Intramolecular [^{18}O] Isotopic Exchange in the Gas Phase Observed during the Tandem Mass Spectrometric Analysis of Peptides

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Abstract: Fast atom bombardment/tandem mass spectrometry of protonated and metal cationized peptides has revealed evidence for novel intramolecular rearrangements in the gas phase. Previous studies utilizing [$^{18}O_2$] analogues (in which the isotopic labels reside in the C-terminal carboxyl group) elucidated a fragmentation mechanism in which the C-terminal amino acid residue is lost with retention of an original carboxyl oxygen at the C-terminus of the truncated peptide product ion. New evidence indicates that in some instances both of the original C-terminal carboxyl oxygens are retained to some degree in this process, demonstrating concomitant isotope exchange and rearrangement. Second generation product ion scanning (MS/MS/MS) analyses of the protonated [$^{18}O_2$] analogues established that both isotope labels are located at the new C-terminus formed via the rearrangement. The kinetic energies released during the formation of the [$^{18}O_1$]- and [$^{18}O_2$]-labeled rearrangement products are indistinguishable, consistent with the involvement of a common intermediate. First generation product ion spectra (MS/MS) of protonated [$^{18}O_2$]-peptides include fragments arising from simple peptide bond cleavage which show isotope enrichments consistent with immediate precursor ions which incorporate [^{18}O] at the peptide bond between the penultimate and C-terminal amino acid residues. Several lines of evidence eliminate the possibility of such incorporation of label during solution-phase preparation of the [$^{18}O_2$]-labeled analogues. Thus, the combined data are in accord with an isotope exchange process occurring in the gas phase, most likely through a mechanism involving the reversible formation of a cyclic intermediate. These studies, combined with previous data, emphasize the importance of the conformations adopted by gas-phase peptide ions in influencing fragmentation pathways.

Introduction

The utility of tandem mass spectrometry, incorporating either low- or high-energy collisional activation of precursor $[M + H]^+$ ions, for the structure elucidation of peptides is now widely recognized.[1,2] In many instances the principal fragmentations observed represent cleavages of the peptide backbone, facilitating sequence analysis. High-energy collisional activation additionally promotes fragmentations of the amino acid side chains, and this may facilitate discrimination between isomeric and isobaric residues.[3] The structural features which predispose to particular fragmentations are, however, imperfectly understood, and more complex dissociation pathways are sometimes observed. Recent work in this laboratory,[4,5] for example, elucidated a rearrangement process of peptide $[M + H]^+$ ions in which loss of the C-terminal amino acid residue is accompanied by transfer of an OH group from the C-terminal carboxylic acid moiety to the carbonyl group of the final peptide bond, giving rise to a product ion which may be formally designated as $[B'_{n-1} + OH]^+$ (using the Roepstorff and Fohlman nomenclature[6] for an n-residue peptide). This ion is mass spectrometrically indistinguishable from the $[M + H]^+$ ion of the corresponding peptide shortened by one amino acid residue at the C-terminus, as judged by second generation product ion scanning experiments.[5] The rearrangement process is apparently analogous to that previously observed by other groups for metal cationized peptides, in which a $[B_{n-1} + Cat + OH]^+$ ion is formed.[7-10] Our earlier studies[5] indicated that the proclivity of protonated and metal cationized peptides to fragment via C-terminal rearrangement was determined by the amino acid composition both at the C-terminus and at points in the sequence remote from the C-terminus. The latter observation suggests the influence of long-range interactions, presumably determined by the conformation of the peptide ion adopted in the gas phase.

The importance of the conformations of peptides and proteins in solution is, of course, widely recognized as a determinant of biological activity.[11] Much attention has focussed, for example, on the conformationally flexible enkephalin pentapeptides,[12] including their apparent adoption of a favored conformation in a biomembrane-like environment.[13] Recent interest has been attached to the potential use of mass spectrometry to characterize the solution conformations of small proteins via inspection of the charge distribution of gas-phase ions generated during electrospray introduction of protein solutions.[14,15]

In principle, mass spectrometry (and, particularly, tandem mass spectrometry) provides the opportunity for the study of intramolecular (intra-ionic) conformational effects in the absence of solvation. Little explicit attention has been focussed, however, on the influence of the gas-phase conformation of peptide ions on their modes of fragmentation. In their studies of the metastable and collision-induced decompositions of metal cationized small peptides, Russell and co-workers[16] proposed polydentate interactions between cations and peptides, implying that particular gas-phase conformations could be induced. Analogous proposals involving polydentate interactions (though with differences in the suggested sites of metal cation attachment) have been made by Teesch and Adams.[17,18] Leary and co-workers[19] have described

(1) Biemann, K. *Biomed. Environ. Mass Spectrom.* **1988**, *16*, 99-112.
(2) Hunt, D. F.; Yates, J. R.; Shabanowitz, J.; Winston, S.; Hauer, C. R. *Proc. Natl. Acad. Sci. USA* **1986**, *83*, 6233-6237.
(3) Johnson, R. S.; Martin, S. A.; Biemann, K. *Int. J. Mass Spectrom. Ion Proc.* **1989**, *86*, 137-154.
(4) Thorne, G. C.; Gaskell, S. J. *Rapid Commun. Mass Spectrom.* **1989**, *3*, 217-221.
(5) Thorne, G. C.; Ballard, K. D.; Gaskell, S. J. *J. Am. Soc. Mass Spectrom.* **1990**, *1*, 249-257.
(6) Roepstorff, P.; Fohlman, J. *Biomed. Mass Spectrom.* **1984**, *11*, 601.
(7) Tang, X.; Ens, W.; Standing, K. G.; Westmore, J. B. *Anal. Chem.* **1988**, *60*, 1791-1799.
(8) Renner, D.; Spiteller, G. *Biomed. Environ. Mass Spectrom.* **1988**, *15*, 75-77.
(9) Grese, R. P.; Cerny, R. L.; Gross, M. L. *J. Am. Chem. Soc.* **1989**, *111*, 2835-2842.
(10) Grese, R. P.; Gross, M. L. *J. Am. Chem. Soc.* **1990**, *112*, 5098-5104.
(11) Rivier, J. E.; Marshall, G. R., Eds. *Peptides: Chemistry, Structure and Biology*; ESCOM Science Publishers: Leiden, The Netherlands, 1990.
(12) Hruby, V. J.; Kao, L.-F.; Pettit, B. M.; Karplus, M. *J. Am. Chem. Soc.* **1988**, *110*, 3351-3359.
(13) Behnam, B. A.; Deber, C. M. *J. Biol. Chem.* **1984**, *259*, 14935-14940.
(14) Chowdhury, S. K.; Katta, V.; Chait, B. T. *J. Am. Chem. Soc.* **1990**, *112*, 9012-9013.
(15) Loo, J. A.; Loo, R. R. O.; Udseth, H. R.; Edmonds, C. G.; Smith, R. D. *Rapid Commun. Mass Spectrom.* **1991**, *5*, 101-105.
(16) Russell, D. H.; McGlohon, E. S.; Mallis, L. M. *Anal. Chem.* **1988**, *60*, 1818-1824.

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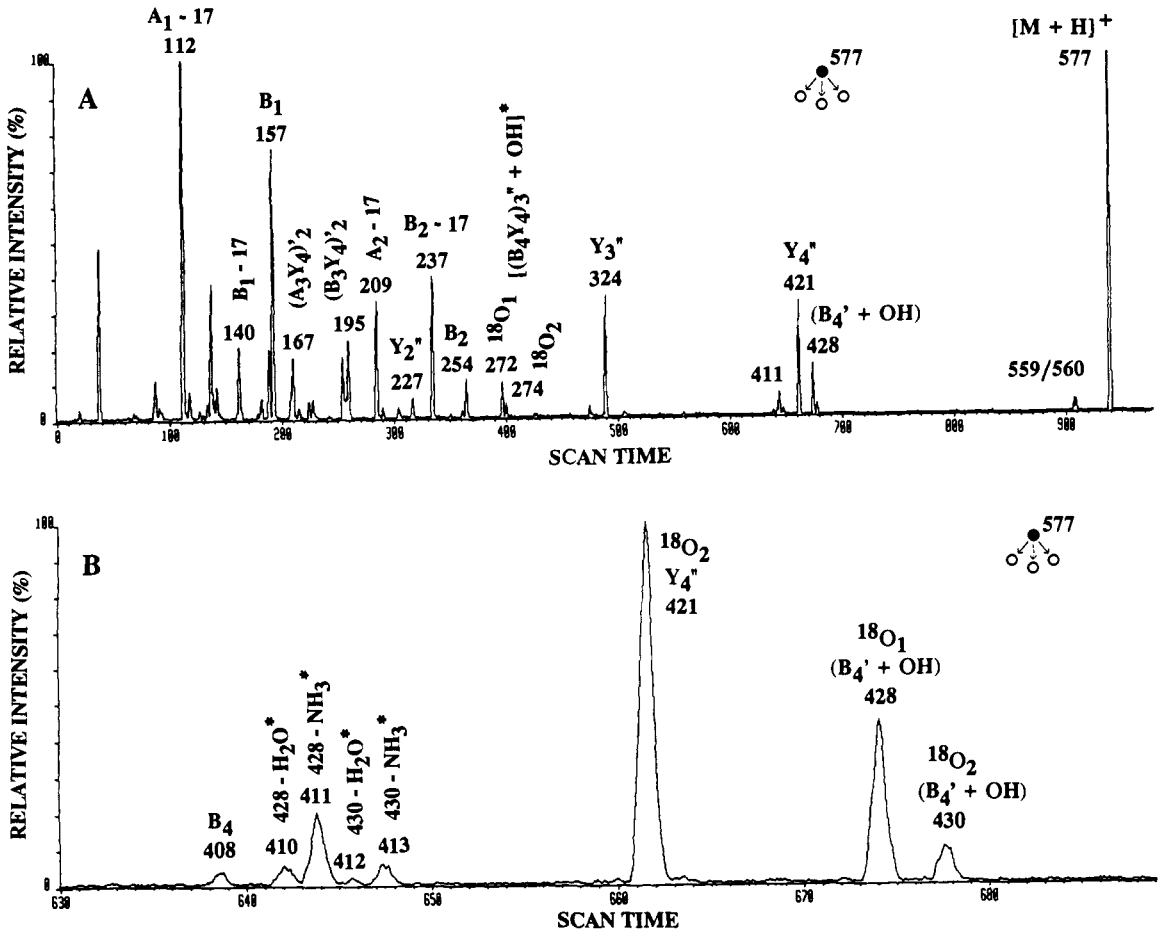
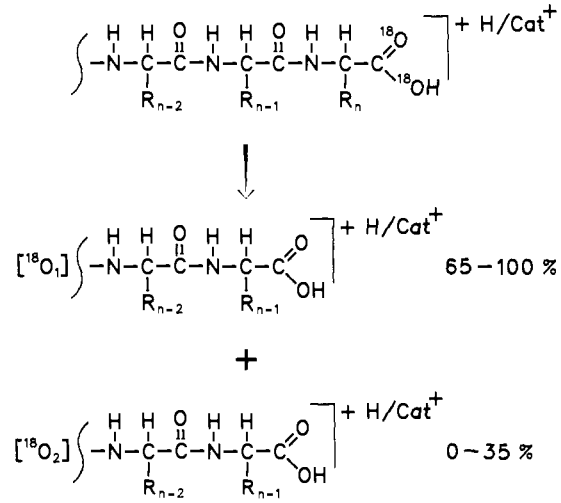


Figure 1. (A) Low-energy CAD (43 eV) first generation product ion spectrum of the $[M + H]^+$ ion of $[^{18}O_2]$RPPGF. (B) Expanded portion of part A encompassing the region of the $[B'_4 + OH]^+$ rearrangement ion. All of the figures employ the symbolism introduced by Cooks and co-workers[21] for designating the type of tandem MS experiment depicted. The isotope labeling patterns and sequential MS experiments (not shown) support the assignments designated by an asterisk.

experimental and theoretical (MNDO calculation) studies of lithium cationized small peptides which suggest the influence of cationization on peptide conformation in the gas phase.

The study of rearrangement and/or isotope exchange processes provides an additional approach to probing gas-phase ion conformations. In our earlier studies[4,5] of the C-terminal rearrangement process occurring in both protonated and metal cationized peptides, the transfer of carboxyl oxygen from the original C-terminus to the new C-terminus during the rearrangement was demonstrated using $[^{18}O_2]$-labeled peptides, with both ^{18}O atoms located in the C-terminal carboxylic acid group. Thus, in the product ion spectra of these compounds, the $[M + H]^+$ ions of $[^{18}O_2]$-labeled peptides gave rise to rearrangement species which retained one of the ^{18}O labels ($[B'_{n-1} + {}^{18}OH]^+$). Also observed (but not further studied) during the previous investigations were less intense signals apparently corresponding to rearrangement species which retained both of the ^{18}O labels. Such signals were present in the previously published product ion spectra of protonated $[^{18}O_2]$angiotensin III and sodium cationized $[^{18}O_2]$-methionine enkephalin.[4] These observations are summarized in Scheme I. The structure of the $[^{18}O_2]$-labeled rearrangement ions and the mechanism of their formation are the subjects of the present study. The mechanisms which have previously been proposed for the rearrangement process for both metal cationized[7-10] and protonated[5] peptides do not account for the retention of both ^{18}O atoms in the rearrangement products. It was unclear, therefore, whether the observation of the $[^{18}O_2]$-labeled rearrangement species required a modified mechanism, incorporating gas-phase $[^{18}O]$-isotope exchange from the C-terminal carboxyl group, or represented a distinct rearrangement process. The present studies were undertaken to address these issues using a variety of tandem MS approaches.

Scheme I

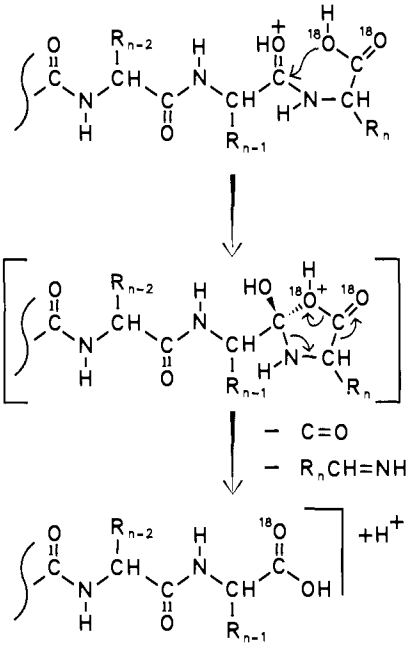


Results and Discussion

When a peptide is ^{18}O labeled by acid catalyzed exchange in the presence of $H_2{}^{18}O$, both of the labile C-terminal oxygens undergo exchange due to their equivalence, as do both oxygens in any carboxylic acid side chains.[20] The pentapeptide RPPGF

(17) Teesch, L. M.; Adams, J. *J. Am. Chem. Soc.* **1990**, *112*, 4110-4120.
(18) Teesch, L. M.; Adams, J. *J. Am. Chem. Soc.* **1991**, *113*, 812-820.
(19) Leary, J. A.; Zhou, Z.; Ogden, S.; Williams, T. D. *J. Am. Soc. Mass Spectrom.* **1990**, *1*, 473-480.

Scheme II



has a free C-terminal carboxylic acid group and four peptide bond carbonyl functions, but no side chain oxygens. The low-energy collisionally activated decomposition (CAD) product ion spectrum of [$^{18}O_2$]-labeled RPPGF is presented in Figure 1A, with an expanded portion of the spectrum encompassing the rearrangement species depicted in Figure 1B. The B_n, Y_n'', and internal cleavage ions observed in Figure 1A are consistent with the expected labeling pattern, so that the Y_n'' ions are shifted in mass by 4 u as compared with the unlabeled material, and the ions of the B_n series and the internal cleavage products are not shifted. The signal corresponding to the $[B'_4 + OH]^+$ rearrangement ion, observed at m/z 426 with the unlabeled material, is shifted primarily by 2 u to m/z 428, consistent with the transfer of one of the C-terminal ^{18}O atoms during the rearrangement. However, a signal approximately 20% as intense as that at m/z 428 is observed at m/z 430 (Figure 1B), suggesting that two ^{18}O atoms were retained in a significant proportion of the rearrangement ions. The retention of one of the ^{18}O atoms during the rearrangement process is readily explained by a mechanism such as that depicted in Scheme II, which is analogous to that proposed by Westmore and co-workers[7] for the rearrangement of sodium cationized peptides. This mechanism differs from others proposed for both protonated[5] and metal cationized[8-10] peptides in that the rearrangement is depicted in Scheme II as charge-directed, as opposed to charge-remote. Definitive evidence to distinguish between the alternative mechanisms is lacking, but Adams and co-workers have recently reported[21] data that tend to favor the Westmore mechanism[7] for the rearrangement of metal cationized peptides. None of the alternative mechanisms adequately accounts for the observation of rearrangement species which retain two ^{18}O atoms if both of these atoms were originally located at the C-terminus.

Many of the peptides which have been observed to undergo this rearrangement process also undergo a second stage of rearrangement, i.e. the $[B'_{n-1} + OH]^+$ ions further rearrange to yield $[B'_{n-2} + OH]^+$ ions. With [$^{18}O_2$]-labeled peptides, the [$^{18}O_1$]-labeled $[B'_{n-1} + OH]^+$ ions formed during the first rearrangement would be expected to give rise to both unlabeled and [$^{18}O_1$]-labeled $[B'_{n-2} + OH]^+$ ions in a ratio of 1:1 (in the absence of an isotope effect). For the [$^{18}O_2$]-labeled $[B'_{n-1} + OH]^+$ ions, if both labels are located at the C-terminus, then these ions would be expected to give rise to [$^{18}O_1$]-labeled $[B'_{n-2} + OH]^+$ ions but no unlabeled $[B'_{n-2} + OH]^+$ ions. If the second label is located somewhere other than at the C-terminus (e.g. in one of the preceding peptide bonds), then both [$^{18}O_1$]- and [$^{18}O_2$]-labeled $[B'_{n-2} + OH]^+$ ions would be expected in a 1:1 ratio. Sodium cationized leucine and methionine enkephalin (YGGFL and YGGFM, respectively) and protonated TRKR all undergo at least two stages of rearrangement. In order to examine directly the sequential rearrangement processes, the [$^{18}O_1$]- and [$^{18}O_2$]-labeled first generation rearrangement ions from these peptides were analyzed by second generation product ion scanning using the sequential MS capability of the BEqQ hybrid mass spectrometer.[4,5,22,23] The first generation rearrangement species, either [$^{18}O_1$] or [$^{18}O_2$] labeled, formed in the first field-free region of the mass spectrometer, were selected with the BE portion of the instrument (achieving an effective mass resolution of 800 for the first generation product ions) and introduced into the rf-only quadrupole. The quadrupole mass analyzer was then scanned over a narrow range to detect the second generation rearrangement ions formed in the rf-only quadrupole. The results of these analyses are presented in Figure 2. Figure 2A shows a portion of the first generation product ion spectrum of sodium cationized [$^{18}O_2$]-leucine enkephalin obtained with metastable decomposition conditions (no collision gas) employed in the rf-only quadrupole, demonstrating the relatively high abundance of the [$^{18}O_2$]-labeled rearrangement ion with this peptide. Figure 2B presents the second generation product ion scan obtained from the [$^{18}O_1$]-labeled rearrangement ion. The unlabeled and [$^{18}O_1$]-labeled second generation rearrangement ions were detected in roughly equal abundance, with the ratio distorted somewhat in favor of the [$^{18}O_1$]-labeled species. The second generation product ion scan obtained from the [$^{18}O_2$]-labeled first generation rearrangement ion is presented in Figure 2C. The major signal detected corresponded to the [$^{18}O_1$]-labeled second generation rearrangement ion, although a small but significant (and reproducible) signal corresponding to the [$^{18}O_2$]-labeled second generation rearrangement ion was also detected. Similar analyses of protonated TRKR are presented in Figure 2d-f. Again, the [$^{18}O_1$]-labeled first generation rearrangement ion gave rise to both unlabeled and [$^{18}O_1$]-labeled second generation rearrangement species, with a slight preponderance of the [$^{18}O_1$]-labeled species. The [$^{18}O_2$]-labeled first generation ion again gave rise primarily to [$^{18}O_1$]-labeled second generation ions, but with a small proportion of [$^{18}O_2$]-labeled species detected. Similar results were obtained with sodium cationized methionine enkephalin. These data strongly suggest that the two ^{18}O atoms in the [$^{18}O_2$]-labeled first generation rearrangement ions were located in the C-terminal carboxyl group, so that both of these atoms were transferred from the original C-terminus to the C-terminus of the first generation rearrangement ion. The distortion from a 1:1 ratio in Figure 2, parts B and E and the presence of detectable amounts of [$^{18}O_2$]-labeled second generation rearrangement species (Figure 2, parts C and F) suggest that both of the carboxyl oxygens can be transferred to the new C-terminus even through two generations.

One hypothesis to explain these data is that two separate mechanisms exist, involving different intermediates, both of which result in the formation of a $[B'_{n-1} + OH]^+$ rearrangement species. These two mechanisms would give rise to the same product for unlabeled peptides, with the differences manifested only through ^{18}O labeling. One mechanism would involve a cyclic intermediate such as that depicted in Scheme II; the other would involve an intermediate with formal equivalence of the three oxygens derived from the peptide bond carbonyl group and the C-terminal carboxyl group. If separate mechanisms involving different intermediates exist, then the kinetic energy released during the gas-phase rearrangement may be different for the two processes. Kinetic energy release measurements are most often performed by mass analyzed ion kinetic energy scanning (MIKES) using a BE ge-

(20) Murphy, R. C.; Clay, K. L. *Biomed. Mass Spectrom.* **1979**, *6*, 309-314.
(21) Teesch, L. M.; Orlando, R. C.; Adams, J. *J. Am. Chem. Soc.* **1991**, *113*, 3668-3675.
(22) Louris, J. N.; Wright, L. G.; Cooks, R. G.; Schoen, A. E. *Anal. Chem.* **1985**, *57*, 2918-2924.
(23) Ballard, K. D.; Raftery, M. J.; Jaeschke, H.; Gaskell, S. J. *J. Am. Soc. Mass Spectrom.* **1991**, *2*, 55-67.

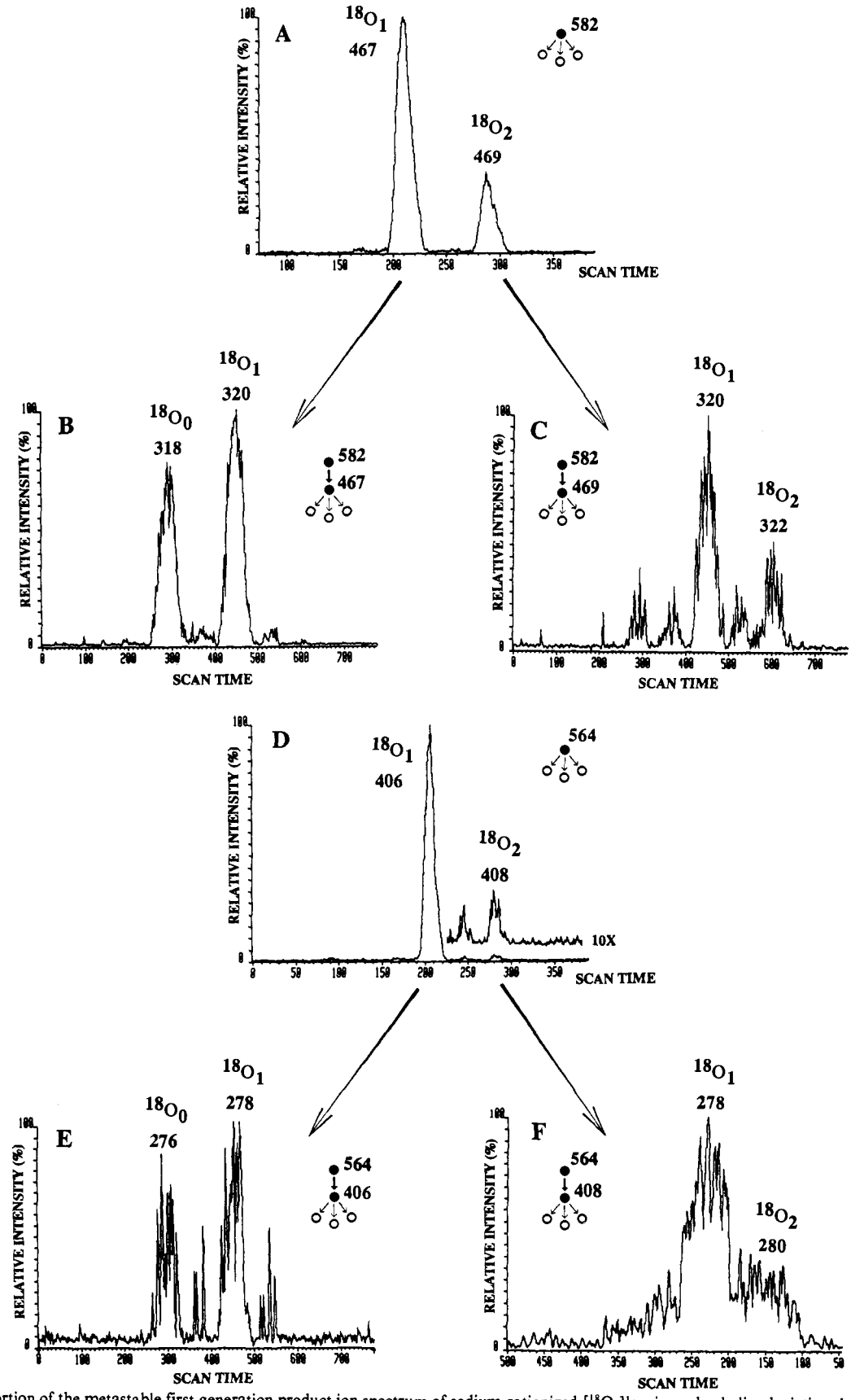


Figure 2. (A) Portion of the metastable first generation product ion spectrum of sodium cationized [$^{18}O_2$]leucine enkephalin, depicting the rearrangement ion region. (B) Second generation product ion spectrum of [$^{18}O_2$]leucine enkephalin, depicting the second generation rearrangement species derived through CAD of the [$^{18}O_1$]-labeled first generation rearrangement ion (m/z 467). (C) As in part B, depicting second generation rearrangement products derived from the [$^{18}O_2$]-labeled first generation rearrangement ion (m/z 469) from [$^{18}O_2$]leucine enkephalin. (D) Portion of the metastable first generation product ion spectrum of the $[M + H]^+$ ion of [$^{18}O_2$]TRKR, depicting the rearrangement ion region. (E) Second generation product ion spectrum of [$^{18}O_2$]TRKR depicting the second generation rearrangement species derived through CAD of the [$^{18}O_1$]-labeled first generation rearrangement ion (m/z 406). (F) As in part E, depicting second generation rearrangement products derived from the [$^{18}O_2$]-labeled first generation rearrangement ion (m/z 408) from [$^{18}O_2$]TRKR. The resolution of Q was intentionally degraded to 1.5 u for this scan in order to enhance sensitivity.

ometry mass spectrometer,[24] but the effective mass resolution of product ions using this technique (100–300) is insufficient to permit adequate estimation of the kinetic energy released during the formation of two product ions which differ in mass by only 2 u. This goal can be achieved, however, with a BEqQ hydrid through mass deconvoluted MIKES analysis.[22,25] Such analyses were performed for metastable sodium cationized [$^{18}O_2$]leucine enkephalin. In these experiments, the precursor ion was selected with B, and the mass analyzer quadrupole was set to transmit either the [$^{18}O_1$]- or the [$^{18}O_2$]-labeled first generation rearrangement ion at unit mass resolution. The electric sector was then scanned over the region corresponding to the rearrangement species in order to generate kinetic energy profiles of the rearrangement ions formed in the second field-free region. On the basis of the peak width at half maximum,[24] the kinetic energy released during the rearrangement was 96.0 ± 1.6 meV (mean ± standard deviation, $n = 4$) for the [$^{18}O_1$]-labeled rearrangement ion and 97.6 ± 1.7 meV for the [$^{18}O_2$]-labeled species. Mass deconvoluted MIKES analyses of a series of peptides of similar molecular mass have indicated kinetic energy release values for B and Y fragmentations (representing cleavages of the peptide backbone) in the range 20–35 meV. Thus, the close similarity of the kinetic energy release values for the [$^{18}O_2$]- and [$^{18}O_1$]-labeled rearrangement products, while not conclusive, is consistent with the involvement of a common (or very similar) intermediate in the formation of these two product ions from sodium cationized [$^{18}O_2$]leucine enkephalin. (The lower abundance of [$^{18}O_2$]-labeled rearrangement ions derived from protonated peptides precluded the recording of precise kinetic energy release data in these instances.)

An alternative hypothesis which would explain the formation of the [$^{18}O_2$]-labeled rearrangement ions would involve a reaction mechanism in which the steps prior to peptide bond cleavage are reversible, enabling intramolecular isotopic exchange to occur without peptide bond cleavage. One possible such mechanism is depicted in Scheme III. According to this mechanism the formation of a cyclic intermediate effects the initial transfer of an ^{18}O atom from the C-terminal carboxyl group to the carbon of the final peptide bond. A carbon–^{18}O bond is broken as a carbon–^{16}O bond is formed, and these two structures of the cyclic intermediate are in equilibrium. The cyclic intermediate then reopens to generate the protonated peptide that has undergone isotopic exchange. This peptide could then undergo rearrangement again (via the mechanism shown in Scheme II, or its equivalent), with transfer of the second ^{18}O atom and subsequent bond cleavage to generate the [$^{18}O_2$]-labeled rearrangement ion. A similar mechanism can be envisaged for the metal cationized species. A mechanism such as that depicted in Scheme III predicts the formation of a discrete protonated peptide in the form of structure II. If this is the case, this ion would be expected to undergo a variety of fragmentations in a fashion similar to that of the original protonated peptide, structure I. In particular, structure II would be predicted to give rise to a Y_1'' ion which is 2 u lower in mass than that generated from structure I. Peptides which incorporate a C-terminal arginine residue often give rise to an intense Y_1'' ion of m/z 175 for unlabeled material. Many such peptides also undergo the rearrangement,[5] and some of these show significant [$^{18}O_2$] labeling in the rearrangement ion and thus would be predicted from Scheme III to generate structure II type ions in the ion beam of the mass spectrometer. Two peptides which meet all of these criteria are TRKR and dynorphin A(1–7) (YGGFLRR); portions of the metastable and low energy CAD product ion spectra of the $[M + H]^+$ ions of these compounds ([$^{18}O_2$] labeled) are presented in Figure 3. Signals corresponding to the presence of both [$^{18}O_2$]- and [$^{18}O_1$]-labeled Y_1'' ions are generated by both of these peptides, and the [$^{18}O_1$] analogues increase in relative abundance under low-energy CAD conditions

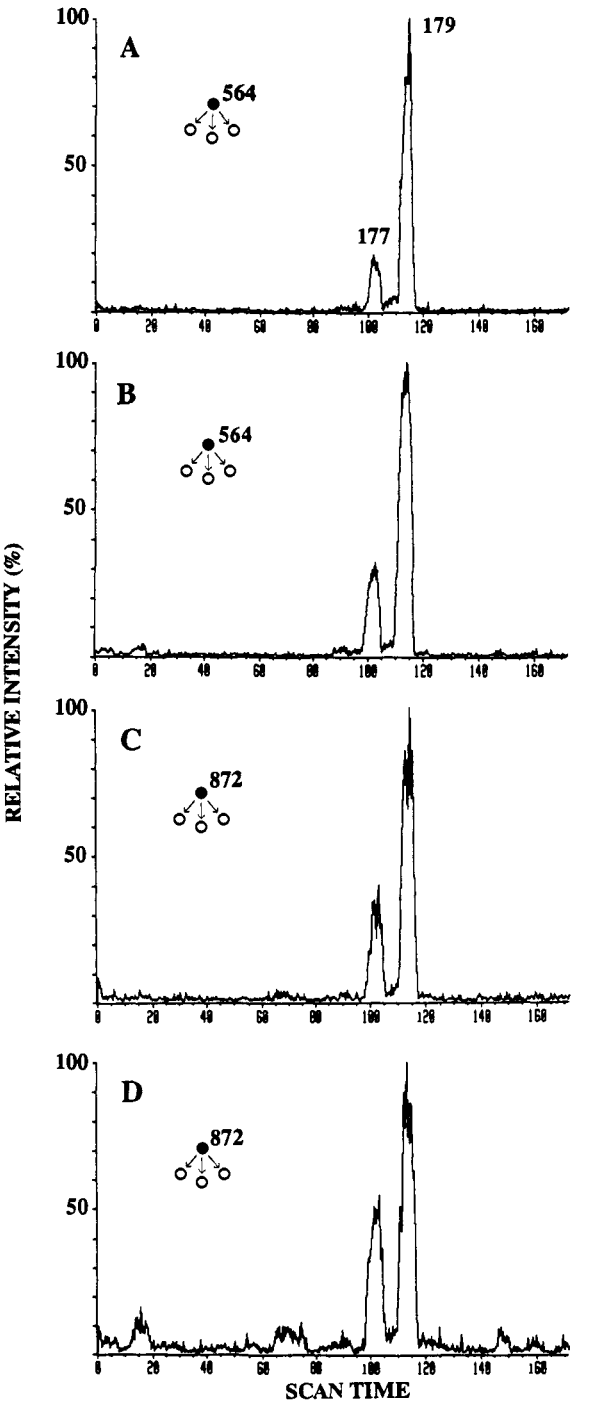


Figure 3. (A) Portion of the metastable first generation product ion spectrum of the $[M + H]^+$ ion of [$^{18}O_2$]TRKR encompassing the region of the Y_1'' ion. (B) As in part A, obtained under 33-eV CAD conditions. (C) Region of the Y_1'' ion of the metastable first generation product ion spectrum of the $[M + H]^+$ ion of [$^{18}O_2$]YGGFLRR. (D) As in part C, obtained under 33-eV CAD conditions.

as compared to metastable decomposition conditions. The observation of the [$^{18}O_1$]-labeled Y_1'' ions strongly supports the existence of structure II ions. Further support is obtained from the full-scan low-energy CAD product ion spectrum of [$^{18}O_2$]-TRKR, shown in Figure 4. The [$^{18}O_1$]- and [$^{18}O_2$]-labeled Y_1'' ions are again present at m/z 177 and 179. The [$^{18}O_2$]-labeled Y_2'' ion is evident at m/z 307, with no signal corresponding to [$^{18}O_1$]-labeled Y_2'' ions. The only oxygen atom in the $(B_3Y_2)_1'$ internal cleavage product is from the carbonyl group contributed by the lysine residue, and a significant signal corresponding to [$^{18}O_1$]-labeled $(B_3Y_2)_1'$ ions is present at m/z 131. Taken together, these data pinpoint the carbonyl function penultimate to the

(24) Cooks, R. G.; Beynon, J. H.; Caprioli, R. M.; Lester, G. R. *Metastable Ions*; Elsevier: Amsterdam, 1973; pp 57–62.
(25) Harris, F. M.; Keenan, G. A.; Bolton, P. D.; Davies, S. B.; Singh, S.; Beynon, J. H. *Int. J. Mass Spectrom. Ion Proc.* **1984**, *50*, 273–292.

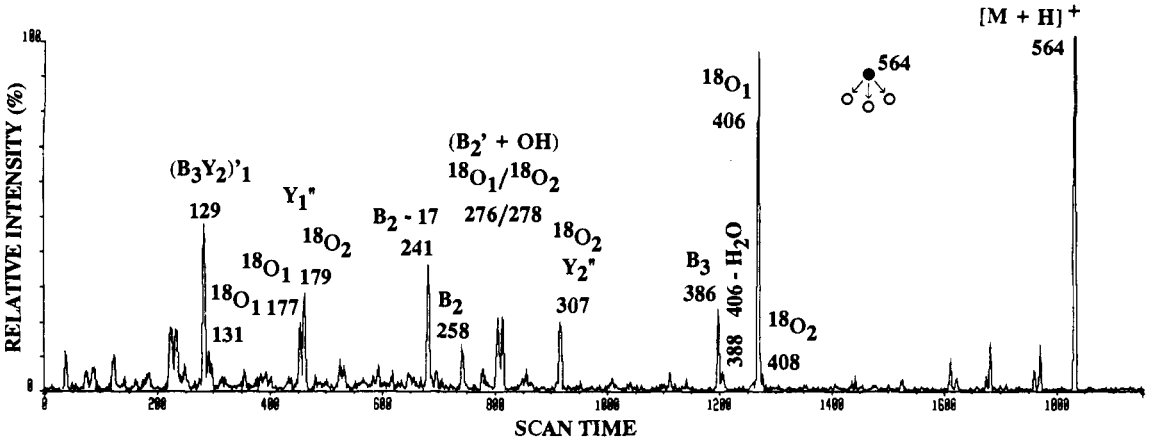
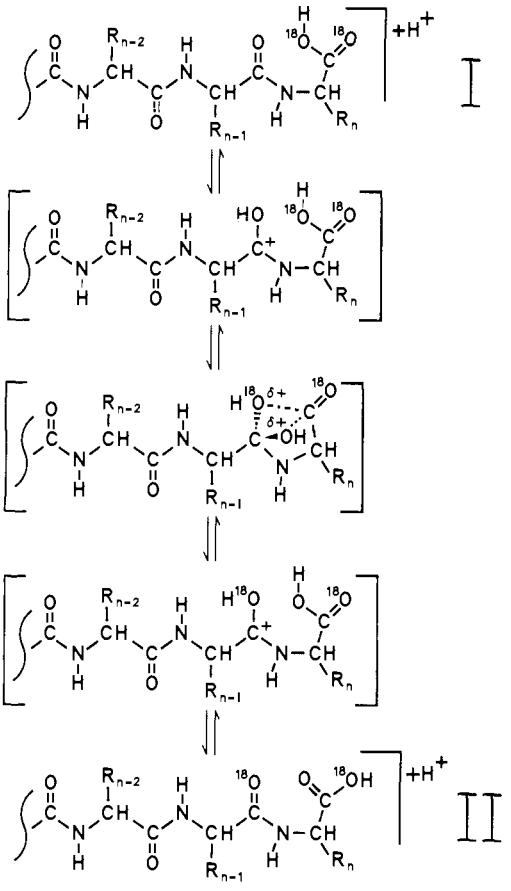


Figure 4. Full scan first generation product ion spectrum of the $[M + H]^+$ ion of [$^{18}O_2$]TRKR (CAD, 33 eV).

Scheme III



C-terminus as a site of ^{18}O enrichment.

The product ion scanning data of Figures 3 and 4 indicate that structure II ions are fragmenting in the rf-only quadrupole of the BEqQ hybrid mass spectrometer. The second generation product ion scanning data in Figure 2 strongly suggest that these ions are formed from structure I ions in the gas phase. It could be postulated, however, that some of the [$^{18}O_2$]-labeled peptide molecules synthesized by acid catalyzed exchange could incorporate one ^{18}O atom at the carbonyl group in the final peptide bond and one in the C-terminal carboxylic acid group. In other words, one could postulate that the structure II species was formed in the solution phase rather than in the gas phase in the mass spectrometer. If such material could be generated by acid catalyzed ^{18}O exchange in solution, one would certainly predict that significant amounts of [$^{18}O_3$]-labeled peptide would also be generated due to the ready exchangeability of the carboxylic acid oxygens. The [$^{18}O_3$]-labeled peptide would then be expected to give rise to [$^{18}O_2$]-labeled Y_1'' ions just as the [$^{18}O_2$]-labeled peptide does. In order to test for this possibility, unlabeled and [^{18}O]-labeled TRKR and dynorphin A(1–7) were analyzed by precursor ion scanning, with the BEqQ hybrid instrument set to detect precursors which give rise to unlabeled (m/z 175) or [$^{18}O_2$]-labeled (m/z 179) Y_1'' ions. The results for dynorphin A(1–7) are presented in Figure 5. A scan for precursors of m/z 175 for the unlabeled material is shown in Figure 5A. The characteristic isotope pattern due to the natural abundance of ^{13}C is apparent. The corresponding scan to detect precursors of m/z 179 for the [^{18}O]-labeled material is presented in Figure 5B. The signal at m/z 874, which would correspond to the postulated [$^{18}O_3$]-labeled peptide, can be entirely accounted for by the natural abundance of ^{13}C. Similarly conclusive results were obtained with TRKR. These data provide strong evidence against the synthesis of [$^{18}O_3$]-labeled peptides by acid catalyzed exchange in solution. Thus, the solution-phase synthesis of [$^{18}O_2$]-labeled peptides having structure II is unlikely.

The preparation of [$^{18}O_2$]-labeled peptides with the isotopic label exclusively in the carboxyl group is consistent with the literature on [^{18}O] exchange. Thus, facile acid- and enzyme-catalyzed exchange of peptide carboxyl oxygens has been reported;[26,27] under the same conditions, no exchange of carbonyl oxygens of the peptide bonds was observed. Slow exchange of the carbonyl oxygen of amides has, however, been reported[28] to occur under basic conditions and efficient acid-catalyzed incorporation of ^{18}O in the C-4 carbonyl of uracil and related compounds has been demonstrated.[29,30] To our knowledge, there is no direct precedent in solution chemistry for the intramolecular [^{18}O] exchange observed in the gas phase in the present work.

Conclusions

The evidence presented above indicates that intramolecular (intra-ionic) isotopic exchange occurs in the gas phase in certain protonated and sodium cationized [$^{18}O_2$]-labeled peptides through a mechanism such as that depicted in Scheme III. The resulting ions of the form of structure II can then undergo the rearrangement process to generate [$^{18}O_2$]-labeled rearrangement species. The structure II ions appear to undergo the same cleavage and rearrangement processes as the original precursor ions (structure I), but the relative abundances of the various product ions attributable to the two precursor forms are different. For instance, in the product ion spectrum of [$^{18}O_2$]TRKR in Figure 4, the [$^{18}O_1$]-labeled Y_1'' ion is twice as intense as the [$^{18}O_1$]-labeled $(B_3Y_2)_1'$ internal cleavage ion, and both of these ions are more intense than the [$^{18}O_2$]-labeled rearrangement ion; the latter,

(26) Desiderio, D. M.; Kai, M. *Biomed. Mass Spectrom.* **1983**, *10*, 471–479.
(27) Rose, K.; Savoy, L.-A.; Simona, M. G.; Offord, R. E.; Wingfield, P. *Biochem. J.* **1988**, *250*, 253–259.
(28) Bender, M. L.; Ginger, R. D.; Unik, J. P. *J. Am. Chem. Soc.* **1958**, *80*, 1444–1448.
(29) Wang, S. Y.; Hahn, B. S.; Fenselau, C.; Zafiriou, O. C. *Biochem. Biophys. Res. Commun.* **1972**, *48*, 1630–1635.
(30) Puzo, G.; Schram, K. H.; McCloskey, J. A. *Nucleic Acids Res.* **1977**, *4*, 2075–2081.

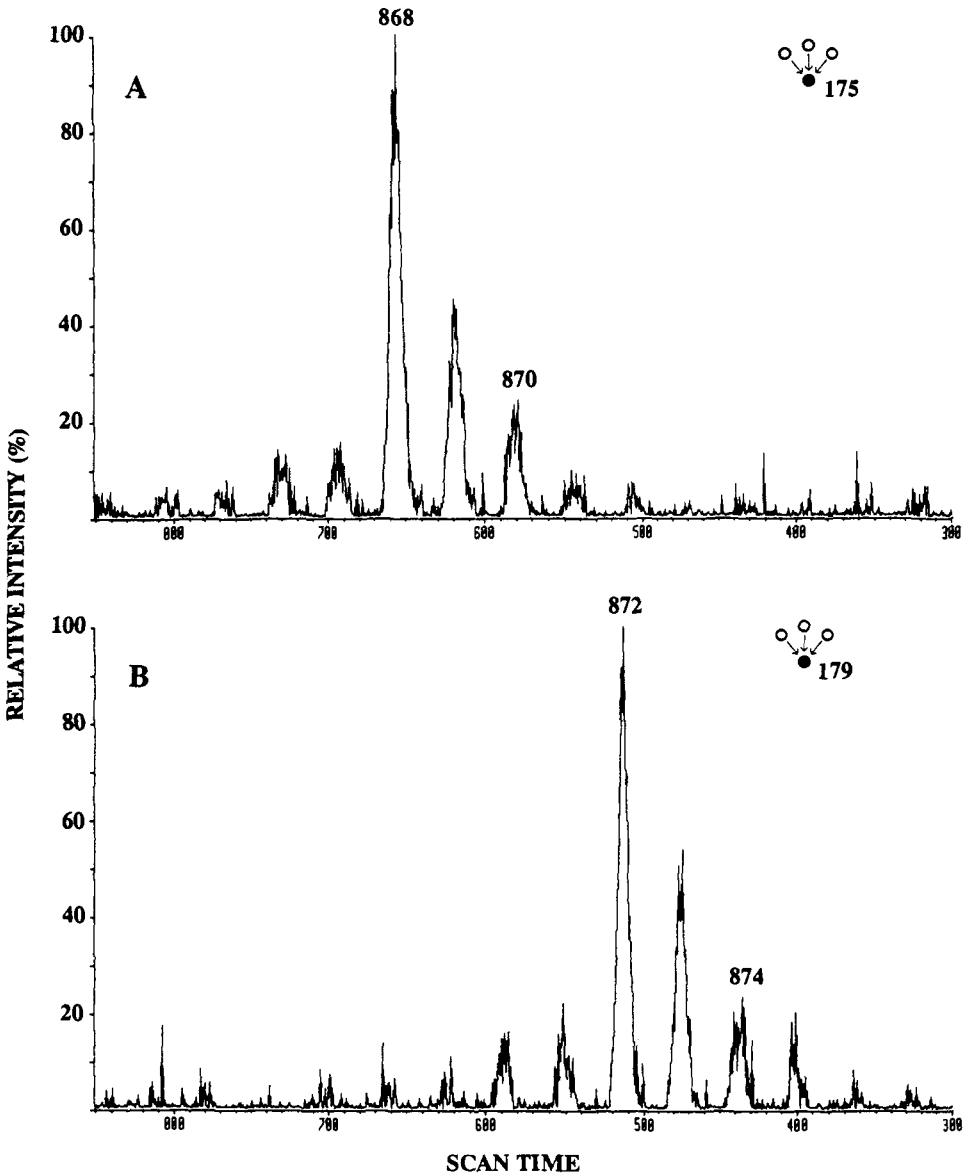


Figure 5. (A) Narrow mass range precursor ion scan to detect precursors of m/z 175 (the Y_1'' ion) for unlabeled YGGFLRR. (B) Similar scan to A to detect precursors of m/z 179 for ^{18}O-labeled YGGFLRR.

however, represents only half of the total rearrangement attributable to structure II since an equal amount of $[^{18}O_1]$-labeled rearrangement ions would be formed from structure II. In contrast, the unlabeled $(B_3Y_2)_1'$ ion is roughly twice as intense as the $[^{18}O_2]Y_1''$ ion, and both of these are considerably less intense than the $[^{18}O_1]$-labeled rearrangement ion. Apparently the relative propensities of the structure I and structure II ions to undergo the various cleavages are different. This could be due in part to an isotope effect, with the presence of an ^{18}O atom at the carbonyl group penultimate to the C-terminus influencing the tendency for various cleavages to occur; the magnitude of the effect, however, appears to be too large to be fully accounted for in this manner. An alternative explanation is that the formation of structure II ions results in a three-dimensional conformation which favors particular cleavage pathways. The effects of three-dimensional conformation in the solution-phase chemistry of peptides and proteins are widely recognized, and it is likely that these effects are also influential in the gas-phase chemistry of peptide ions.

Experimental Section

Materials. The unlabeled peptides bradykinin(1-5) (RPPGF), antikentsin (TRKR), dynorphin A(1-7) (YGGFLRR), leucine enkephalin (YGGFL), and methionine enkephalin (YGGFM) were purchased from Bachem (Torrance, CA) and were used as received. These peptides were labeled at their C-termini with ^{18}O by acid catalyzed isotopic exchange. Each peptide (100–200 μg) was dissolved in [^{18}O]water (25 μL, Cambridge Isotope Laboratories, Woburn, MA) and hydrochloric acid (12 M, 1 μL) was added. These mixtures were allowed to stand at room temperature for 72 h, and aliquots (1–2 μL) were used for tandem MS analysis.

Mass Spectrometry. Tandem MS analyses were performed with a VG ZAB-SEQ (VG Analytical Ltd, Manchester, UK) hybrid instrument of BEqQ configuration (B = magnetic sector, E = electric sector, q = rf-only quadrupole, and Q = quadrupole mass filter). Ionization was by FAB using 8 keV xenon for the primary atom beam. The liquid matrix was a 1:1 mixture of 2,2′-dithiodiethanol and thioglycerol, saturated with oxalic acid. All scan data were acquired using the multichannel analyzer (MCA) mode of the VG 11/250J data system, with 6–8 scans typically accumulated. It should be noted that the apparent resolution of full scan plots of MCA data may appear artifactually degraded due to compression algorithms incorporated in the plotting routines of the data system.

First generation product ion spectra were obtained by manual selection of the precursor ion with the double focusing portion of the instrument (resolution 1200–1500), with subsequent scanning of Q (1–1.5 unit mass resolution) to detect the first generation product ions formed in q. For collisionally activated decomposition (CAD) experiments, argon was used as the target gas at an estimated pressure in the collision region of 1.8×10^{-4} mbar; the collision energy ranged from 8 to 45 eV in the laboratory frame of reference. For precursor ion scans, Q was set to transmit the product ion of interest and B was scanned over the appropriate range sequentially to introduce the source-formed precursor ions into q; CAD conditions for these experiments were again 1.8×10^{-4} mbar of argon

in q, with a collision energy of 14 eV.

For second generation product ion spectra (sequential MS), appropriate settings of B and E were established to select first generation product ions (effective mass resolution 800) formed under metastable conditions in the first field-free region of the mass spectrometer[23] using a previously described instrument modification that permits independent control of E[4]. First generation product ions so selected were subjected to CAD in q (1.8×10^{-4} mbar of argon, 8 eV collision energy), with subsequent mass analysis of the second generation product ions with Q; 50-100 scans were accumulated by the data system in the MCA mode.

Mass deconvoluted MIKES analyses were obtained with the precursor ion selected by B, and with Q set to transmit the product ion of interest at unit mass resolution. E was then scanned over a narrow range, and 6-8 scans were accumulated. Metastable decomposition conditions were employed for these experiments.

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Reactions of $(OC)_2Fe^{\cdot -}$ and $(OC)_3Mn^-$ with Cyclopropane: C-H Bond Activation, Dehydrogenation, and Deuterium Isotope Effects

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Abstract: The reactions of cyclopropane with $(OC)_2Fe^{\cdot -}$ dihydrido-(c-C_3H_4) $(OC)_3Mn^-$ are unique among the hydrocarbons studied in that hydrido-(c-C_3H_5) and dihydrido-(c-C_3H_4) adduct products are observed accompaning those of dehydrogenation. The adduct and (adduct-H_2) product negative ion distributions are 35/65 from $(OC)_2Fe^{\cdot -}$ and 80/20 from $(OC)_3Mn^-$. The Fe-centered product ions were shown to be unsaturated and were characterized as $(OC)_2Fe(H)(c\text{-}C_3H_5)^{\cdot -}$ for the adduct, and $(OC)_2Fe(\pi\text{-}c\text{-}C_3H_4)^{\cdot -}$ or $(OC)_2Fe{=}\overline{CCH_2CH_2}^{\cdot -}$ for the (adduct-H_2) based on the results of their reactions with neutrals, e.g., D_2, Me_3SiH, and H_2S. The Mn-centered product ions did not react with these neutrals, suggesting that these ions are coordinatively saturated. The 18-electron dihydrido-(π-cyclopropene) structure likely represents the adducts, and $(OC)_3Mn(H)(\eta^3\text{-}c\text{-}C_3H_3)^-$ may represent the (adduct-H_2) ions. The extent of dehydrogenation was significantly reduced in the reactions with cyclopropane-d_6; general product structure (% from $(OC)_2Fe^{\cdot -}$, % from $(OC)_3Mn^-$): $(OC)_xM(D)(c\text{-}C_3D_5)^-$ ($\approx$80, $\approx$20), $(OC)_xM(D)_2(c\text{-}C_3D_4)^-$ ($\approx$9, $\approx$80), and $(OC)_xM(C_3D_4)^-$ (11, 0). The much smaller than expected kinetic isotope effect measured for the $(OC)_2Fe^{\cdot -}$ reactions (1.2 ± 0.1) is the result of the increased collisional quenching efficiency of the excited adducts $[(OC)_2Fe(D)(c\text{-}C_3D_5)^{\cdot -}]^*$ by the buffer gas. This secondary isotope effect reduces the return of the adducts to the starting ion and c-C_3D_6 that increases the rate of the cyclopropane-d_6 reaction masking the primary isotope effect for the insertion step. The larger isotope effect observed for the $(OC)_3Mn^-$ reactions (1.9 ± 0.3) is due to the normal isotope effect for the additional intramolecular deuterium shift step required to give the major adduct product ions. The reactivity of the cyclopropane C-H bonds suggest that the M-(c-C_3H_5) bond energy is greater than that for a secondary alkyl group in these negative ion complexes.

Intermolecular C-H bond activation with unsaturated transition-metal complexes is now a relatively common process with a number of examples in the literature.[1-10] However, success in intermolecular C-C bond activation is rare. It appears that both kinetic and thermodynamic factors are important for the cleavage of C-C bonds. A larger kinetic barrier is assumed for C-C bond activation than for the oxidative insertion into C-H bonds by transition-metal centers.[1b,11] Several indirect approaches using cyclopentadienes and even 1,1-dimethylcyclopentane[12] have led to products of C-C bond cleavage. In each case, the generation of the metal-alkyl bond occurs by rearrangement of a cyclopentadiene intermediate complex by an alkyl shift forming the corresponding Cp derivative; aromatization of the C_5 ring is the driving force.

Direct C-C bond activation has required strained alkanes, e.g., cyclopropane, where relief of the ring strain may facilitate the reaction,[1b,12] but depends on the strain in the product metallocycloalkane. Periana and Bergman[13] have shown that formation of $(\eta^5\text{-}C_5Me_5)(Me_3P)\text{-}\overline{Rh\text{-}CH_2CH_2CH_2}$ in the reaction of $(\eta^5\text{-}C_5Me_5)(Me_3P)Rh$ and cyclopropane proceeds by thermal rearrangement of the hydrido-cyclopropyl intermediate. The gas-phase reactions of Sc^+,[14] Y^+,[14] La^+,[14] Gd^+,[15] and $(\eta^5\text{-}c\text{-}C_5H_5)Co^+$[16] and cyclopropane occur predominately or exclusively by dehydrogenation.

C-H bond activation studies and dehydrogenation of alkanes containing C-H bonds β to the insertion site, cyclopentane, and

(1) For some recent reviews on this topic, see: (a) Ryabov, A. D. *Chem. Rev.* **1990**, *90*, 403. (b) Crabtree, R. H. *Chem. Rev.* **1985**, *85*, 245. (c) Halpern, J. *Inorg. Chim. Acta* **1985**, *57*, 1897. (d) Green, M. L. H.; O'Hare, D. *Pure Appl. Chem.* **1985**, *57*, 1897. (e) Rothwell, I. P. *Polyhedron* **1985**, *4*, 177. (f) Shilov, A. E. *The Activation of Saturated Hydrocarbons by Transition Metal Complexes*; Reidel: Dordrecht, 1984. (g) Parshall, G. W. *CHEMTECH* **1984**, *14*, 628. (h) Muetterties, E. L. *Chem. Soc. Rev.* **1983**, *12*, 283. (i) Brookhart, M.; Green, M. L. H. *J. Organomet. Chem.* **1983**, *250*, 395.

(2) (a) Janowicz, A. H.; Bergman, R. G. *J. Am. Chem. Soc.* **1982**, *104*, 352. (b) Janowicz, A. H.; Bergman, R. G. *J. Am. Chem. Soc.* **1983**, *105*, 3929.

(3) Hoyano, J. K.; Graham, W. A. G. *J. Am. Chem. Soc.* **1982**, *104*, 3723.

(4) (a) Jones, W. D.; Feher, F. J. *J. Am. Chem. Soc.* **1982**, *104*, 4240. (b) Jones, W. D.; Feher, F. J. *J. Am. Chem. Soc.* **1984**, *106*, 1650.

(5) Janowicz, A. H.; Periana, R. A.; Buchanan, J. M.; Kovac, C. A.; Stryker, J. M.; Wax, M. J.; Bergman, R. G. *Pure Appl. Chem.* **1984**, *56*, 13.

(6) Periana, R. A.; Bergman, R. G. *J. Am. Chem. Soc.* **1986**, *108*, 7332.

(7) Sponsler, M. B.; Weiller, B. H.; Stoutland, P. O.; Bergman, R. G. *J. Am. Chem. Soc.* **1989**, *111*, 6841.

(8) Fryzuk, M. D.; Jones, T.; Einstein, F. W. D. *Organometallics* **1984**, *3*, 185.

(9) Nubel, P. O.; Brown, T. L. *J. Am. Chem. Soc.* **1984**, *106*, 644.

(10) Jones, W. D.; Feher, F. J. *J. Am. Chem. Soc.* **1982**, *104*, 4240.

(11) Blomberg, M. R. A.; Siegbahn, P. E. M.; Nagashima, U.; Wennerberg, J. *J. Am. Chem. Soc.* **1991**, *113*, 424.

(12) Crabtree, R. H.; Dion, R. P.; Gibboni, D. J.; McGrath, D. V.; Holt, E. M. *J. Am. Chem. Soc.* **1986**, *108*, 7222 and references therein.

(13) (a) Periana, R. A.; Bergman, R. G. *J. Am. Chem. Soc.* **1984**, *106*, 7272. (b) Periana, R. A.; Bergman, R. G. *J. Am. Chem. Soc.* **1986**, *108*, 7346 references therein.

(14) Huang, Y.; Wise, M. B.; Jacobson, D. B.; Freiser, B. S. *Organometallics* **1987**, *6*, 346.

(15) Schilling, J. B.; Beauchamp, J. L. *J. Am. Chem. Soc.* **1988**, *110*, 15.

(16) Jacobson, D. B.; Freiser, B. S. *J. Am. Chem. Soc.* **1985**, *107*, 7399.

0002-7863/92/1514-71$03.00/0