


[5] Collision-Induced Dissociation (CID) of Peptides and Proteins

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Abstract

The most commonly used activation method in the tandem mass spectrometry (MS) of peptides and proteins is energetic collisions with a neutral target gas. The overall process of collisional activation followed by fragmentation of the ion is commonly referred to as collision-induced dissociation (CID). The structural information that results from CID of a peptide or protein ion is highly dependent on the conditions used to effect CID. These include, for example, the relative translational energy of the ion and target, the nature of the target, the number of collisions that is likely to take place, and the observation window of the apparatus. This chapter summarizes the key experimental parameters in the CID of peptide and protein ions, as well as the conditions that tend to prevail in the most commonly employed tandem mass spectrometers.

Introduction

Evidence for the gas-phase CID of molecular ions is apparent in the first mass spectra recorded by Sir J. J. Thomson with his parabola mass spectrograph, and the phenomenon was a subject of study throughout the development of MS during the first half of the twentieth century. A summary of the early work on CID has been published (Cooks, 1995). The modern application of CID to the detection, identification, and structural
analysis of organic molecules, to complex mixture analysis, and to biopolymer sequencing can be traced to multiples works (Beynon et al., 1973; Futrell and Tiernan, 1972; Jennings, 1968; McLafferty et al., 1973; Wachs et al., 1972) on CID itself and on the closely allied topic of metastable ion dissociation (Cooks et al., 1973). Detailed study of CID during the latter half of the twentieth century has resulted in an understanding of the energy transfer mechanisms and dissociation chemistry of small ions (<500 Da) (Cooks, 1978; McLafferty, 1983, 1992; Shukla and Futrell, 2000; Turecek and McLafferty, 1996). Tandem mass spectrometers, which use gas-phase collision regions to produce product ions from precursor ions, have proven extremely useful for the identification and characterization of ions and for complex mixture analysis. This usefulness is due to several factors, including the ease of implementation of CID (relative to alternative methods such as photo- or surface-induced dissociation [SID]), the fact that CID is universal (i.e., all ions have a collision cross section), and the fact that CID cross sections are typically high.

The last 20 years has seen a revolution in the application of MS and tandem MS to biological problems, due to the advent of ionization methods such as matrix-assisted laser desorption ionization (MALDI) (Hillenkamp and Karas, 2000; Karas et al., 1985) and electrospray ionization (ESI) (Cole, 1997; Whitehouse et al., 1985), capable of producing ions from biologically derived samples such as peptides, proteins, and nucleic acids. Given the popularity and wide application of CID for use in tandem MS of small ions, it is not surprising that CID has been extended to the tandem MS of these larger biological ions (Biemann and Papayannopoulos, 1994; Hunt et al., 1986). Indeed, tandem MS with CID has become an important tool in the growing field of proteomics, the effort to elucidate the protein complement for a given species as a function of physiological conditions (Aebersold and Goodlet, 2001; Larsen and Roepstorff, 2000; Yates, 1998). As is often the case in science, the application of the technique has outpaced the understanding of the underlying phenomena that dictate the appearance of the resulting data. The energy-transfer mechanisms that operate during collisions of large (>1000 Da) possibly multiply charged ions with small target gas atoms or molecules are not as well understood as they are for smaller ions. The dissociation chemistry of large multiply charged biological ions upon activation by collisions is also the subject of continuing study. The intent of this chapter is to describe the important experimental variables that affect the CID of biologically derived macroions and to describe what is known about how such ions behave as a function of those variables, with reference to examples from the literature. The discussion is largely focused on the behavior of peptide and protein ions, as these species constitute by far the most widely studied biological
ions by CID. The ranges of the relevant variables that can be accessed with available instrumentation is discussed. Note that in terms of instrumentation, the discussion is kept narrowly focused on CID behavior; other characteristics of tandem mass spectrometers, such as precursor and product mass resolution, efficiency of ion transfer between mass analysis stages, sensitivity, and others, are not discussed. The reader should be aware that although a given instrument may access a range of CID variables, which yields useful tandem mass spectra in terms of the dissociation that occurs, other instrument criteria must also be considered when evaluating the applicability of a tandem mass spectrometer to a particular problem.

Experimental Variables that Influence the CID Behavior of Biological Ions

A wide variety of conditions has been used to effect CID of biological ions as new types of tandem mass spectrometers have been developed or as existing types have been fitted with ion sources capable of producing biological ions. Some activation methods have been developed specifically to improve the quality of tandem MS data for biological ions. A set of figures of merit for CID are outlined here to aid in the discussion of how peptides and proteins dissociate as a function of the CID conditions used:

1. The time over which the activation occurs relative to the time for unimolecular dissociation or rearrangement.
2. The amount of energy that can be deposited during the activation.
3. The distribution of the deposited energy.
4. The variability of the deposited energy.
5. The form of the deposited energy (vibrational vs. electronic).
6. The time scale of the instrument used (the kinetic window within which dissociation reactions must occur in order to be observed).
7. The efficiency of the CID process, in terms of cross section or rate constant.

The first figure of merit, the time scale of the activation, refers to how fast energy is deposited in the ion relative to how fast energy equilibrates through the degrees of freedom of the ion, how fast the ion dissociates or rearranges, and how fast energy is removed by cooling processes such as deactivating collisions or photon emission. McLuckey and Goeringer (1997) have distinguished three time regimens for activation methods used in tandem MS, including collisional methods: fast, slow, and very slow (slow heating). Figure 1 illustrates typical ranges of activation times for a number of activation methods, with the collision-based methods of interest here highlighted.
Fast methods are those in which the input of energy occurs rapidly relative to unimolecular dissociation, such as single-collision CID at kilo-electron volts or electron volts (in the laboratory frame of reference) kinetic energy. Slow CID methods are those in which multiple collisions can occur, with long (tens to hundreds of microseconds) intervals between individual collisions. In this regimen, chemistry can occur between collisions, meaning that ions can dissociate or isomerize during the activation. This type of CID is effected in collision cells, such as the central collision quadrupole of triple quadrupole tandem mass spectrometers and other instruments that use multipole collision regions, which are operated at pressures of a few millitorr to as high as a torr (Dodonov et al., 1997). Very slow activation methods, or slow heating methods, are distinguished from slow methods by the fact that deactivating processes, such as cooling collisions or photon emission, can occur between activation events. In this

![Activation time scale](image-url)
regimen, a steady state between activation and deactivation can be achieved. If the dissociation rate is low relative to the activation/deactivation rate (i.e., if the ions are in the so-called “rapid energy exchange” condition), then the ion internal energy can be described with Boltzmann statistics. To a good approximation, the ions can be considered to have an internal temperature, and ion dissociation can be described by the Arrhenius theory. Quadrupole ion trap collisional activation in approximately 1 millitorr of helium bath gas (Goeringer et al., 1999; Louris et al., 1987) and sustained off-resonance irradiation (SORI) (Gauthier et al., 1991) in Fourier transform–ion cyclotron resonance (FT-ICR) MS are examples of slow heating CID methods. Direct heating of the helium bath gas is another form of slow heating that can be used for CID in quadrupole ion traps (Asano et al., 1999a; Butcher et al., 1999). Multiple excitation collisional activation (MECA) (Williams and McLafferty, 1990) and very-low-energy collisional activation (VLE-CA) (Boering et al., 1992a,b) are other forms of slow heating that have been used in FT-ICR. Slow heating methods lead to similar protein and peptide dissociation as slow methods, although an important difference is that for slow heating methods, the product ions are generally not subject to further activation, and hence less sequential dissociation is observed.

The amount of energy that can be deposited in an ion depends on the relative collision energy of the colliding ion/neutral pair. Most CID experiments can be roughly categorized into one of two energy regimens: “high-energy” CID with kiloelectron volt kinetic energy in the laboratory frame of reference and “low-energy” CID with 1–100 eV kinetic energy in the laboratory frame. It bears mentioning here that as discussed throughout this section, there are many variables that determine the amount, distribution, form, and so on, of internal energy deposited into the ion, and further, there are a number of variables in addition to those pertaining to the internal energy that determine the appearance of a CID spectrum. Hence, the historic distinction between “high”- and “low”-energy CID based only on the laboratory kinetic energy is an oversimplification that should probably be avoided. Some statement regarding the collision energy, together with an indication of the number of collisions, the mass of the collision partner, and the activation time is probably a minimum in describing a particular CID experiment.

The kinematics for collisions of small ions with neutrals have been carefully described (Cooks, 1978; McLuckey, 1992; Shukla and Futrell, 2000) and show that it is the relative kinetic energy of the collision partners that is available for transfer to internal energy of the ion. This center-of-mass collision energy, $KE_{com}$, is a fraction of the laboratory kinetic energy, $KE_{lab}$, as given by Eq. (1) if the velocity of the neutral is ignored:
\[ KE_{\text{com}} = \left( \frac{N}{m_p + N} \right) KE_{\text{lab}}, \]  

where \( N \) is the mass of the neutral target and \( m_p \) is the mass of the ion. 

Equation (1) shows that the energy available for transfer into internal modes for a collision of a high \( m/z \) ion with a small neutral is very low and suggests that CID of high \( m/z \) ions should be very inefficient. However, Marzluff et al. (1994a) have shown that at least for low center-of-mass kinetic energy, the energy transfer process in collisions of large ions with small targets can be extremely efficient. In this study, a homologous series of deprotonated peptides, which all dissociate via the same well-characterized mechanism (Marzluff et al., 1994b), was used to study the efficiency of dissociation as a function of peptide size. The results indicate that the extent of dissociation of these peptides upon SORI-CID in an FT-ICR using nitrogen collision gas increased with the size of the peptide. Rice-Ramsperger-Kassel-Marcus (RRKM) statistical dissociation rate calculations (Forst, 1973; Holbrook and Robinson, 1972; Lorquet, 1994) for one of the peptides studied, gly-gly-ile, show that at the observed dissociation rate, approximately 55% of the available kinetic energy was transferred to internal energy during each collision. Trajectory calculations conducted with a molecular mechanics force field support this experimental observation and indicate that for a larger system such as bradykinin (nine amino acid residues and 444 internal degrees of freedom), as much as 90% of the available kinetic energy could be transferred.

Douglas (1998) has developed an approximation for the internal energy deposited into large ions upon electron volt collisions in a linear quadrupole, which supports the observations described earlier. Using experimental dissociation cross sections (see discussion of efficiency below) measured as a function of center-of-mass collision energy, Douglas showed that electron volt collisions of bromobenzene cations with nitrogen lead to approximately 70% conversion of center-of-mass energy to internal energy. Further work by Douglas aimed primarily at measuring collision cross sections for protein ions in a triple-quadrupole instrument provided further evidence for the efficiency of energy transfer (Chen et al., 1997). The collisions of large ions with small target gas atoms were shown to be most accurately modeled with a diffuse scattering model, wherein a small target atom collides with the ion at the center-of-mass collision energy, and then leaves with a thermal energy distribution; the result is that 90–95% of the collision energy is converted to internal energy of the ion during the relatively long interaction between the ion and the neutral. Experimental measurement of the dissociation threshold for the loss of heme from
holomyoglobin supported this large energy transfer of more than 90% (Douglas, 1998).

A number of authors have developed an impulsive collision theory (ICT) to describe the energy-transfer process for collisions of large ions with small neutrals (Cooper et al., 1993; Uggerud and Derrick, 1991). In this model, direct momentum transfer between a constituent atom of the ion and the collision partner occurs, resulting in much larger transfer of internal energy to the ion than would be predicted based only on consideration of the overall mass of the large ion (Eq. [1]). Such binary collisions between portions of a large ion and the small target have been examined by other researchers (Boyd et al., 1984; Douglas, 1982; Futrell, 1986). Bradley et al. (1994) compared the energy transfer to large organic ions consisting of many small atoms (C, N, O, H) with that to ions of similar m/z consisting of large atoms (CsI clusters) and showed that the behavior was consistent with the prediction of the ICT, viz. more energy transfer to the organic ion during collisions with helium, due to the closer similarity in mass between the small atoms of the organic ion and the helium atoms. The reverse was found to be true for collisions with argon, because of a closer mass match between the Cs and I atoms of the cluster and the argon target atoms.

Turecek (1991) has shown that in principle, the center-of-mass limit on the amount of energy deposited can be overcome by invalidating the assumption made in Eq. (1), viz. that the velocity of the neutral collision partner is zero. By colliding ions with fast (kiloelectron volts) rather than thermal targets, much more energy is available for transfer to internal energy during the collision. However, the practical difficulties of crossing a fast beam of ions with a fast beam of neutrals or of colliding a fast beam of neutrals with a small stationary ion cloud, with sufficient cross section to yield measurable product signals may limit the analytical applicability of this form of CID. Note that crossed beams have been used for fundamental studies of ion–molecule reactions at very low kinetic energy (Futrell, 2000).

The number of collisions occurring during the activation period also determines the amount of energy deposited. Most analytical applications of CID use collision regions operated at pressures high enough to ensure that multiple collisions occur to increase internal energy and hence yield extensive dissociation. The disadvantage to using higher pressure is that increased scattering may result in a loss of sensitivity; however, Douglas and French (1992) have shown that in quadrupole transmission devices used to carry ions from an ESI source to the mass analyzer, collisional cooling of ions at elevated pressure (~8 millitorr) leads to improved sensitivity and resolution (by removal of kinetic energy spread in the ions). This concept has also been applied to quadrupole collision cells of tandem mass spectrometers, leading to improved product ion collection efficiency.
and resolution when the cells are operated at 8 millitorr compared to the 1–2 millitorr normally used (Douglas et al., 1993; Thomson et al., 1995).

This discussion relates to the magnitude of the energy that can be deposited; the third and fourth figures of merit relate to the distribution of energies that can be deposited and the variability of the energy distribution. The distribution of energies plays an important role in determining the appearance of CID spectra (Vekey, 1996). Ideally, the energy distribution is narrow, well defined, and variable over a wide energy range, so dissociation channels having a wide range of critical energies can be accessed. CID methods lead to energy distributions that are typically fairly broad and ill defined, at least for fast and slow CID methods. In kilo-electron-volt collisions, a distribution having a maximum at only a few electron volts, with a long tail out to higher energy (tens of electron volts), is often obtained for small ions, allowing some high critical energy channels to be observed. For electron-volt collisions, the maximum of the distribution is similar, but the high-energy tail is not present, so that only lower critical energy processes are accessed. Douglas (1998) has presented a method for estimating the internal energy distribution for multiple collision CID in a quadrupole from a measure of the dissociation cross section as a function of collision energy. For ion trap collisional activation, it has been shown that the internal energy distribution can be modeled as a Boltzmann or truncated Boltzmann distribution, depending on the relative rates of activation/deactivation and dissociation (Goeringer et al., 1999). Schnier et al. (1999) have shown that for FT-ICR SORI, leucine enkephalin and bradykinin ions achieve effective temperatures of between 500 and 700 K, although the authors state that actual distribution of internal energy is not well characterized and may not be Boltzmann. As discussed earlier, the maximum of the internal energy distribution can be varied to some extent by varying the collision energy; CID spectra acquired at low laboratory kinetic energies (electron volts) typically show greater sensitivity to changes in energy than spectra acquired at kiloelectron-volt energy (Busch et al., 1988).

The fifth figure of merit relates to whether the internal energy is present in the activated ion in electronic or vibrational modes. Statistical theories of activated ion dissociation assume that internal energy equilibration through all vibrational degrees of freedom is faster than dissociation (Forst, 1973; Holbrook and Robinson, 1972; Lorquet, 1994). Experimental evidence shows that this holds for most small organic ions; however, exceptions where dissociation from an electronic excited state is faster than relaxation of energy into vibrational modes have been reported (Shukla et al., 1990). There is little evidence for or against the statistical hypothesis for large peptide and protein ions. However, it seems reasonable, at least
under multiple collision conditions where energy is deposited in small increments at a variety of collision sites on the ion, that collisional activation effectively gives rise to a statistical ion energy distribution.

The sixth figure of merit relates to how fast the dissociation reactions must occur in order to be observed on the time scale of the instrument used. The kinetic window varies widely across different instrument types from a few tens to hundreds of microseconds for magnetic sector–based instruments operating at kiloelectron-volt ion kinetic energies, milliseconds in triple-quadrupole and quadrupole-TOF instruments, and from hundreds of milliseconds up to seconds in trapping instruments. The length of the kinetic window is important for large biological ions, because dissociation of these ions may be relatively slow because of their large number of degrees of freedom. The amount of energy above the critical energy for dissociation required to drive a dissociation reaction at a rate that is observable on the time scale of the instrument used is called the kinetic shift and may be very large (up to or even exceeding 100 eV) for large biological ions. This obviously makes the previous discussion regarding the amount of energy that can be deposited into the ions upon collisional activation very important (e.g., at kiloelectron-volt kinetic energies, dissociation rates must be driven to at least $10^4 \text{s}^{-1}$ in order to be observed), and hence inherently slow processes may not be observable in this regimen, due to an inaccessibly high kinetic shift.

The efficiency of the CID process relates to how much of the available precursor ion is converted to product ions. For beam-type instruments, such as sectors and triple quadrupoles, this is most conveniently represented with a Beer’s law type of relationship, as shown in Eq. (2):

$$[M^+_p] = [M^+_p]_0 e^{-nsl},$$

where $[M^+_p]_0$ is the precursor ion flux without collision gas, $[M^+_p]$ is the precursor ion flux after the introduction of collision gas, $l$ is the path length, $n$ is the target number density, and $s$ is the total ion loss cross section. If no processes for precursor ion loss other than CID operate, then $s$ represents the cross section for CID. For trapping instruments, an analogous expression in terms of rate constant and reaction times can be used:

$$[M^+_p]_t = [M^+_p]_0 e^{-nkt},$$

where $[M^+_p]_0$ is the precursor ion abundance before any reaction, $[M^+_p]_t$ is the precursor ion abundance after reaction time $t$, and $k$ is the rate constant for all precursor ion loss processes. If no precursor ion loss processes other the CID operate, then $k$ represents the rate constant for CID. Equation (2) shows that the only variable available to increase the efficiency of CID in
beam-type instruments is \( n \), the number density of the neutral collision partner (increasing the path length is possible but is usually not straightforward over a wide range). The advantages and disadvantages of operating at higher collision gas pressure were discussed earlier. At higher pressure, ion loss processes such as scattering may degrade sensitivity, although in quadrupole collision cells, the collisional cooling afforded by higher collision gas pressure has been shown to give the opposite effect. It is easier to increase the efficiency of CID in ion-trapping instruments by increasing the reaction time, \( t \). Reaction times as long as seconds can be used to completely drive precursor ions to product ions. Note that operation at either higher collision gas pressure or longer activation time increases the likelihood of multiple collisions occurring and, hence, implies that the activation is either slow or very slow (slow heating), with the attendant possibility for chemistry to occur during the activation, as discussed earlier.

Summary of Commonly Used CID Conditions

The values for any individual figure of merit discussed earlier may vary widely with the type of instrument used and the design of the experiment, and the various figures are not independent but influence one another. In this section, three commonly used regimens for effecting CID of gas-phase biological ions are described in terms of the figures of merit outlined earlier. Table I summarizes the information given in this section with typical ranges of values used for the operating parameters and figures of merit for the three regimens. CID spectra for a common ion, the doubly protonated ion of the nine residue peptide bradykinin (RPPGFSPFR), are used throughout this section to illustrate the influence of the figures of merit on CID behavior.

High-Energy CID (Fast Activation)

As discussed earlier, the collision energy is only one of a number of variables that must be specified to adequately describe a CID regimen. The term “high-energy” CID is usually used to describe CID effected at kilo-electron-volt precursor ion kinetic energies, with a target gas pressure that is low enough that only single or at most a few (fewer than five) collisions can occur. Because of the high kinetic energy of the ions, the time scale for dissociation is usually on the order of a few microseconds. The vast majority of CID experiments under this regimen have been carried out using sector-based instrumentation. However, kilo-electron-volt collisions are seeing increasing use in tandem time-of-flight (TOF) instruments, as discussed later in this chapter.
<table>
<thead>
<tr>
<th>Figure of merit</th>
<th>“High-energy” CID (fast activation)</th>
<th>“Low-energy” CID (slow activation)</th>
<th>Trapping CID (very slow activation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Instruments used</td>
<td>Magnetic/electric sectors, TOF/TOF</td>
<td>Tandem quadrupoles, quadrupole</td>
<td>Quadrupole ion traps, FT-ICR traps</td>
</tr>
<tr>
<td>Collision energy</td>
<td>2–10 keV</td>
<td>1–200 eV</td>
<td>1–20 eV</td>
</tr>
<tr>
<td>Collision number</td>
<td>1–5</td>
<td>10–100</td>
<td>100 s</td>
</tr>
<tr>
<td>Activation time scale</td>
<td>1–10 μs</td>
<td>0.5–1 ms</td>
<td>10–100 ms</td>
</tr>
<tr>
<td>Instrument time scale (kinetic</td>
<td>10–100 μs/10⁶–10⁴ s⁻¹</td>
<td>0.1–1 ms/10⁴–10³ s⁻¹</td>
<td>10 ms–1 s/10²–1 s⁻¹</td>
</tr>
<tr>
<td>window/minimum observable reaction rate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Distribution of internal energy</td>
<td>Centered at a few electron volts, high-energy tail to tens of electron volts</td>
<td>Centered at few eV, no high energy tail</td>
<td>Centered at a few electron volts, may be Boltzmann or Boltzmann-like</td>
</tr>
<tr>
<td>Variability of internal energy</td>
<td>Relatively invariable, scattering angle provides some energy resolved info.</td>
<td>Readily variable with collision energy to obtain energy resolved info.</td>
<td>Some variability with collision energy and number</td>
</tr>
<tr>
<td>Efficiency</td>
<td>&lt;10%</td>
<td>5–50%</td>
<td>50–100%</td>
</tr>
<tr>
<td>General results</td>
<td>High-energy channels may be accessed together with lower energy processes, sequential dissociation observed</td>
<td>Lower energy processes only, isomerization of precursor may occur, sequential dissociation observed</td>
<td>Low-energy processes only, extensive isomerization of precursor, very slow processes can be observed, typically little sequential dissociation</td>
</tr>
</tbody>
</table>
Double-focusing mass spectrometers based on magnetic and electric sectors were the first instruments used for CID studies of small organic ions (Beynon et al., 1973; Futrell and Tiernan, 1972; Jennings, 1968; McLafferty et al., 1973; Wachs et al., 1972), and were among the first used for CID of peptide ions approximately a decade later (Biemann and Papayannopoulos, 1994). Two-sector MS instruments may be used to record MS/MS spectra by using a variety of linked scans (Busch et al., 1988) of the magnetic (B) and electric (E) fields, albeit with relatively poor precursor ion or product ion resolution, depending on the type of scan. A number of three-sector instruments were constructed to help overcome this limitation (Gross, 1990). Four-sector instruments, true tandem mass spectrometers that combine two double focusing instruments for high resolution of precursor and product ions, were also constructed and were commercially available from a number of manufacturers (Gross, 1990). The use of these instruments for tandem MS of biological ions was demonstrated throughout the 1980s and early 1990s, but they have now been largely superseded by tandem quadrupoles, hybrid quadrupole/TOF instruments, ion-trapping instruments, and increasingly TOF/TOF instruments.

Readily obtainable field strengths for sectors are such that these instruments must operate on ion beams accelerated to kiloelectron-volt kinetic energies; hence, most CID work with sector instruments is done with collisions in this regimen. As discussed earlier, kiloelectron-volt collisions deposit on average a few electron volts of internal energy, but the distribution can include a tail to higher internal energies, so that higher critical energy or lower rate processes (requiring large kinetic shifts) can be observed. High-energy CID spectra are relatively insensitive to changes in the kinetic energy of the precursor ion, and so obtaining energy-resolved CID spectra with high-energy CID is difficult. However, it has been shown that higher energy collisions lead to larger scattering angles, so recording MS/MS data as a function of angle (angle-resolved MS [ARMS]) yields information on dissociation as a function of internal energy (McLuckey and Cooks, 1983). It is possible to decelerate and then reaccelerate the beam to also access low-energy collisions; however, high-energy CID spectra are very reproducible on a given instrument and even between instruments of different configuration, and this is often cited as a major advantage so that operation at low energy is usually not considered to be a desirable alternative.

The transit time of a 10-keV beam of ions at m/z 1000 through a 10-cm collision cell is 2.3 ms, and 23 ms along a 1-m flight path from the collision cell to the detector. The former value establishes the maximum amount of time between activating collisions, if the cell is operated at a pressure at which multiple collisions in this time frame are likely. High-energy CID is
normally performed using pressures chosen to yield a given attenuation of the ion beam, which is related to the number of collisions. Single or at most a few (5–10) collisions are typically used; beyond this, scattering of the beam leads to unacceptable losses in sensitivity. Increasing the number of collisions, of course, increases the energy deposited. At least for small ions, internal rearrangements can occur on a time scale of nanoseconds or less, hence isomerization can occur between collisions even at high kinetic energy (Holmes, 1985). The transit time through the whole instrument of some tens of microseconds establishes a lower limit on the observable rates of approximately $10^4$ to $10^6$ s$^{-1}$.

For peptides, the figures of merit described lead to CID spectra, which differ from those obtained using other commonly employed conditions primarily in that abundant dissociation of amino acid side chains is observed in addition to peptide bond cleavage. This is illustrated in Fig. 2, the MS/MS spectrum of $+2$ bradykinin (Downard and Biemann, 1994) obtained on a four-sector instrument using 8-keV collisions with helium at a pressure at which the primary beam intensity reduced by 70%, corresponding to between two and four collisions (Holmes, 1985). The ions labeled $w_n$ and $d_n$ correspond to side-chain cleavage (Biemann, 1988; Roepstorff and Fohlman, 1984). It has been suggested that these cleavages are charge remote and, hence, require higher critical energies to be accessed, implicating the high-energy tail of the internal energy distribution as the cause for these cleavages. This is supported by the absence of

![Fig. 2. Collision-induced dissociation (CID) spectrum of the $(M + 2H)^{+2}$ of bradykinin collected on a four-sector tandem mass spectrometer using 8-keV collisions with helium at a pressure that reduced the primary beam intensity by 70%, corresponding to between two and four collisions. Note the abundant side-chain cleavage ions (labeled $w_n$ and $d_n$) and the immonium ions of individual amino acids (labeled with capital letters). (Reproduced with permission from Downard and Biemann, 1994.)](image-url)
side-chain cleavages in CID spectra collected at electron-volt collision energy (see later discussion). These side-chain cleavages have been shown to be useful for the distinction of isomeric and isobaric amino acids in peptide-sequencing applications (Biemann and Papayannopoulos, 1994). The upper limit for CID at high energy is approximately m/z 3000, beyond which dissociation is not readily observed, probably because of the large kinetic shift required to drive dissociations at a rate of at least 10^4 s^{-1}. The efficiency of high-energy CID is typically only a few percent even for smaller molecules because of this short time scale and because of the product ion collection efficiency.

The advantages of CID conducted at kiloelectron-volt energy and low collision numbers, viz. reproducibility and access to side-chain cleavages for isomeric and isobaric ion distinction, are driving interest in an alternative instrument, the tandem TOF (TOF/TOF), for accessing this regimen. An advantage of the TOF/TOF over sector-based instruments is the well-established compatibility of TOF with the MALDI source. A number of forms of TOF/TOF have been described for CID and photodissociation of small molecules and cluster ions (Cornish and Cotter, 1993a; Hop, 1998; Jardine et al., 1992). At least two groups have demonstrated the use of TOF/TOF instruments to dissociate peptide ions with kiloelectron-volt collisions (Cornish and Cotter, 1993b; Medzihradszky, 2000). Figure 3 shows a CID spectrum for a tryptic peptide dissociated using 3-keV collisions with argon (pressure unspecified) (Medzihradszky, 2000). The abundant immonium ions of individual amino acids and the w_n ions resulting from side-chain cleavage are characteristic of the high collision energy used and were helpful in establishing the peptide composition and distinguishing the isomeric leucine and isoleucine residues, respectively.

Low-Energy CID (Slow Activation)

The term “low-energy” CID is typically used to refer to CID conducted in quadrupole or other multipole collisions cells and is characterized by collision energy of up to 100 eV, target gas pressures selected to allow multiple (tens to hundreds) collisions, and a time scale on the order of a few hundred microseconds to a few milliseconds. For simplicity, throughout this discussion the term quadrupole, represented with the letter q, will be used to describe a multipole collision cell that passes all m/z ratios above a certain lower limit, with the understanding that higher multipoles such as hexapoles and octapoles are in some cases used instead of quadrupoles. The electron-volt energy regimen is used in tandem quadrupole instruments (usually referred to as triple quadrupoles [QqQ], where Q is a mass-resolving quadrupole) and in hybrid instruments that combine quadrupole
Biological mass spectrometry
collision cells with other types of mass analyzers for precursor selection and/or product mass analysis (Yost and Boyd, 1990). A hybrid instrument that is increasing in popularity is the quadrupole-quadrupole-TOF (QqTOF) (Morris et al., 1996; Shevchenko, 1997), where precursor selection is done with the first quadrupole, CID occurs in the second quadrupole, and the products are mass analyzed via TOF. The attractive mass analysis characteristics of orthogonal acceleration, reflectron TOF are driving interest in this instrument, and two commercial versions are available.

The advantage of using a radiofrequency (RF) quadrupole (or other multipole) as a collision cell is that the RF field provides a strong focusing force toward the ion optical axis, so that losses of precursor and product ions are minimized, even at relatively high collision numbers. However, to enjoy this advantage, ions must move through the quadrupole slowly enough to be influenced by the rapidly changing RF field; hence, kinetic energies less than 100 eV are typically used. For small organic ions, low-energy collisions lead to internal energy distributions that have a peak at a few electron volts of internal energy, but without the long tail to higher energy, which characterizes kiloelectron-volt CID. The observed differences between electron-volt and kiloelectron-volt dissociation of peptides is that electron-volt CID lacks the side-chain cleavage peaks often observed at higher energy. This is illustrated in Fig. 4 for the MS/MS spectrum of +2 bradykinin collected on a triple-quadrupole instrument using electron-volt collisions (between 70 and 200 eV) with argon at a pressure that yielded a target-gas thickness of 2–5 \times 10^{14} \text{atoms/cm}^2 (Tang et al., 1993), where target-gas thickness is equal to the product of the number density of the gas and the length of the collision cell (Thomson et al., 1995). The absence of w- and d-type ions in this electron-volt kinetic energy spectrum supports the suggestion that side-chain cleavages leading to these ions are high-energy processes. Some sequential dissociation, leading to internal fragments (e.g., y_{7}\text{b}_{3}) and immonium ions (P and F), is observed.

As discussed earlier in this chapter, Douglas (1998) has estimated that collisions of large ions with small targets can be very efficient for the transfer of available kinetic energy to internal energy; therefore, provided that sufficient collisions occur, even large proteins having many degrees of

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**Fig. 3.** Collision-induced dissociation (CID) spectrum for an \(^{16}\text{O}^{18}\text{O}\)-labeled tryptic peptide (DLEEGIQTLMGGR) collected on a tandem time-of-flight (TOF)/TOF instrument using 3-keV collisions with argon (pressure unspecified) for activation. Peak intensities in the full spectrum (lower panel) were multiplied eight times relative to the immonium ion spectrum (upper left panel). (Reproduced with permission from Medzihradzky et al., 2000.)
FIG. 4. MS/MS spectrum of +2 bradykinin collected on a triple‐quadrupole instrument using electron volt collisions (between 70 and 200 eV) with argon at a pressure that yielded a target‐gas thickness of $2 - 5 \times 10^{14}$ atoms/cm$^2$. Note at this lower collision energy, only $b$‐ and $y$‐type ions were formed, and that sequential dissociation led to ammonia loss (e.g., $y_{13} - 17$) and internal dissociation (e.g., $y_7 - b_5$). Ammonium ions were observed for proline (P) and phenylalanine (F). (Reproduced with permission from Tang et al., 1993.)
freedom, and hence large kinetic shifts can be driven to dissociate at observable rates. This is illustrated in the data in Fig. 5, which shows the dissociation of the \((M + 20H)^{+20}\) ion of apomyoglobin collected on a triple-quadrupole instrument at 2-keV collision energy using collisions with argon at a pressure that yielded a target-gas thickness of \(1 \times 10^{14}\) atoms/cm\(^2\) (Smith et al., 1990).

Note that this experiment is at the interface between the low-energy and high-energy regimens, highlighting the danger of describing an experiment based only on the collision energy. Because the force acting on an ion is proportional to the charge on the ion, a higher collision energy can be used for this highly charged protein ion while still enjoying the focusing advantages of the linear quadrupole discussed earlier in this chapter. At the pressure used in this study, multiple collisions were likely, so the experiment was a slow activation experiment with ample time for rearrangement before dissociation. Dissociation of this large precursor was aided by the fact that the ions were already “heated” in the electrospray interface by use of large potential differences between the lenses used to transport ions into the mass spectrometer (see the discussion of “nozzle/skimmer” dissociation below).

![CID of peptides and proteins](image-url)

**Fig. 5.** Collision-induced dissociation (CID) spectrum of \((M + 20H)^{+20}\) ions of horse heart apomyoglobin collected on a triple-quadrupole instrument at 2-keV collision energy using collisions with argon at a pressure that yielded a target-gas thickness of \(1 \times 10^{14}\) atoms/cm\(^2\). (Reproduced with permission from Smith et al., 1990.)
CID spectra collected at electron-volt collision energy can be more sensitive to changes in the collision energy than CID at kiloelectron-volt energy, allowing the effects of internal energy on ion dissociation to be probed in this regimen. This technique is referred to as energy-resolved MS (ERMS) (McLuckey and Cooks, 1983). ERMS is used to generate break-down graphs, or plots of the relative contributions of ion dissociation channels as a function of ion internal energy. Such graphs can be useful for elucidating reaction mechanisms and for distinguishing isomeric species.

At 100 eV, an m/z 1000 ion will travel through a 20-cm quadrupole in approximately 1.5 ms. This time scale means that extensive isomerization of the ion of interest may occur between collisions if multiple collision conditions are used. Processes with rate constants of $10^3 \text{s}^{-1}$ or greater can be observed in this relatively slow CID regimen. Collision gas pressures of 1–2 millitorr, leading to some tens of collisions, have traditionally been used to effect CID in a quadrupole. However, a major innovation in the use of quadrupoles for CID of larger biological ions is the demonstration that collisional damping of radial motion in the quadrupole allows higher pressures, and hence more collisions, to be used without excessive ion losses due to scattering (Douglas and French, 1992). Operation at approximately 8 millitorr has been shown to improve the efficiency of triple-quadrupole MS/MS from only a few percent to 30–50% (Thomson, 1995). Note that much of this improvement is due to the improved collection and transmission of product ions by the mass analyzing quadrupole (Q3) after the collision cell due to the removal of the spatial and kinetic energy spread of the products. At higher pressure, transit times through collision cell can be very long (tens of milliseconds) unless an additional electric field in the direction of ion motion through the quadrupole is added to “pull” the ions through the gas. When such a field is added, pressures of tens to hundreds of millitorr and as high as 3 torr may be used to effect CID (Dodonov et al., 1997; Javahery and Thomson, 1997; Lock and Dyer, 1999; Mansoori et al., 1998). Under these conditions, dissociation reactions with rates as low as a few tens per second can be accessed.

Another form of CID conducted at electron-volt collision energy is the so-called “in-source,” “cone-voltage,” or “nozzle/skimmer” CID effected in the interface region of ESI sources (Bruins, 1997; Katta et al., 1991; Loo et al., 1998). The lens voltages that guide ions through the intermediate pressure region between the atmospheric pressure source and the high vacuum of the mass analyzer region may be manipulated to cause ions to undergo collisions with residual background gas at collision energies up to a few hundred electron volts. The time scale for this type of dissociation is on the order of a few hundred microseconds to a few milliseconds. Although this is not a true MS/MS experiment because there is no prior
selection of the precursor ion, nozzle/skimmer CID can provide efficient dissociation of peptides and proteins on simple instruments. For example, the nozzle/skimmer CID spectrum of +2 bradykinin collected at 520 eV in the approximately 1–10 torr region of an ESI source interfaced to a single quadrupole is shown in Fig. 6 (bottom panel) (Katta et al., 1991). The spectrum is similar to that collected via true MS/MS with a triple-quadrupole instrument (Fig. 4), in that only $b$- and $y$-type ions are observed. Nozzle/skimmer dissociation has also been demonstrated for whole protein ions. Figure 7 shows the dissociation of multiply charged carbonic anhydrase B cations in an electrospray source interfaced to an FT-ICR instrument (Senko et al., 1994a). Nozzle/skimmer CID has also been used for ERMS of small peptides, with comparable results to those obtained for quadrupole ERMS (Dono et al., 1997; Harrison, 1999).

**Fig. 6.** Spectra of bradykinin recorded as a function of the potential difference between interface lenses of the electrospray source. (A) The potential difference was 80 V, and only the $(M+2H)^{+2}$ ions and $(M+2H)^{+2}$ with solvent molecules attached were observed. (B) The potential difference was increased to 160 V, so that the $(M+2H)^{+2}$ was efficiently desolvated. (C) The potential difference was further increased to 260 V, and abundant $b$- and $y$-type product ions were observed due to nozzle/skimmer collision-induced dissociation (CID). (Reproduced with permission from Katta et al., 1991.)
Ion-Trapping CID (Very Slow Activation, Slow Heating)

The two forms of ion traps used in MS, electrodynamic quadrupole ion traps and electrostatic/magnetic ICR ion traps, vary in the mechanism by which they store ions and perform mass analysis; however, their behavior with respect to CID is quite similar. CID in both instruments is characterized by many collisions (hundreds) at a few electron-volt (tens) collision energy and, as such, is qualitatively similar to the “low-energy” quadrupole CID discussed in the previous section. An important difference is that the trapping nature of these instruments means that very long time scales can be used to achieve very high dissociation efficiency (75–100%) and to access very slow dissociation channels.
Another consequence of the long time scale accessible with trapping instruments is that deactivating processes can also occur, so that a steady-state internal energy can be achieved. Details of CID in a quadrupole ion trap are discussed first in this section, followed by discussion of CID in an FT-ICR trap.

Quadrupole Ion Trap CID. Quadrupole ion traps store ions in the rapidly changing potential well generated by the application of an RF voltage to the trap electrodes. Ion motion in the field depends, *inter alia*, on the frequency and amplitude of the RF voltage and on ion \( m/z \). For a given RF frequency and amplitude, a selected precursor \( m/z \) will have a characteristic frequency of motion in the trap, normally referred to as the *secular frequency*. Application of a supplementary voltage matching this secular frequency will excite the precursor ions, causing them to undergo energetic collisions with background gas or deliberately added collision gas (usually helium, see below), thereby increasing their internal energy. This process, commonly called *resonance excitation*, was first demonstrated by Louris *et al.* in 1987. The depth of the trapping potential well is such that the ions can only be excited to a few electron volts of kinetic energy before they are ejected from the trapping volume by the supplementary resonant voltage. For this reason, multiple collisions over a relatively long time (tens to hundreds of milliseconds) are required to build up sufficient internal energy to lead to dissociation. Clearly, extensive rearrangement of the precursor ion can occur during the activation and before dissociation. Another consequence of this long slow buildup of internal energy is that deactivating processes such as IR emission and/or cooling collisions can also occur. Goeringer and McLuckey (1996a,b) have developed a collisional energy-transfer model for resonance excitation, which shows that competition between activation and deactivation leads to a steady-state internal energy that can be characterized by a Boltzmann distribution and an internal temperature, provided the dissociation rate is low compared to the activation/deactivation rate. If dissociation is rapid enough to deplete the high-energy tail of the distribution, a truncated Boltzmann distribution results and the temperature is more correctly described as an “effective” internal temperature (Vekey, 1996). For small peptides (e.g., leucine enkephalin, 556 Da), effective temperatures of approximately 650 K can be achieved (Goeringer *et al.*, 1999). The available temperature decreases with increasing \( m/z \) as the difference between the mass of the ion and the collision partner increases. Another factor that limits the temperature to which high \( m/z \) ions can be raised is that for a given RF amplitude, higher \( m/z \) ions are stored in shallower potential wells and so must be excited with lower resonance voltage amplitudes to avoid ion losses through ejection from the trap. Current instrumentation limits
the protein ion $m/z$ on which CID can be performed to approximately 8600 Da/charge (+1 ubiquitin) (Reid et al., 2001), although the upper limit for newer ion traps (e.g., the Finnigan LCQ, Bruker Esquire, or Hitachi M-8000) has not yet been evaluated. Note that this limitation is stated in terms of mass/charge. Proteins with masses greater than ubiquitin can be readily dissociated with quadrupole ion traps as multiply charged ions with $m/z$ lower that 8600 Da/charge; for example, a study of the dissociation of $(M + 2H)^{+2}$ to $(M + 21H)^{+21}$ ions of apomyoglobin (mass 16,950 Da) has appeared (Newton et al., 2001).

Shown in Fig. 8 is a CID spectrum for +2 bradykinin acquired with 200 ms of resonance excitation in an ion trap having a background pressure of 1 millitorr of helium. Note that only $b$- and $y$-type ions are formed, in similar fashion to the electron-volt collision CID performed in a quadrupole collision cell (Fig. 4) and in an ESI interface (Fig. 6). Whole-protein ions can also be dissociated via resonance excitation in a quadrupole ion trap. Figure 9 shows the CID spectrum of $(M + 8H)^{+8}$ ion of ubiquitin. The precursor ion was excited for 300 ms in the presence of 1 millitorr of helium.

![Figure 8. Collision-induced dissociation (CID) spectrum for +2 bradykinin acquired with 200 ms of resonance excitation in a quadrupole ion trap having a background pressure of 1 millitorr of helium. Note that only $b$- and $y$-type ions are formed, in similar fashion to the electron-volt collision CID performed in a quadrupole collision cell (Fig. 4) and in an ESI interface (Fig. 6). The abundance scale was multiplied three times relative to the abundance of the remaining precursor ion.](image-url)
(Reid et al., 2001). Although the internal temperature accessed during ion trap CID of this large ion is relatively low, the trapping nature of the instrument allows for long reaction times and hence low reaction rates, so relatively efficient dissociation is observed. An important point about quadrupole ion trap CID is that all product ions below a certain low-mass cutoff (LMCO), determined by the RF amplitude, are not trapped. For CID of large ions, it is desirable to employ a relatively high RF amplitude to maximize the potential well depth and avoid ion losses by ejection; however, a high RF amplitude means that low m/z products are not trapped and so are not observed.

As discussed earlier in this chapter, ion trap CID is always carried out under multiple collision conditions, hence extensive rearrangement can occur during the activation period and before dissociation. The collision gas used is almost always helium, because helium bath gas is necessary to improve the resolution of ion trap mass analysis (Stafford et al., 1984) and so is already present in the system at a static background pressure of 1 millitorr. The effect of adding higher mass collision gas has been studied, and it has been shown that the deposited internal energy is increased (Charles et al., 1994; Gronowska et al., 1990; Morand et al., 1992), as predicted by Eq. (1). Several groups have reported that addition of a small fraction (5%) of, for example, argon, krypton, or xenon can improve the CID efficiency of peptide ions by allowing higher internal energy deposition (Vachet and Glish, 1996). This has the effect of increasing the extent of dissociation and accessing higher critical energy dissociation channels (Vachet and Glish, 1996). Addition of heavy gases also allows CID to be carried out at lower RF-trapping voltages, which helps to overcome the LMCO limitation discussed earlier (Doroshenko and Cotter, 1996a). Note that the upper m/z limit for CID in the ion trap given earlier (8600 Da/charge) was probably achieved at least in part because of the significant background pressure of air (~1.5 × 10⁻⁴ torr) present in the ion trap (Reid et al., 2001).

An alternative to raising the internal temperature of the ions in a quadrupole ion trap by increasing the kinetic energy of the ions via resonance excitation is to increase the kinetic energy of the neutral collision partner by heating the helium bath gas. Asano et al. (1999a) have shown that ions reach thermal equilibrium with the bath gas, so if the temperature of the bath gas is known, and if the rate of activation/deactivation is large relative to the rate of dissociation, then Arrhenius activation parameters can be derived from measurements of ion dissociation rate as a function of bath gas temperature. In addition, they have demonstrated the measurement of Arrhenius parameters for leucine enkephalin and
FIG. 9. Collision-induced dissociation (CID) spectrum of (M + 8H)^+ ubiquitin ions excited in a quadrupole ion trap for 300 ms in the presence of 1 millitorr of helium. (A) The
bradykinin (Butcher et al., 1999) and have used leucine enkephalin as a thermometer ion to derive the temperature achieved via resonance excitation (Goeringer et al., 1999) and boundary-activated dissociation (BAD) (Asano et al., 1999b). Figure 10 shows the CID spectrum obtained for +2 bradykinin at a bath gas temperature of 486 K and pressure of 1 millitorr, with a reaction time of 10 s (Butcher et al., 1999). At this temperature, the facile cleavage at the N-terminal of proline dominates, with loss of a water molecule and formation of one other complementary pair also observed at low abundance.

A number of other alternatives for ion heating in quadrupole ion traps have also been demonstrated, such as direct current (DC) pulse activation

![CID spectrum for (M + 2H)^+ bradykinin](image)

**FIG. 10.** Collision-induced dissociation (CID) spectrum for (M + 2H)^+2 bradykinin obtained by storing the ions in quadrupole ion trap at a helium bath gas temperature of 486 K and pressure of 1 millitorr for 10 s. (Reproduced with permission from Butcher et al., 1997.)

Product ions shown have charge states ranging from unity to +8. (B and C) Ion/ion proton transfer reactions with anions of perfluorodimethycyclohexane have been used after CID to reduce the charge states of the product ions largely to +1 to simplify interpretation of the spectrum. Only b- and y-type ions are formed, and 50% sequence coverage is obtained from this single charge state. (Reproduced with permission from Reid et al., 2001.)
(which yields a mixture of CID and SID products) (Lammert and Cooks, 1992); low-frequency square-wave activation (Wang et al., 1996); irradiation with broadband waveforms such as filtered noise fields (FNFs) (Asano et al., 1995; Volmer and Niedziella, 2000), stored waveforms generated with inverse Fourier transforms (SWIFT) (Doroshenko and Cotter, 1996b; Soni et al., 1995), and random white noise (McLuckey et al., 1992); BAD (Creaser and O’Neill, 1993; Glish and Vachet, 1998; Paradisi et al., 1992); and off-resonance activation with a single frequency selected to be lower than the secular frequency of the precursor by approximately 5% (Qin and Chait, 1996). The last two techniques have been studied fairly extensively for their applicability to CID of peptide ions. The BAD technique involves applying a DC voltage to the ion trap electrodes to move ions close to the boundaries of stability in the trap, thereby increasing the amplitude of their oscillations and causing them to undergo energetic collisions with the bath gas. Glish and Vachet (1998) have shown that BAD can be used to dissociate peptides with the advantage that it is not necessary to tune the excitation to the exact frequency of ion oscillation to achieve maximum efficiency. However, the BAD technique suffers from relatively poor overall efficiencies due to the competition between ion ejection and dissociation. This limitation can be overcome somewhat by the combination of BAD with heavier collision gases (Glish and Vachet, 1998). Asano et al. (1999b) used leucine enkephalin to estimate that BAD can elevate ions to an effective internal temperature of approximately 700 K during collisions with helium, comparable to that obtained via normal resonance activation. Qin and Chait (1996) have shown that irradiating ions with a large-amplitude (21 V_{pp}) alternating current (AC) voltage at a frequency approximately 5% below the secular frequency of the ion could more efficiently dissociate large peptides than normal on-resonance excitation. They attributed the increase in efficiency to the ability to deposit larger amounts of internal energy without ion ejection from the trap.

**Fourier Transform–Ion Cyclotron Resonance CID.** FT-ICR MS uses the combination of a high magnetic field (from 3 to as high as 20 Tesla) with small DC potentials to trap ions. Ion motion in the magnetic field exhibits a characteristic frequency, the cyclotron orbital frequency, which is m/z dependent; hence, a resonance excitation method analogous to that described earlier for quadrupole ion traps can be employed in FT-ICR traps to increase ion kinetic energy and thus cause internal energy deposition via energetic collisions (Cody and Freiser, 1982). However, it has been shown that irradiating trapped ions at frequencies slightly (<1%) above or below the cyclotron frequency greatly improves the efficiency of CID in FT-ICR traps (Gauthier et al., 1991), because the ions can be held at
elevated kinetic energy for long periods without ejection from the trap. This technique is referred to as SORI. The mechanism for SORI essentially involves elevation of the excited ions to a steady-state kinetic energy after the first 10–20 ms of excitation due to dephasing of the ion motion with respect to the applied excitation. Ion kinetic energies are comparable to those achieved via quadrupole ion trap resonance excitation (i.e., some few electron volts and hence many multiple collisions are required to increase ion internal energy enough to cause dissociation). The maximum kinetic energy accessible depends on the strength of the trapping magnetic field. Schnier et al. (1999) have used Arrhenius activation parameters measured via blackbody infrared radiative dissociation (BIRD) for leucine enkephalin and bradykinin ions to show that effective temperatures of between 500 and 700 K are achieved during SORI using nitrogen as the collision gas. However, the authors are careful to state that the actual distribution of internal energy is not well characterized and may not be Boltzmann (Schnier et al., 1999). Laskin and Futrell (2000) have shown that smaller organic ions, such as bromonaphthalene, achieve effective temperatures from 1300 to 4000 K during SORI (Laskin and Futrell, 2000). Preliminary attempts to use master equation modeling to develop a thermal model of SORI analogous to that developed for quadrupole ion trap CID have shown that the initial internal energy of the ions before activation and radiative relaxation during activation both play significant roles in determining the ion internal temperature (Marzluff and Beauchamp, 1996).

![CID of peptides and proteins](image)

**Fig. 11.** Collision-induced dissociation (CID) spectrum of \((M + 2H)^{2+}\) bradykinin obtained via Fourier transform–ion cyclotron resonance (FT-ICR) sustained off-resonance irradiation (SORI) with irradiation for 500 ms at a nitrogen pressure of between 1 and \(8 \times 10^{-8}\) torr. Note the similarity between this spectrum and those shown in Figs. 8 and 10 for quadrupole ion trap resonance excitation and bath gas heating to 486 K, respectively. (Reproduced with permission from Schnier et al., 1999.)
Figure 11 shows the CID spectrum of +2 bradykinin obtained via SORI with irradiation for 500 ms at a nitrogen pressure of between 1 and $8 \times 10^{-6}$ torr (Schnier et al., 1999). Note the similarity between this spectrum and those shown in Figs. 8 and 10 for quadrupole ion trap resonance excitation and bath gas heating to 486 K, respectively.

As discussed earlier in this chapter, Marzluff et al. (1994a) has shown that transfer of available kinetic energy to internal energy is efficient at the kinetic energies accessed via SORI. Therefore, provided that sufficient collisions occur, even large protein ions can be made to dissociate. Figure 12 shows the SORI-CID spectrum of $(M+17H)^{+17}$ ion of apomyoglobin

![Figure 12](image)

**Fig. 12.** Sustained off-resonance irradiation (SORI)–collision-induced dissociation (CID) spectrum of $(M+17H)^{+17}$ apomyoglobin obtained by exciting the ions for 2 s in the presence of nitrogen. (A) The activation was shifted to the low $m/z$ side of the precursor ion, and (B) it was shifted to the high $m/z$ side. Product ions having $m/z$ ratios that cause them to be on resonance with the applied activation voltage may be further dissociated or ejected from the ion cyclotron resonance (ICR) cell, so that dissociation on both sides of the precursor ion is necessary to observe all dissociation channels accessed by the precursor. (Reproduced with permission from Senko et al., 1994b.)
obtained by exciting the ions for 2 s in the presence of nitrogen (pressure unspecified) (Senko et al., 1994b). In analogy to quadrupole ion trap CID, the internal temperature of the ions is expected to be relatively low, but the trapping nature of the ICR instrument allows for very long excitation times, so processes with rates as low as 10/second or less can be observed.

The effects of the pressure and nature of the collision gas used in SORI-CID have not been widely studied. FT-ICR operates at very low (10⁻⁹ torr) pressure during mass analysis to achieve ultrahigh resolution; therefore, collision gas for CID is pulsed into the cell. Because the collision gas is not present at a constant pressure during the CID event, characterization of pressure effects is difficult. Gorshkov et al. (1999) have presented a model for estimating the average laboratory-frame kinetic energy, which accounts for the presence of collision gas, which can cool the ions if the pressure is too high. They have shown that there is an optimum gas pulse length and, hence, pressure peak during the CID event, which yields maximum internal energy deposition and CID efficiency. At shorter pulse times (lower pressure), an insufficient number of collisions occurs, and CID yields are reduced; at longer pulse times (higher pressure), collisional damping lowers ion kinetic energy, again limiting internal energy deposition and subsequent CID yields.

Two other methods for exciting ions for CID in an FT-ICR trap are also used, both with the same goal as SORI to increase ion kinetic energy without causing ion ejection from the trapping cell. MECA (Williams and McLafferty, 1990) relies on a number (hundreds) of low-amplitude on-resonance excitation periods, each of which increases ion kinetic energy, but not to the extent that ions are ejected. Ion kinetic energy decreases between each activation period, but internal energy does not (at least not all the way back to the starting point), and so by using several excitation periods, internal energy can be increased until ions dissociate without causing ion injection. VLE-CA also uses on-resonance activation (Boering et al., 1992a,b), but with rapid 180° phase shifts of the applied excitation voltage. These phase shifts prevent the applied voltage from increasing the ion kinetic energy to the ejection point. Senko et al. (1994b) have evaluated the applicability of SORI, MECA, and VLE-CA (and the related resonant amplitude modulated collisional activation [RAM-CA]) to the dissociation of protein ions in an FT-ICR cell.

An important distinction between CID in both types of ion traps and the other types of CID discussed above is that because of the resonant nature of the excitation in ion traps, the product ions are not themselves subject to further activation after their formation. Product ions are formed with the same internal energy as the precursor, on a “per degree-of-free-
dom” basis, and so they may still undergo further dissociation if the dissociation rate is faster than the rate of deactivation; however, they will not receive any further internal energy from the resonance excitation voltage, unless they have \( m/z \) values that place them very near the precursor ion or on-resonance with the excitation voltage in off-resonance excitation experiments such as SORI. For example, in Fig. 12, the \( y_{149}^{+16}, y_{147}^{+16}, y_{93}^{+10}, \) and \( y_{126}^{+14} \) ions are abundant products having \( m/z \) values above that of the precursor ion. These products are observed when the activation is shifted below the \( m/z \) of the precursor (Fig. 12A) but are dissociated or ejected from the trap when the activation is shifted above the \( m/z \) of the precursor (Fig. 12B). In the quadrupole ion trap, Goeringer et al. (1999) have shown that the helium bath gas actively cools product ions at a fairly high rate so that further dissociation is minimized in the quadrupole ion trap. Vachet and Glish (1996) and Doroshenko and Cotter (1996a) have reported an increase in sequential dissociation when heavier bath gases such as argon, krypton, or xenon are added to the helium during CID. The general lack of sequential dissociation observed in ion trap CID may or may not be regarded as a positive result. In terms of peptide and protein sequencing, the absence of internal fragments from the amino acid chain makes deduction of the sequence from the MS/MS dissociation easier. On the other hand, some use has been made of the immonium ions of individual amino acids that appear from sequential dissociation of the precursor to help determine the amino acid content of the peptide under study (Biemann and Papayannopoulos, 1994; Hunt et al., 1986).

Summary and Conclusions

This chapter has focused exclusively on the effects of experimental variables, described in terms of the set of figures of merit, on CID. At least an equal amount could be said about the effects of ion structure on CID behavior, even if the discussion were limited to what is known about peptides and proteins. An exhaustive summary of what is known about biological ion structural effects is beyond the scope of this chapter. The interested reader is referred to the literature on the effects of primary structure, secondary structure and conformation, and charge state on the dissociation reactions of peptides and proteins (O’Hair, 2000; Polce et al., 2000; Schlosser and Lehmann, 2000; Tsapralis et al., 1999, 2000; Wysocki et al., 2000). The reader should also note that MS and tandem MS is seeing increasing use in the study of other important biological molecules such as nucleic acids (Hofstadler and Griffey, 2001), lipids (Murphy et al., 2001), and carbohydrates (Harvey, 2001).
CID is a widely applicable technique that can be used to obtain sequence and structural information from biologically derived ions. A wide variety of activation conditions lead to complementary information from different CID methods. CID conducted on sector or TOF/TOF instruments with kiloelectron-volt collision energy and low collision numbers leads to efficient dissociation of the amide bonds in peptides to yield sequence information, while also accessing amino acid side-chain cleavages to aid in the distinction of isomeric and isobaric amino acids. Dissociation spectra recorded in this high-energy regimen are extremely reproducible. A disadvantage of operation in this regimen is that dissociation rates must be driven at $10^4$–$10^6$/s, which has proven to be very difficult for ions more than approximately 3000 Da. Lower energy CID in the electron-volt regimen is effected in collision quadrupoles, where time scales can be greater by at least three orders of magnitude than at high energy. This, coupled with the high efficiency of energy transfer for collisions at lower energy, allows efficient dissociation of large ions, but without the side-chain cleavages observed at higher energy. Ion-trapping instruments can use even longer dissociation times (up to several seconds) to access very slow (1/s or less) dissociation processes. This allows ion trap slow heating methods to efficiently dissociate biomolecules of increasing size, even up to very large proteins.

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References


