Study of Target Gas Excitation Accompanying Collisional Activation of Large Polyatomic Ions

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Abstract: Studies on the dynamics of collision-induced dissociation (CID) of the $[M + H]^+$ ion of chlorophyll *a* are reported. The dynamical information derived from this study is based upon measurements of the energy loss accompanying CID, i.e., the change in the translational energy of the $[M + H]^+$ ion upon collision with the target gas. When helium is used as the target gas, the energy loss is quite large (ca. 24 eV), but the measured energy loss decreases as the ionization energy of the target gas is decreased. For example, the energy loss decreases from 24 to 9 eV when He, Ne, Ar, and Kr target gases are used. In addition, the energy loss decreases as the energy of the incident ion is decreased. We interpret the trend in the energy loss data as evidence that target gas excitation and collisional activation are competitive processes. The general conclusions from this work are supported by studies of ion-molecule reactions between translationally excited (keV energies) polyatomic ions (e.g., $C_6H_6^+$) and thermal neutral atoms and molecules. The latter studies were performed by Fourier transform mass spectrometry (FTMS). Based on these studies a general model for keV energy collisional activation is proposed.

Tandem mass spectrometry (TMS)[1] is a potentially useful method for structural characterization of large, biologically important molecules.[2-5] The TMS experiment involves mass-selecting a specific ion m_1^+ with MS-I, exciting the m_1^+ ion to an energy above the dissociation threshold, and mass-analyzing (with MS-II) the ionic products m_n^+ formed by dissociation of m_1^+. Typically, the incident ion m_1^+ is activated to energies above the dissociation threshold by an ion-neutral collision (collisional activation (CA) step), whereby a small fraction of the ions translational energy is converted into internal energy.[6-9] The dissociation of the collisionally activated ion (referred to as collision-induced dissociation (CID)) is useful for structural characterization and for trace level analysis of organic compounds.[1] Although the dynamics of the CID process have been studied quite thoroughly for small ionic systems[7-9] (less than 200 amu), there have been few studies on the dynamics of CID of large (greater than 1000 amu) molecular ions. The only studies reported to date which deal with the dynamics of CID of large molecular ions were limited to peptides.[10,11] Two important points emerge from the previous studies: (i) the energy loss accompanying CA increases as the mass of the incident ion (m_1^+) increases, and (ii) the efficiency of CID (per cent conversion of incident ions to fragment ions) decreases with increasing mass. Thus, the following question emerges: is the reduced efficiency for CID of large molecular ions related to the dynamics of the CA step or the dynamics of dissociation of large molecular ions?

Collision-induced dissociation (CID) is usually described in terms of a two-step mechanism, i.e., the keV energy ion/neutral collision converts some fraction of the translational energy of the incident ion m_1^+ into internal energy (collisional activation (CA)), and subsequently the ion dissociates by vibrational predissociation.[12] A tandem mass spectrometer (such as shown in Figure 1) can be used to probe the details of the CA process and the masses of the CID product ions.[13,14] In this experiment a mass-selected (by MS-I) ion beam is passed through a high-pressure collision cell where CA occurs. The subsequent dissociation reactions of the collisionally activated ions occur in the region between the high-pressure collision cell and the entrance to MS-II.[14] The transient time of the ion over this distance is a few microseconds, depending upon the instrument configuration. In the instrument shown in Figure 1, MS-I is a high-resolution magnetic sector analyzer and MS-II is a spherical (90°) electrostatic analyzer.[14] In this type of instrument the CID product ions are energy analyzed and the *m/z* ratios of the product ions calculated by

$$m_n^+ = m_1^+(E_n/E_0) \tag{1}$$

In eq 1, E_0 is the potential applied to the electrostatic analyzer to focus the stable ion beam, and E_n is the potential necessary to bring the fragment ion m_n^+ to focus at the final collector slit. Since the ions subjected to CA are analyzed according to their kinetic energies, the amount of translational energy which is converted into internal energy (some fraction of the total energy loss) as a result of the high-energy ion-neutral collision can be estimated by the methods described by Derrick[10,11] and Cooks.[12] The energy loss (ΔE) accompanying CA can then be used to estimate the internal energy of the ions which are stable (nondissociating) or nonstable (dissociating) on the microsecond time scale.[12] The energy loss (ΔE) is obtained from the energy shift ($E_1 - E_2$) for the ion (eq 2), and the amount of translational energy

$$\Delta E = e(V/E_0)(m_1/m_n)(E_1 - E_2) \tag{2}$$

converted to internal energy of the incident ion (Q) is obtained by solving simultaneously eq 3 and 4. The terms in these

$$E_g = m_1 Q^2/(4 m_g E) \tag{3}$$

$$\Delta E = Q + E_g \tag{4}$$

equations are defined as follows: V is the ion source accelerating potential, E_0 is the potential of the electrostatic analyzer required to focus the normal ion beam, m_1^+ and m_n^+ are the *m/z* ratios of the incident and fragment ions, and the term ($E_1 - E_2$) is the energy shift of the ions in electrostatic analyzer volts. The terms E_g and m_g are the energy and mass of the collision gas, and E

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[11] Neumann, G. M.; Sheil, M. M.; Derrick, P. J. *Z. Naturforsch., A: Phys., Phys. Chem., Kosmophys.* **1984**, *39*, 584.

[12] Cooks, R. G. In *Collision Spectroscopy*; Cooks, R. G., Ed.; Plenum Press: New York, 1978; pp 357-450.

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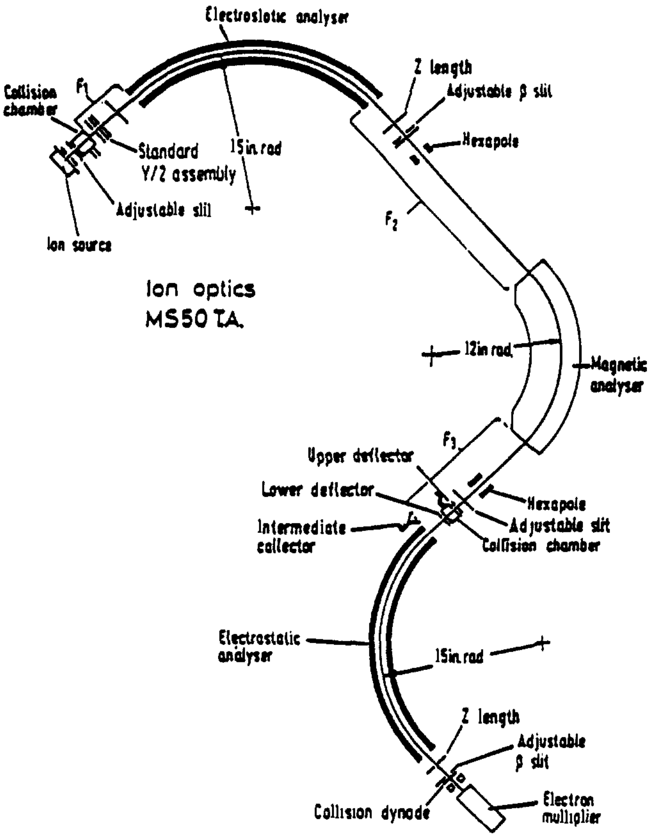

Figure 1. Schematic diagram of the Kratos MS-50TA tandem mass spectrometer.

is the energy of the incident ion prior to collision.

Using the treatment described above to determine Q, it is assumed that the energy of the target atom following CA is negligible and that the scattering angle of the incident ion approaches zero.[10-12] Thus, *assuming the excitation of the target atom can be neglected*, a direct measure of ΔE can be used to evaluate Q for large molecules subjected to CA. Derrick reported energy loss values in excess of 50 eV for large molecular ions of peptides.[10,11] Since it is generally accepted that keV energy CA involves electronic excitation of the incident ion, such large energy loss values are unexpected.[8,12] Thus, the large energy loss values reported by Derrick suggest that (i) CA of large molecular ions does not occur by electronic excitation (i.e., presumably, energy transfer involves translational-to-vibrational/rotational excitation processes[8]), and/or (ii) appreciable excitation of the target gas occurs and only a small fraction of the measured energy loss appears as internal energy of the incident ion. Previous studies have shown that low-energy CID (10–100 eV translational energies), which involves predominantly vibrational excitation, gives high yields for product ions.[5,8] Whether keV energy CA involves predominantly vibrational excitation, as opposed to electronic excitation, is of little direct consequence. That is, regardless of the mode of excitation, dissociation of large polyatomic ions can be described by a vibrational predissociation mechanism. On the other hand, important considerations arise if target gas excitation is in competition with formation of CID product ions; i.e., the term E_g in eq 4 is not negligible, as generally assumed, and a limiting factor in improving the efficiency for CID of large molecular ions is related to the relative cross section for target gas excitation vis-à-vis CA of the incident ion.

In this paper we describe the results of studies on the chlorophyll *a* $[M + H]^+$ molecular ion ionized by fast-atom bombardment (FAB).[15] In order to probe the dynamics of the CA process, the energy loss accompanying CA was measured for various target gases (e.g., He, Ne, Ar, and Kr). Although the results of these studies suggest that a large amount of energy is lost upon collision, it appears that a large fraction of the energy loss goes into excitation of the target gas atoms. These ideas and general conclusions are supported by CA studies of small polyatomics systems.

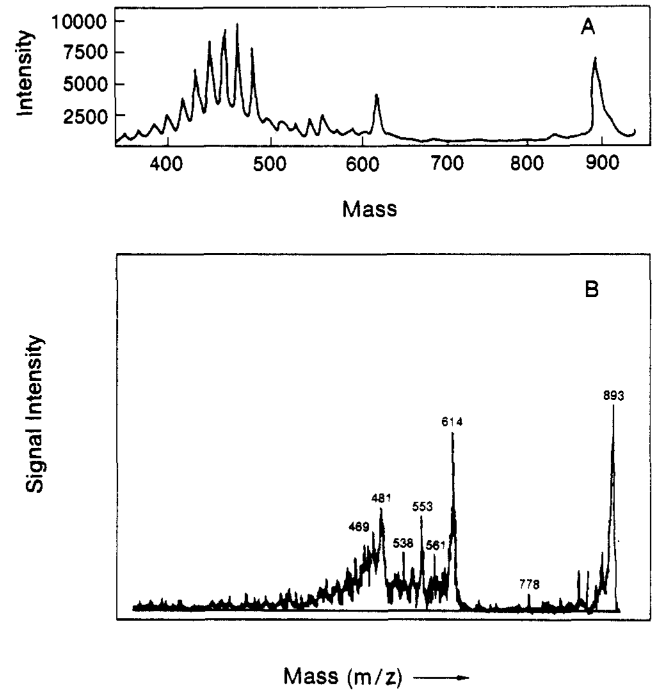

Figure 2. (A) Time-of-flight (^{252}Cf fission fragment ionization) and (B) collision-induced dissociation (CID) (He target gas) mass spectra of chlorophyll *a*. The time-of-flight spectrum was reproduced from ref 16a. The *m/z* scale for B was calculated by using eq 2 and an average energy loss value of 24 eV.

Experimental Section

The work described in this report was performed on a triple analyzer Kratos MS-50TA mass spectrometer.[13,14] The chlorophyll *a* samples (obtained from Aldrich) were dissolved in chloroform to give a solution which was ca. 1 μg/μL. Approximately 3 μL of sample was mixed with 2–4 μL of *o*-nitrophenyl octyl ether (liquid matrix) on the probe tip. The fast-atom bombardment (FAB) ionization was performed by keV energy argon or xenon neutrals produced by the Ion Tech Saddle field FAB gun.[15] The FAB gun was operated at a potential of 6–10 keV and a neutral beam current equivalent to ca. 10–20 μA as measured by the FAB source control unit (Ion Tech Model B-50).

The tandem MS studies were performed by using standard methods described previously.[14] In the experiments involving measurements of the energy loss accompanying collisional activation, an energy resolution for the normal ion beam of ca. 1000 was employed. That is, for an ion beam energy of 8000 eV the width (fwhm) of the normal ion beam (measured by scanning the electric sector voltage; see Figure 6) did not exceed 8 eV. The electric sector potentials were measured with a 5.5 digit multimeter (Fluke Model 8840A), and the accelerating voltage was measured by using the same multimeter to monitor the accelerating voltage reference power supply. The energy loss accompanying CA was measured by using the methods described by Cooks and Derrick (see text for discussion).[10-12] For the chlorophyll *a* system an intense signal is observed in the metastable ion spectrum at *m/z* 614; this ion arises by loss of the phytyl side chain (see Figure 4).[16] The *m/z* 614 ion was used to calibrate the energy axis for the CID spectra.[17] The collision gases used were obtained from Matheson Gas Products (high purity grade) and used without further purification. The collision gas was admitted to the collsion cell by using a variable leak valve. The pressure in the collision cell was unknown; however, the gas pressure was adjused to give a 10–25% attenuation of the incident ion beam. At these collision cell pressures no effects due to multiple collisions were detectable.[7]

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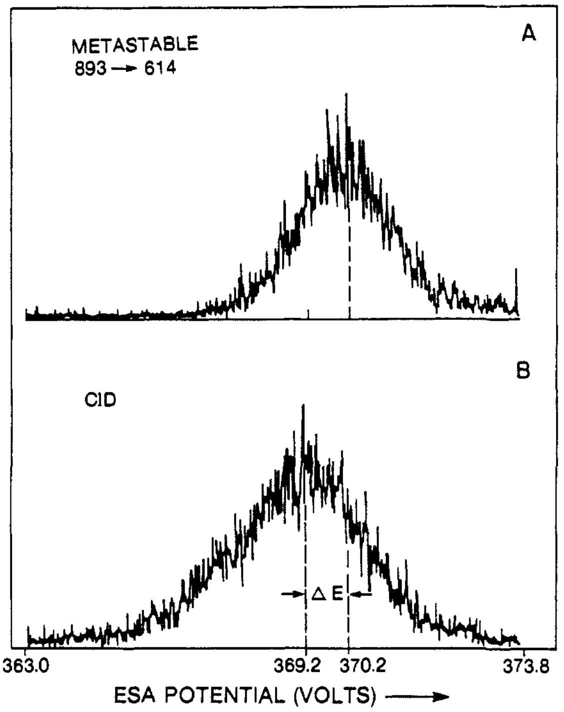

Figure 3. Expanded (A) metastable ion and (B) collision-induced dissociation spectra of chlorophyll *a* showing the region between *m/z* 602 (363.0 V) and *m/z* 620 (373.8 V). The electric sector potential required to transmit the normal ion beam is 538.42 V. Note the shift in energy for the ion at *m/z* 614 when collision gas is added to the collision cell.

The experimental apparatus and the procedure used for the FTMS CID studies have been described previously.[18]

Results

The collision-induced dissociation (CID) spectrum of chlorophyll *a* is contained in Figure 2. This spectrum is qualitatively similar to the ^{252}Cf plasma desorption ionization mass spectrum reported by Hunt.[16] The major difference between the CID and ^{252}Cf PD spectra are the relative abundance of the fragment ions, in particular *m/z* 614. Also, the mass assignment of the CID product ions made by using eq 1 differ from those reported by Hunt[16] and by Chait and Field[19] by as much as 2–3 mass units. For instance, the measured *m/z* ratios in the CID spectrum are lower by 2–3 mass units than the values obtained by time-of-flight measurements.

In the unimolecular metastable ion spectrum of the chlorophyll *a* $[M + H]^+$ ion an intense signal is observed at *m/z* 614 (see Figure 3A) which corresponds to loss of the phytyl side chain from the $[M + H]^+$ ion (Figure 4). Upon addition of the collision gas the *m/z* 614 signal is shifted (corresponding to $E_1 - E_2$ in eq 2 given above) to *m/z* 612 (see Figure 3B). To ensure that the observed shift in the signal was not due to a change in the composition of the product ions (i.e., successive losses of H atoms), the CID spectrum of perdeuterated chlorophyll *a* was also investigated. At the maximum available mass resolution for the CID spectrum (ca. 250 at *m/z* 600) no detectable structure (corresponding to loss of H (or D)) in the spectrum could be detected. Thus, the shift in apparent *m/z* value corresponds to an energy loss (calculated using eq 2) of approximately 24 eV.[20] If this energy loss value is then used to calculate the masses of the CID product ions, i.e., *m/z* values calculated by using eq 2, letting $(E_1 - E_2)$ equal 1.0 V, good agreement (less than 0.25 amu error) is obtained between the ^{252}Cf-PD and CID spectra. The excellent agreement for the mass assignment suggests that the energy loss accompanying CA is constant for each of the product ions, i.e., on the average, the $[M + H]^+$ ions of chlorophyll *a* have undergone an energy loss of ca. 24 eV.

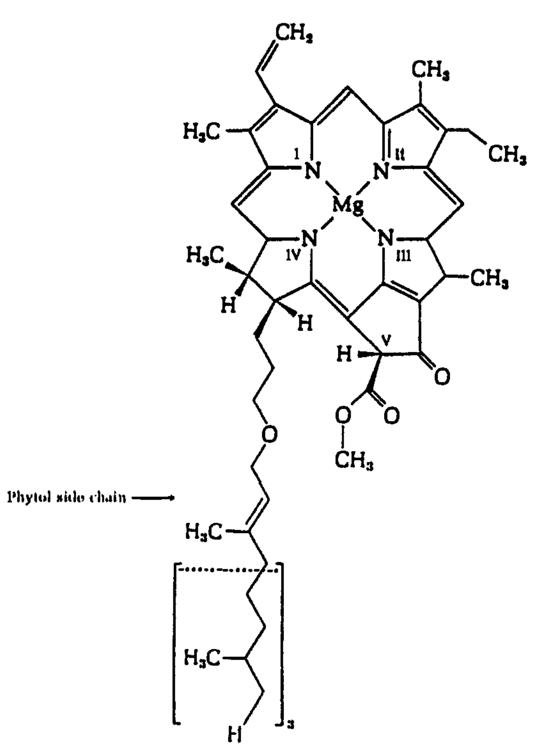

Figure 4. Structure of chlorophyll *a* showing the cleavage reaction which gives rise to the ion at *m/z* 614. The molecular weight of chlorophyll *a* is 892 amu; thus the $[M + H]^+$ ion appears at *m/z* 893.

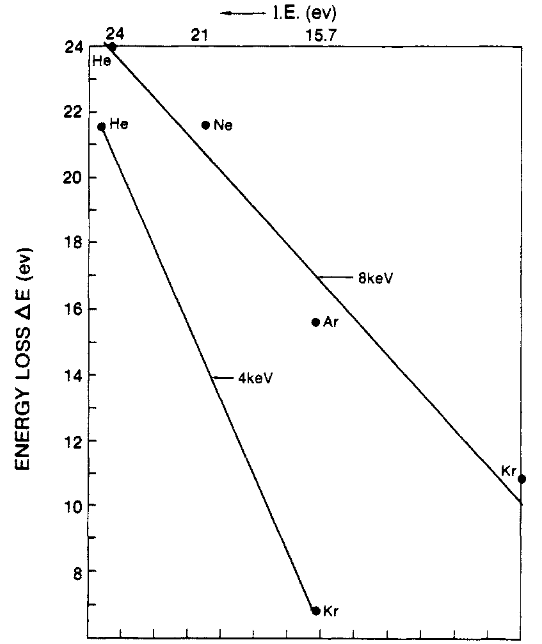

Figure 5. Plot of the energy loss accompanying collisional activation of the $[M + H]^+$ ion of chlorophyll *a* as a function of center-of-mass energy (lower axis) and ionization energy of the target gas (upper axis). The solid line is for $[M + H]^+$ ions with 8-keV translational energy, while the dotted line is for ions with 4-keV translational energy.

The effect of different target gases on the energy loss, as well as the general appearance of the CID spectra, was examined by

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[20] The energy loss value of 24 eV is calculated by using this peak maximum to obtain the energy shift in electrostatic analyzer volts (e.g., $E_1 - E_2$). As can be seen from Figure 3, the energy loss value calculated from the peak maxima underestimates the energy loss (note tailing of the peak to lower energy, indicating that energy losses greater than 24 eV are apparent). Thus, the error bars associated with absolute energy loss measurements are quite large; estimated to be ±25%. Although the error bars associated with absolute measurement are quite large, the relative errors and precision of the measurements are quite good (less than ±1–2%).

Table I Relative Abundances[a] for Selected Fragment Ions in the CID Spectrum of Chlorophyll *a* $[M + H]^-$ Ions

target neutral	*m/z* value of fragment ions				
	614	553	481	469	456
He	0.41	0.17	0.20	0.12	0.09
Ar	0.35	0.18	0.20	0.16	0.10
N_2[b]	0.38	0.11	0.21	0.14	0.16

[a] The precision in the measured values is ca. ±5%. These values were obtained by adding collision gas until the $[M + H]^+$ ion signal was attenuated by 25%. The relative abundance values are independent of collision gas pressure over a beam attenuation range of 25–75%. [b] Data for N_2 collision gas is included in this table to indicate the lack of dependence of the CID product ions on the nature of the target gas. *Note*, however, the strong dependence for the energy loss on the nature of the target gas (Figure 5).

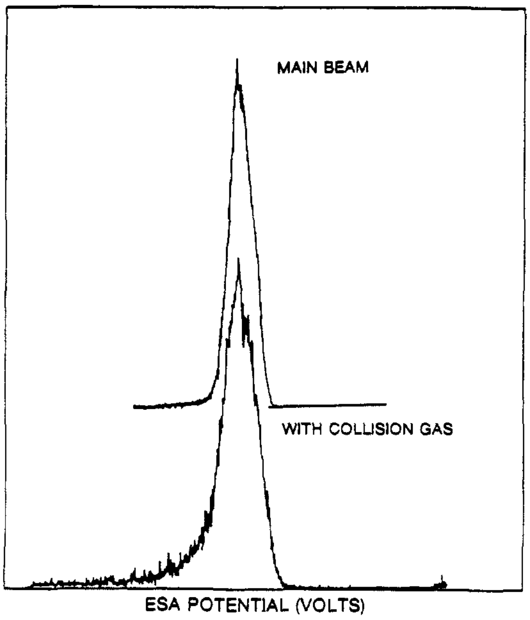

Figure 6. Peak profile for the $[M + H]^+$ ion of chlorophyll *a* without added collision gas (upper trace) and with added collision gas (lower trace). *Note* the tailing of the peak profile to lower energy when collision gas is present. The tailing to low energy arises as a result of the $[M + H]^+$ ions losing energy upon collision, but the energy of the collisionally activated ion is not sufficient to result in dissociation on the microsecond time scale. The peak profile was recorded at an energy resolution of ca. 3000 (see Experimental Section), i.e., a peak width (fwhm) of 2.6 eV for an 8-keV ion beam.

using Ne, Ar, Kr, and N_2. The energy loss values obtained from these measurements are displayed graphically in Figure 5. In the series of noble target gases the center-of-mass collision energy (denoted E_{com}) increases from ~36 eV (He) to about 650 eV (Kr). It should be noted, however, that the measured energy loss decreases as the E_{com} increases. Also given in Figure 5 is the ionization energy of the noble target gas. These data show clearly that the energy loss increases as the ionization energy of the target atom increases. The energy loss accompanying CA was also measured with incident beam energies of 4 keV. These data are similar to those obtained for 8 keV energy incident ions, but the absolute values for the energy loss of 4 keV beam energy are significantly different.

Although the energy loss is strongly dependent upon the target gas, viz. the ionization energy of the target gas, and the energy of the incident ion beam, the relative abundance of the CID product ions is independent of these parameters. In Table I selected CID product ions and their relative abundances are tabulated for different target gases.

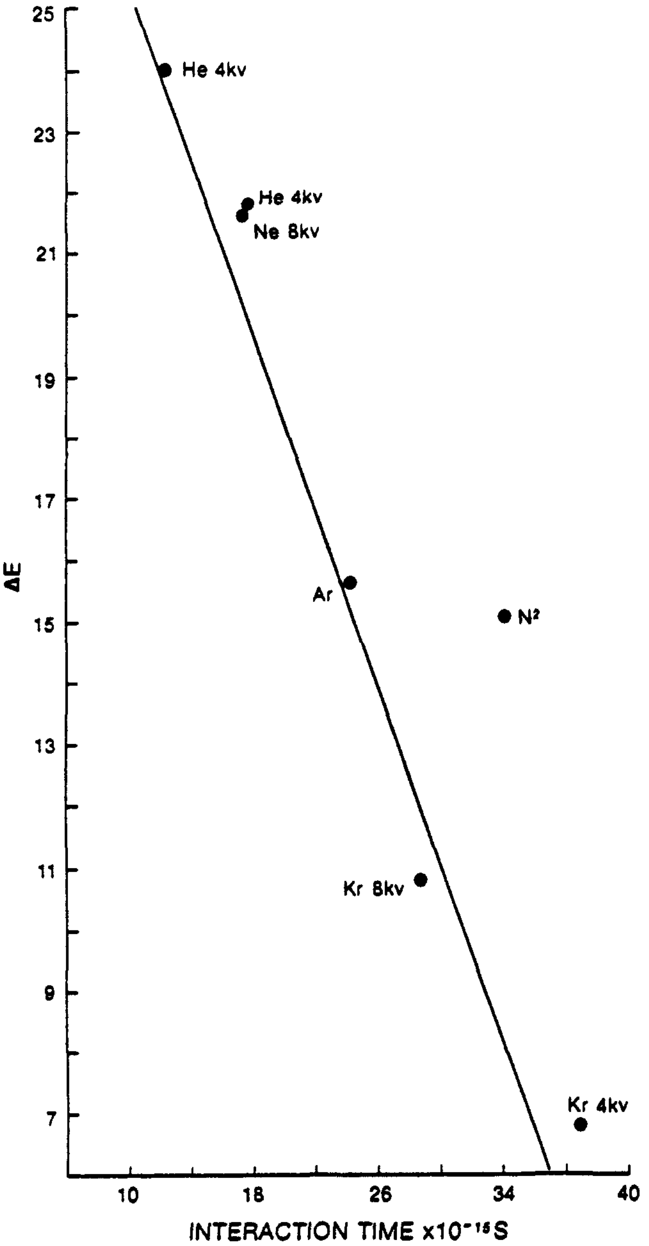

Figure 7. Plot of the energy loss accompanying collision activation of the $[M + H]^+$ ion of chlorophyll *a* as a function of the interaction time of the ion and the target neutral. (See ref 21 for discussion of the interaction time.)

The peak profile for the $[M + H]^+$ ion was also examined as collision gas was added to the collision chamber. Figure 6 shows the peak profile for the $[M + H]^+$ obtained without added collision gas and at a collision gas (He target gas) pressure corresponding to 25% attenuation of the main beam signal. The tailing in the peak profile to low energy arises from $[M + H]^+$ ions which have undergone energy loss as a result of an ion-neutral collision but which are stable on the time scale required to reach the ion detector (i.e., ca. 1×10^{-5} s). If we estimate the energy corresponding to zero signal intensity in Figure 6 (arbitrarily chosen as $\log I/I_0 = -2$), an energy loss value of 12.5 eV (±0.25 eV) is obtained. This measurement suggests that chlorophyll *a* $[M + H]^+$ ions which undergo an energy loss of ~12.5 eV or less are stable for periods greater than 10 μs, *but this is not the important point*! The more important point relates to the internal energy of an ion which undergoes an energy loss of 12.5 eV; i.e., what fraction of the 12.5 eV energy loss appears as internal energy of the $[M + H]^+$ ions? In the following sections we will argue that the fraction of the total energy loss which appears as internal energy of the $[M + H]^+$ ion is small (10–25%), and that the dominant fraction of the total energy loss is partitioned as excitation of the target gas.

Discussion

The energy loss dependence on the target gas (Figure 5) suggests that excitation of the target gas and CA are competitive

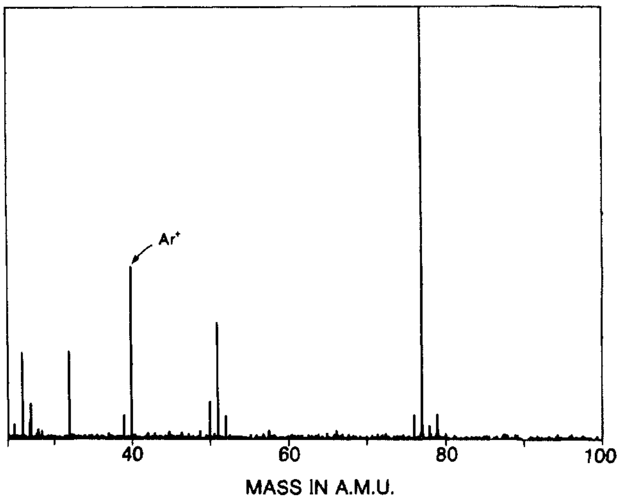

Figure 8. Fourier transform mass spectrum showing the product ions formed by translationally excited $C_6H_6^+$ (benzene) ions reacting with argon. The maximum translational energy of the $C_6H_6^+$ ions is 500 eV.

reactions. The energy loss dependence on incident ion energy (Figure 5) is also consistent with the occurrence of target gas excitation. To further illustrate this point, in Figure 7 the experimentally measured energy loss is plotted against the time of interaction of the incident ion with the target atom.[21] Although it is not possible to unequivocally ascertain the mode of target gas excitation, i.e., electronic excitation or ionization of the target gas, results of studies of the keV energy CA of polyatomic ions [$C_6H_6^+$ (benzene) and $C_7H_8^+$ (toluene)] by Fourier transform mass spectrometry (FTMS) reveal that the cross section for ionization of the target gas is quite large.[22] For example, if $C_6H_6^+$ (benzene) is accelerated to energies in excess of 500 eV and then allowed to react with argon, abundant ions of m/z 40 (Ar^+) are observed (Figure 8). If the $C_6H_6^+$ ion is accelerated to energies in excess of 1 keV and then allowed to react with Ar, Ar^{2+} is observed in the FT mass spectrum. Similar reactions are observed for Ne target gas. Studies could not be performed with He target gas because the low mass range of our FTMS system is limited to m/z 17.

The high efficiency for charge-transfer ion–molecule reactions between $C_6H_6^+$ ions and neutral Ar and the corresponding reaction for $C_6H_6^+$ ions and Ne are rather surprising. For example, charge transfer between $C_6H_6^+$ (benzene) ions and argon is endothermic by approximately 6 eV, whereas the corresponding reaction with Ne is endothermic by greater than 11 eV.[23] In studies of well-defined systems, "charge transfer collisions conform to the adiabatic theory, ... which predicts that the cross-section (for reaction) is small under the adiabatic condition that the time of collision (a/v) is much larger than the time of transition ($h/\Delta E$)" [where a is the sum of the atomic diameters, v is the velocity of the incident ion, and E' is the difference in the ionization energy of the collision partners].[24,25] In terms of an adiabatic model, one would expect the charge density within the "collision-complex", $[(C_6H_6 \cdots Ar)^+]^*$, to be centered predominantly around the benzene ion. Even if a substantial fraction of the charge density were to develop around the Ar atom, as the two pairs of the "collision complex" begin to separate, the charge density would rapidly develop around the lower ionizing energy partner. However, if the collision-complex is short-lived, charge distribution may not follow an adiabatic model.

The energy loss dependence on interaction time of the incident ion and target neutral, i.e., lifetime of the collision-complex, is not inconsistent with an adiabatic model. That is, as the interaction time between the ion and neutral is increased, the energy loss value decreases. With longer interaction times the energy loss value decreases because the cross section for target gas excitation decreases: i.e., at low translational energies a larger fraction of the total energy loss (ΔE) appears as internal energy of the incident ion. Conversely, the cross section for charge transfer increases sharply with increasing energy (velocity) of the incident ion. Other work suggests that the cross section for charge transfer is proportional to $m(\Delta E'^{2})$.[25] Thus, the relative cross section for CA and charge transfer will depend upon the interaction time or lifetime of the collision-complex, the mass of the incident ion, and the energy difference between the initial and final states ($\Delta E'^{2}$) of the collision pairs.

To amplify these ideas, consider an ion of m/z 100 with a translational energy of 8 keV, i.e., a velocity of 1.24×10^5 ms^{-1}. Assuming an interaction distance between the ion and neutral of 10 radii, the ion is in the "collision sphere" of a helium atom for ca. 4×10^{-15} s. Thus, the collision partners can be viewed as a collision complex having a lifetime of ca. 4×10^{-15} s. The lifetime of the collision-complex is insufficient for complete energy randomization. That is, the energy converted from translational modes will not be partitioned statistically into all degrees of freedom of the collision complex. Although the lifetime of the collision complex increases with mass of the incident ion (ν decreases as $m^{-1/2}$) and size of the target atom,[26] the lifetime of the collision-complex is still short with respect to energy randomization. While increasing the mass of the incident ion by an order of magnitude (m/z 1000) increases the lifetime of the collision complex by a factor of 3.16, i.e., 1.3×10^{-14} s, such a short-lived collision complex is still unlikely to behave adiabatically. For example, field-ionization kinetics data on a large number of systems suggest that complete energy (and presumably charge) randomization requires times of greater than 10^{-12} to 10^{-11} s.[27]

The FTMS data on model polyatomic systems suggest that target gas excitation (a large fraction involving target gas ionization) is an important reaction and that this reaction is in competition with CA. In fact, the increasing values for the energy loss and decreased efficiency of CID with increasing mass of the incident ion[10,11] are both consistent with target gas excitation. If polyatomic/atomic ionic collisions follow the same general trend as atomic/atomic and diatomic/atomic ion/neutral collisions,[24,25] charge-transfer reaction cross section should increase with mass, with a concomitant decrease in the CA cross section. Thus, the reduction in the CID efficiency for large molecular ions is not due solely to the dissociation dynamics of large molecules, i.e., dissociation reactions of large polyatomic ions being subject to substantial "kinetic shifts". The suggestion that the low efficiency for CID of large molecular ions has its origin in the dynamics of CA is supported by recent photodissociation studies of Fukuda.[28] Fukuda studied the photodissociation (using photons of 2.41 eV; 514.5 nm) reactions of relatively large porphyrins. The CID and PDS spectra of the porphyrins studied were surprisingly similar, which suggest the averaged-internal energies of the activated ions, i.e., ions activated by absorption of a single photon (2.41 eV) and CA, are quite similar (ca. 2.5 eV). Beynon has also shown that the averaged-internal energies of dissociating n-butylbenzene

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(21) The interaction time given in Figure 7 was obtained by calculating the velocity of the incident ion and assuming an interaction potential between the ion and neutral at a distance corresponding to 10 radii of the target neutral. The interaction distance chosen is totally arbitrary and is not a crucial point in the argument.

(22) Bricker, D. L.; Russell, D. H. *Anal. Chim. Acta* **1986**, *178*, 117.

(23) Rosenstock, H. M.; Draxl, K.; Steiner, B. W.; Herron, J. T. *J. Phys. Chem. Ref. Data* **1977**, *6*, 1.

(24) (a) Hasted, J. B. *J. Appl. Phys.* **1959**, *30*, 25. (b) See also: Hasted, J. B. In ref 12, pp 384–388.

(25) In Hasted's original paper (ref 24a) he used the symbol ΔE to denote the difference in energy between the initial and final states of the collision pairs. To avoid confusion between this value and the energy loss accompanying CA (our symbol ΔE), we denoted Hasted's ΔE as ($\Delta E'$).

(26) The lifetime of the "collision complex" increases to 30×10^{-15} s for an ion of m/z 1000 (8 keV energy) reacting with a krypton atom. Values for the radii of the target neutrals (He, Ne, Ar, and Kr) were taken from: Berry, S. R.; Rice, S. A.; Ross, J. *Physical Chemistry*; Wiley: New York, 1980; p 168.

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molecular ions activated by photon absorption (ca. 241 eV or 514.5 nm) and CA are comparable.[29]

An interesting aspect of the dynamics of CA processes is the recent study by Kenttamaa and Cooks which shows that low-energy CA deposits more internal energy into the incident ion than does high-energy (keV) CA.[30] Kenttamaa and Cooks' results are totally consistent with our data. That is, at incident ion velocities below the threshold for target gas excitation, CA is relatively efficient, but as the velocity of the incident ion is increased above the threshold for target gas excitation, the favored reaction channel is ionization of the target gas.

Conclusions

Derrick's earlier work and the present work have important implications for collision-induced dissociation/tandem mass spectrometry (CID–TMS) for structural characterization of large molecules. The questions concerning the low efficiency for CID–TMS influence the practical utility of the method,[31] especially at high mass (>3000 daltons) where sensitivity is an overriding consideration. Derrick reported large energy losses accompanying CA, inferring that the principal factors influencing the sensitivity of CID–TMS of large molecular ions are associated with the dynamics of dissociation of the collisionally activated ion. On the other hand, our data (both from the ion beam experiments on the $[M + H]^+$ ion of chlorophyll *a* and the endothermic charge-transfer reactions of small polyatomic ionic systems studied by FTMS) suggest that an important factor influencing the sensitivity of CID–TMS is associated with the CA step. That is, although large energy losses accompany CA, the dominant fraction of the energy loss goes into excitation of the target gas rather than excitation of the incident ion.

The available data suggest that the endothermic charge-transfer reaction is a momentum transfer process. That is, the cross section for charge-transfer reactions should increase as both the velocity and mass of the incident ion increases. Derrick has shown that the energy loss accompanying CA increases linearly with mass of the incident ion, and preliminary FTMS results show that the cross section for endothermic charge transfer increases linearly with velocity of the incident ion. Since the velocity of the incident ion decreases as $m^{-1/2}$, the more critical parameter influencing the efficiency for CID is the mass of the incident ion.

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Registry No. $C_6H_6^+$, 34504-50-2; He, 7440-59-7; Ne, 7440-01-9; Ar, 7440-37-1; Kr, 7439-90-9; chlorophyll *a*, 479-61-8.

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The Active Species in Surface-Enhanced Raman Scattering of Flavins on Silver Colloids

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Abstract: The N(3) flavin anion is shown to be the active species in the surface-enhanced Raman (SER) spectra of riboflavin, lumiflavin, FMN, and FAD adsorbed on colloidal silver. Participation of the anion is verified by the pH dependence of the spectra, the near identity of flavin SER spectra in water and deuterium oxide colloids, and the absence of SER for flavins with covalently bound N(3) substituents. The SER spectrum of lumichrome is briefly discussed.

Resonance Raman spectroscopy is now firmly established[1] as a useful tool in the elucidation of the structure of flavins and flavoproteins. For example, resonance Raman spectroscopy can reveal subtle details of the hydrogen bonding interactions in flavoproteins,[2] or charge-transfer interactions between flavins and the substrates or inhibitors of the reactions catalyzed by flavoproteins.[3,4] The technique has also proven useful for identification of the active intermediate in metalloflavoproteins.[5]

The major experimental problem in flavin Raman spectroscopy is the intense fluorescence of the isoalloxazine moiety. The fluorescence is intense enough to completely obscure resonance Raman spectra of free flavins and many flavoproteins. Many approaches to fluorescence rejection and quenching have been applied to flavin Raman spectroscopy.

Tsuboi and Nishimura[6] demonstrated that FMN and FAD Raman spectra could be obtained by excitation into the second or third electronic transition of the isoalloxazine. Spiro and co-workers used coherent Raman spectroscopy to attack the fluorescence problem.[7] Nishina and co-workers[8] relied upon the

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