J. Am. Chem. Soc. **1994,** *116,* 6947–6948

Collisional Activation of Large Molecules Is an Efficient Process

Elaine M. Marzluff, Sherrie Campbell, M. T. Rodgers, and J. L. Beauchamp*

Contribution No. 8936, Beckman Institute
California Institute of Technology
Pasadena, California 91125

Received March 24, 1994

It is a common perception that as molecules become larger they become inherently more difficult to activate and dissociate in the gas phase, especially under low energy collision induced dissociation (CID) conditions.[1] This difficulty has been attributed both to the inefficiency of the collisional activation process[1a,b] and to the requirement of higher levels of internal excitation to induce dissociation.[1c] A serious consequence of this supposition is the implied difficulty of using CID methods to sequence peptides and proteins.

A thorough understanding of the collisional activation dynamics of large molecules has been difficult to obtain due to lack of a simple model system. We have discovered a candidate for such studies in an investigation of the low energy dissociation pathways exhibited by deprotonated peptides.[2] In particular, deprotonated peptides without acidic side chains dissociate at low collision energies to yield exclusively the deprotonated C-terminus amino acid. This system provides a homologous series of molecules which undergo unimolecular dissociation with similar activation parameters. Here the effect of molecular size on low energy collisional activation processes is investigated using Fourier transform ion cyclotron resonance mass spectrometry.

The instrument and methodology of the collisional activation experiments have been described previously in detail.[2] The deprotonated parent peptide was isolated with a series of radio frequency (rf) ejection pulses. Activation of the peptide was initiated by the application of rf excitation slightly off resonance from the cyclotron frequency of the selected species.[3] The ion oscillates with low (typically <5 eV center of mass (COM)) bounded translational energy. In the presence of a collision gas the ion undergoes multiple collisions with the internal energy of the ion increasing over time until it undergoes unimolecular dissociation.[4] Collision gases included nitrogen, carbon dioxide, cyclohexane, and ammonia at static pressures of 0.5–2.0 × 10^{-7} Torr. The extent of dissociation of the activated ion was monitored as the translational energy, excitation time, and collision gas were varied.

The proposed mechanism to account for the dissociation of deprotonated peptides without acidic side chains to give the deprotonated C-terminus amino acid is illustrated in Scheme 1.[2] The reaction is endothermic by approximately 30 kcal mol^{-1} (1.3 eV) and is estimated to have a threshold energy of 2.5 eV and a frequency factor of $10^{13.2}$. Larger molecules will require more internal energy to produce dissociation at the same rate. A simple RRKM rate calculation demonstrates this for a series of deprotonated alanine polymers with an assumed threshold energy of 2.5 eV as shown in Figure 1.[5] Typical rates of dissociation for the time scale of our experiments using off resonance collisional activation are in the range of 1–10 s^{-1}. The excess energy in the activated complex required to obtain equal rates of dissociation increases as the number of degrees of freedom of the molecule increases.

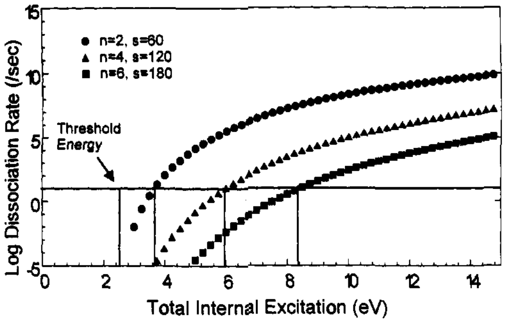


Figure 1. Dissociation rates for deprotonated (ala)$_n$ as a function of total internal energy of the molecule at an assumed threshold energy (E_0) of 2.5 eV and a frequency factor of $10^{13.2}$ where n = 2, 4, 6 and s (the number of internal degrees of freedom) = 60, 120, and 180.

Scheme 1

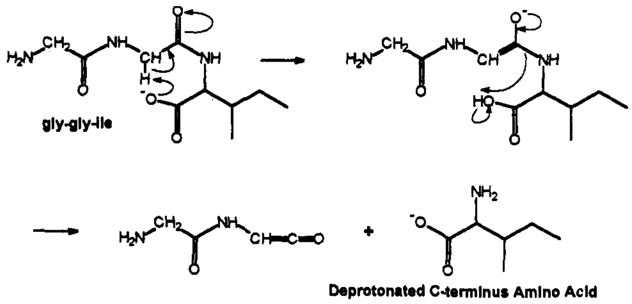


Figure 2a shows the extent of dissociation observed for each peptide. These data clearly demonstrate that the efficiency of collisional activation and dissociation increases with increasing molecular size. Some of this enhanced dissociation can be attributed to increased cross section as a function of size. However, simple estimates of molecular size imply that this can account for at most a factor of 2 difference and does not fully explain the nearly 8-fold difference in dissociation efficiency observed here.

It is concluded that the efficiency of converting relative translational energy into vibrational excitation increases with increasing molecular size. A simple estimate of the collision efficiency can be obtained by considering deprotonated gly-gly-ile which is 50% dissociated with the conditions indicated in Figure 2a. Using a gas kinetic ion–molecule collision rate of 1 × 10^{-9} cm^3 $molecule^{-1}$ s^{-1}, under these conditions the ion undergoes about 6.5 collisions s^{-1}. RRKM calculations with an assumed threshold energy of 2.5 eV indicate that it requires about 4.5 eV of total internal energy to induce dissociation at a rate of 10 s^{-1}. This corresponds to an average energy transfer of 0.69 eV per collision, which is 55% of the average COM collision energy.

Trajectory calculations using a molecular mechanics force field are consistent with our experimental observations.[6] Figure 3 shows the calculated amount of energy transferred per collision for deprotonated gly-gly-ile colliding with nitrogen at a relative

---

* Author to whom correspondence should be addressed.

[1] (a) Alexander, A. J.; Boyd, R. K. *Int. J. Mass. Spectrom. Ion Processes* **1989**, *90*, 211–240. (b) Alexander, A. J.; Thibault, P.; Boyd, R. K.; Curtis, J. M.; Rinehart, K. L. *Int. J. Mass Spectrom. Ion Processes* **1990**, *98*, 107–134. (c) Despeyroux, D.; Wright, A. D.; Jennings, K. R. *Int. J. Mass Spectrom. Ion Processes* **1993**, *126*, 95–99.

[2] Marzluff, E. M.; Campbell, S.; Rodgers, M. T.; Beauchamp, J. L. *J. Am. Chem. Soc.*, in press.

[3] Gauthier, J. W.; Trautman, T. R.; Jacobsen, D. B. *Anal. Chim. Acta* **1991**, *246*, 211–225.

[4] Numerous mass spectrometry experiments have made use of multiple low energy collision conditions to accumulate internal excitation and induce rearrangement and dissociation of ions. See, for example: Jarrold, M. F.; Honea, E. C. *J. Am. Chem. Soc.* **1992**, *114*, 459–464. Nourse, B. D.; Cox, K. A.; Morand, K. L.; Cooks, R. G. *J. Am. Chem. Soc.* **1992**, *114*, 2010–2016.

[5] RRKM calculations were carried out using the Whitten–Rabinovitch approximation to estimate sums and densities of states. Whitten, G. Z.; Rabinovitch, B. S. *J. Chem. Phys.* **1963**, *38*, 2466–2473. See also Appendix 1 of ref 2 for more details.

[6] Trajectory calculations were run using MM+, a version of the MM2 force field supplied with Hyperchem (Autodesk, Inc.) to describe the peptide and an exponential-6 potential to describe the projectile–peptide interaction. It is described more fully by Allinger: Allinger, N. L. *J. Am. Chem. Soc.* **1977**, *99*, 8127. Atomic charges were initially optimized using semiempirical methods.

0002-7863/94/1516-6947$04.50/0

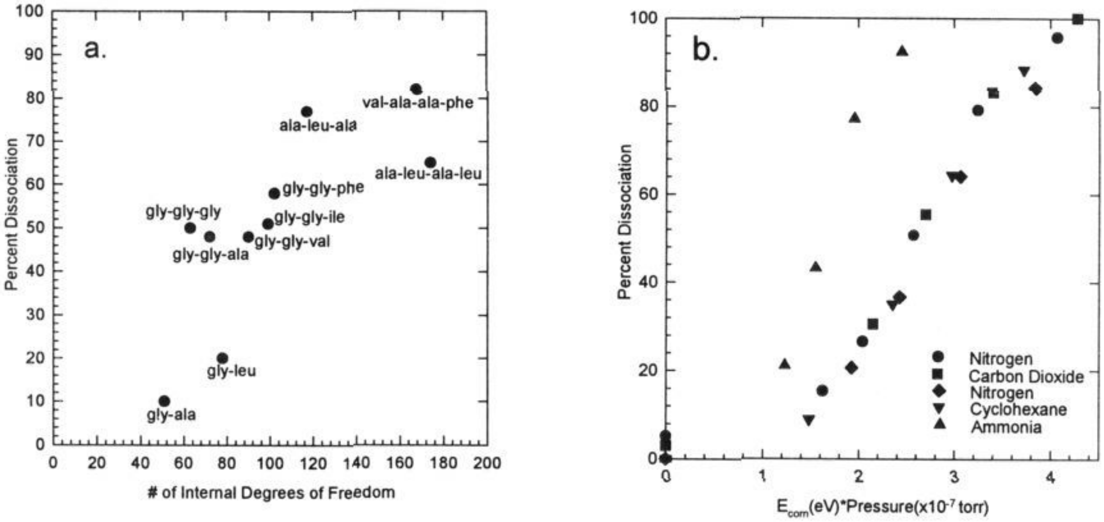
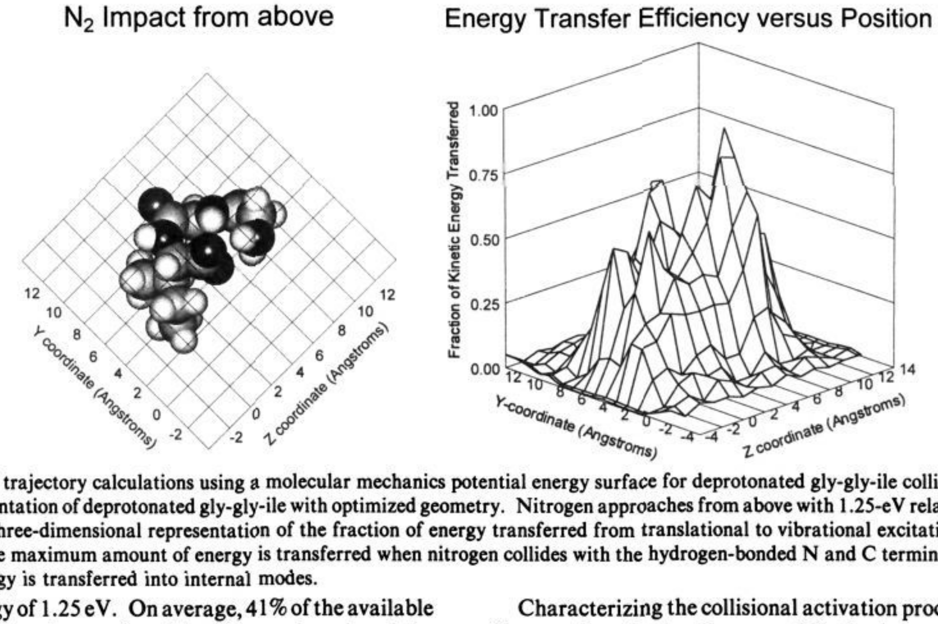


Figure 2. (a) Effect of molecular size on dissociation efficiency. The extent of dissociation as a function of the number of internal modes of the peptide at a constant average center of mass collision energy of 1.25 eV and nitrogen collision gas pressure of 2×10^{-7} Torr is shown. The off resonance excitation was applied for a period of 1 s. (b) Effect of collision gas on dissociation efficiency. The extent of dissociation of deprotonated gly-gly-ala was monitored as the average COM collision energy was varied for nitrogen, carbon dioxide, cyclohexane, and ammonia collision gases. The off resonance excitation was applied for a period of 1 s. Data are given for nitrogen at two different pressures. Ammonia is more efficient at activating large molecules.


N_2 Impact from above

Energy Transfer Efficiency versus Position

Figure 3. Results of trajectory calculations using a molecular mechanics potential energy surface for deprotonated gly-gly-ile colliding with nitrogen. On the left is the orientation of deprotonated gly-gly-ile with optimized geometry. Nitrogen approaches from above with 1.25-eV relative kinetic energy. On the right is the three-dimensional representation of the fraction of energy transferred from translational to vibrational excitation as a function of impact position. The maximum amount of energy is transferred when nitrogen collides with the hydrogen-bonded N and C termini. On average 41% of the collision energy is transferred into internal modes.

translational energy of 1.25 eV. On average, 41% of the available relative kinetic energy is transferred into internal modes of the peptide, which undergoes extensive deformation during the collisions. Energy transfer is insensitive to nitrogen orientation. The peptide behaves like a compressible energy-absorbing medium. Trajectory calculations for nitrogen colliding with protonated bradykinin (a nonapeptide with 444 internal degrees of freedom) typically show energy conversions of greater than 90% even at relative collision energies as high as 100 eV. It should be possible to deposit reasonably well defined amounts of internal excitation in a large molecule under single-collision conditions.[7]

The effect of collision gas on dissociation efficiency was examined for nitrogen, carbon dioxide, cyclohexane, and ammonia. Nitrogen, carbon dioxide, and cyclohexane have similar activation efficiencies (Figure 2b), with the extent of dissociation varying as a function of the product of the average COM collision energy and the pressure, which is a measure of the "energy exposure" for multiple collision conditions. The one polar species investigated, ammonia, is more efficient at transfer of energy into internal modes.

Characterizing the collisional activation process is a necessary first step in understanding more fully the dynamics and energetics of the dissociation of large, complex molecules. The off resonance collisional activation process is amenable to modeling with a master equation analysis which includes the effects of ion deactivation due to collisions and infrared radiative emission.[8] This may yield parameters such as activation energies. The largest species we have examined in the present studies has a molecular weight of 405 amu. It will be of particular interest to extend the present studies to peptides with significantly higher molecular weights.

Acknowledgment. We gratefully acknowledge the financial support of E.M.M. from a Rainin Fellowship and a NIH-NRSA Human Genome Fellowship, of S.C. from a NIH-NRSA traineeship in biotechnology, and of M.T.R. from a California Institute of Technology Consortium grant. We are indebted to the Beckman Foundation and Institute for support of the research facilities. This work was supported in part by the NSF under Grant CHE-9108318. Funds for instrument development have also been provided by ARPA and the DOD-URI program (ONR-N0014-92-J-1901).

---

(7) One way to readily accomplish this is by excitation with a fast neutral colliding with a slow ion. This has been proposed by Turecek: Turecek, F. *Rapid Commun. Mass Spectrom.* **1991**, *5*, 78.

(8) E. M. Marzluff, J. L. Beauchamp, to be published.