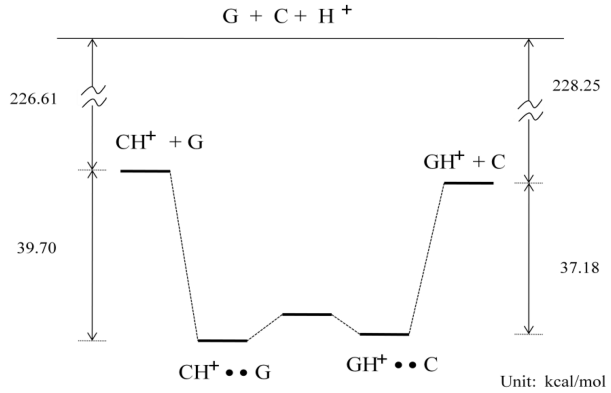

Figure 2. Energy diagram describing the important cluster and individual ion species that are involved in the dissociation of the G••H$^+$••C cluster ions. The energy values given in this diagram are from ref. 12, which were obtained by density functional theory (DFT) calculations (B3LYP/6-31+G(d,p)). All the energies denoted here include zero-point vibrational energy corrections.

sense, the observation of the preferred fragmentation into [CH$^+$] + G is quite puzzling.

The above results imply that when G••H$^+$••C dissociated upon collisional activation, the separation of G and C occurred along the local reaction profile before global thermal equilibrium involving the proton transfer was established. In other words, a little higher population of G••H$^+$C over that of GH$^+$••C due to the thermal stability (0.88 kcal/mol) was reflected directly in the abundances of the two dissociated monomers. The kinetic (or dynamic) factor seems to play a major role in the dissociation of the G••H$^+$••C cluster ions than the thermochemical factor does. It may be possible that the entropy effect explains the unexpected preferred dissociation into the G + [CH$^+$] channel. However, considering that the rigid planar structures of the involved guanine and cytosine molecules, the entropy effect that accompanies structural rearrangement upon the protonation or collisional activation does not seem to be responsible for the observed dissociation anomaly.

To summarize, the above unexpected dissociation of G••H$^+$••C into G + [CH$^+$] implies that the Cooks' kinetic method should be used with some caution as it was originally developed for the systems of weakly bound complexes with simple reaction profiles. It can also be suggested that the kinetic factor or detailed dynamics governing the proton transfer and dissociation should be considered in the application of the kinetic method for proton affinity measurements.

Acknowledgments

This work is supported by Seoul R&BD program (PA090889) and also by a Korea Research Foundation grant funded by the Korean government (MOEHRD, Basic Research Promotion Fund, KRF-2008-314-C00166).

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