Metal Ion-Peptide Interactions in the Gas Phase: A Tandem Mass Spectrometry Study of Alkali Metal Cationized Peptides

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Abstract: Fast atom bombardment combined with tandem mass spectrometry has been used to investigate the gas-phase interactions of alkali metal ions and small peptides. Alkali cations bind to peptides primarily at the C-terminus, promoting the loss of a C-terminal residue to give a peptide having one less amino acid. This novel fragmentation involving migration of an oxygen atom is general, occurs for metastable as well as for collisionally activated ions, and is the basis of a method to identify rapidly the C-terminal amino acid. The mechanism of decomposition is analogous to the cleavage of a C-terminal amino acid by the enzyme carboxypeptidase. In addition to the ions formed by loss of a residue of the C-terminal amino acid, another class of fragment ions of the type $-CONHCHR^1CON^+(Met)=CHR^2$ (Met = alkali metal ion) is produced by collisional activation. The peptide-metal ion complex isomerizes upon collisional activation, forming a molecular species in which the metal ion is coordinated to a deprotonated amide nitrogen. These isomers lose both CO and an amino acid or small peptide, depending on the site of coordination, from the C-terminus to produce the metalated immonium ions. Although these ions are produced at low abundance when ions decompose metastably, they become dominant for collisionally activated peptides containing histidine residues. Formation of a stable six-member chelate ring involving the pyridine nitrogen of the imidazole ring of histidine accounts for their abundance. The final structure has bonding features that are analogous to those of transition metal ions and deprotonated amide nitrogens of peptides in solution.

The combination of fast atom bombardment (FAB) and collisional activation tandem mass spectrometry (MS/CA/MS) has become a powerful method for sequencing peptides and small proteins.[1] FAB by itself provides molecular weight and some sequencing information. The low abundance of fragment ions, the presence of interferences from impurities in the sample, and/or chemical noise from ions of the FAB matrix, however, limit sequence information. Tandem mass spectrometry overcomes these disadvantages by allowing one to isolate the molecular ion of interest from interferences and chemical noise. Collisional activation leads to production of fragment ions from which the primary sequence of a peptide can be deduced.

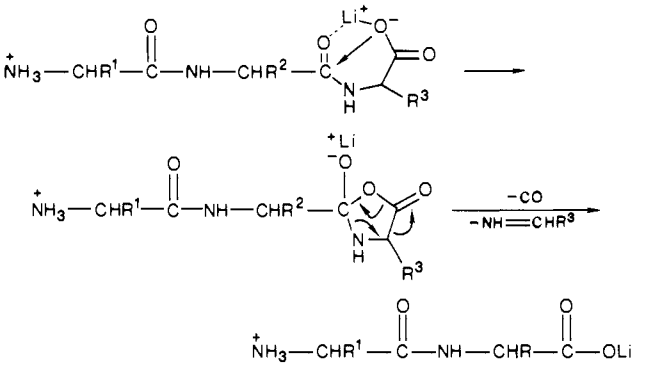
Peptides have been sequenced mass spectrometrically by using almost exclusively protonated species. Although MS/CA/MS spectra of $[M + H]^+$ ions provide a substantial amount of sequence information, the spectra are complicated because the precursor ions are not a single species; instead they exist as a mixture of ions protonated at different sites. Chemical modification was suggested as one way to localize the charge and cause the peptide to fragment in a more predictable way.[2] An alternative, much simpler approach may be to activate by collision peptides cationized with metal ions (i.e., $[M + Cat]^+$).

The approach of activating cationized species has been applied effectively to structural studies of fatty acids and alcohols,[3] sugars,[4] bile salts,[5] nucleotides, and nucleosides.[6] Alkali metal ions interact selectively with the polar functional groups, and the collisional activation leads to "charge-remote" fragmentations.[3] Furthermore, MS/CA/MS of sucrose[7] and nucleosides[8] cationized with alkali metal ions, by way of precedent, gives structural information complementary to collisional activation of the $[M + H]^+$ ions.

Mallis and Russell[9a] were the first to suggest that activating peptide-metal ion complexes produces useful structural information. They proposed that the site of interaction of Na^+ with a small peptide is at highly basic sites such as the N-terminus or at nitrogen in side chains. In contrast, Renner and Spiteller,[10] using a magnetic sector mass spectrometer in the linked scan mode, observed that the principal fragment ion from a peptide $[M + Na]^+$ ion arises by the loss of the C-terminal amino acid residue. They proposed a mechanism in which the metal ion interacts with the C-terminus carboxylate (Scheme I); however, no experimental support was given for the suggestion.

According to the mechanism, complexation at the C-terminal carboxylic group polarizes the carbonyl bond of the adjacent amino acid residue, allowing nucleophilic attack by the negative oxygen. Subsequent cleavage and losses of CO and an imine leads to a "new" peptide of one less amino acid. According to current

Scheme I



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nomenclature conventions,[11] the ion is designated, $[B_{n-1} + Li + OH]^+$.

Using a reflecting TOF mass spectrometer, Tang et al.[12] substantiated a similar mechanism for the metastable loss of the C-terminus from the $[M + Na]^+$ and $[M + Ag]^+$ ions of leucine- and methionine-enkephlin. The latter authors, however, proposed the transfer of hydroxide ion in lieu of O^- attack.

We expect that decomposition reactions of metal ion/peptide complexes in the gas phase will not only be a source of structural information but also reflect intrinsic interactions of the metal ion and the peptide. In solution, coordination with metal ions is known to modify the conformation of peptides, change their reactivity, and be of use for peptide synthesis. Of fundamental importance are the structure and stability of the complex itself.[13] The interactions can be quite complex because of the number of potential binding sites in the peptide. Besides the peptide bond itself, which provides both oxygen and nitrogen binding sites, other possible sites for bonding are the N-terminal NH_2 group, the C-terminal CO_2^- group, and the functional side chains of the amino acid residues.

Complexes of model peptides and transition metals in solution involve strong coordination of the metal ions to deprotonated peptide nitrogens.[14,15] These interactions are influenced by pH and the nature of the side chain. Three types of transition metal ion–peptide complexes result from the interaction.[13] At low pH, the interaction is exclusively with the side chain (type S); at intermediate pH, side-chain and deprotonated amide interactions (type SP); and at higher pH metal ions form a biuret-type complex (type B) with four deprotonated peptide nitrogens. The SP type interactions are only possible when a six-member chelate ring may form with a side-chain donor atom and a deprotonated nitrogen. In contrast, alkali metal ions in solution bind to peptides at the amide bond by direct coordination only to the carbonyl oxygen.[16]

The role of solvent is also important in metal ion–peptide complexes. Water is known to be a coordinating ligand for many metalloenzymes[17] and model metal–peptide complexes.[14] However, water poses a problem for studying metal ion–peptide interactions because it can compete with the peptide as a binding site for metal ions.[13] Thus, model studies in aqueous solutions may be hampered by metal ion hydrolysis and precipitation. Gas-phase studies are not influenced by solvent interferences. For example, we report here evidence that SP-type interactions are predominant for species produced by collisional activation of complexes of small peptides and alkali metal ions. No evidence of this interaction in solution has been reported.

Although the molecular environment of a gas-phase metal ion–peptide complex is different from that of a biological system, it is well known that properties in the gas phase are intrinsic. They have been exceptionally useful in understanding solution acid–base chemistry,[18] for example. Recently, mass spectrometry was used to study hydrogen-bonding interactions within the interiors of peptides[19] and to order the relative gas-phase proton affinities of the basic amino acids.[20]

Evidence is also given here that the metastable and collisional activation decompositions of alkali metal cationized peptides reveal intrinsic interactions of the metal and the peptide. We propose that alkali metal ions generally interact with the carboxylate function of the C-terminus, and that this interaction promotes the metastable loss of the C-terminal amino acid residue. In addition, we demonstrate that the Renner–Spiteller mechanism is general, and provides the basis for a rapid method for identifying the C-terminal amino acid. Upon collisional activation, however, isomerization of the $[M + Cat]^+$ occurs by facile migration of the metal to a deprotonated amide nitrogen. The isomerized species dissociates rapidly, producing $-CONHCHR^1CON^+(Met)=CHR^2$ prevalent in the collisional activation spectra. We also present preliminary evidence that the gas-phase binding affinities of alkali metals to amino acid residues are analogous to the relative binding affinities of transition metals to amino acid residues in solution.

Table I. Relative Abundance of Fragment Ions from CA of GlyGlyLeu $[M + Cat]^+$

fragment ions	Li+	Na+	K+	Ag+	Cu+
$M + Cat - CH_4$	10	17	16		
$M + Cat - H_2O$	7	15	22	93	100
$M + Cat - CO$					50
$X_2 + Cat - H$	23	17	8	53	
$M + Cat - CO_2$	33	43	29	62	71
$Y_2 + Cat + H$ or $M + Cat - C_4H_9$	29	48	25	24	6
$Z_2 + Cat - H$	8	8	6		9
$M + Cat - 88$	46	57	16	29	12
$B_2 + Cat + OH$	100	100	7	100	22
$B_2 + Cat - H$ or $Z_1 + Cat - H$	11	16	16	28	11
$A_2 + Cat - H$	41	29	12	55	19
$C_1 + Cat - H$	18	13	5	26	5
$B_1 + Cat - H$	4		8	9	4
$A_1 + Cat - H$	6		5	13	6
Cat		31	100	16	1

This initial study was limited to interactions of peptides and alkali metal ions even though transition metal ions are more biologically relevant. The coordination chemistry of alkali cations and peptides should be simpler than for transition metals. Furthermore, alkali cations, specifically Na^+ and K^+, are not only ubiquitous in living sytems but also important in mass spectrometry for producing abundant $[M + Cat]^+$ ions. Cationization with alkali metal ions has been used as a principal source of molecular weight information in field desorption,[21] plasma desorption,[22] and laser desorption[23] mass spectrometry, and as a confirmatory source in FAB mass spectrometry.[24]

Results and Discussion

Formation of $[M + Cat]^+$. Fast atom bombardment of peptides dissolved in a matrix of 1:1 glycerol/thioglycerol containing LiI produce as most abundant the $[M + Cat]^+$ species. The yield of $[M + Cat]^+$ is directly influenced by the ratio of peptide and alkali metal salt and the size of the peptide. Small peptides (three to six residues) give abundant $[M + Cat]^+$ ions. As the size of peptide increases, those species containing multiple metal ions (i.e., $[M - H + 2Cat]^+$ or $[M - 2H + 3Cat]^+$) become more dominant.

The mechanism of cationization by FAB is not fully understood. It is unclear whether cationization occurs on the surface or in the gas-phase selvedge region just above the surface. The yield of $[M + Cat]^+$ is influenced by how well the analyte competes with the matrix for the available alkali metal ions. Kebarle[25] and co-workers proposed a "gas-phase collision model" for the pro-

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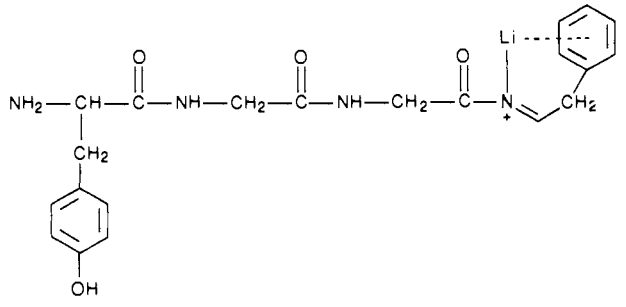
duction of $[M + Cat]^+$ ions by FAB whereby cationization occurs via ion/molecule reactions as the high-temperature, high-density "gas" of the selvedge region as it expands into the vacuum. This is not inconsistent with evidence that $[M + Cat]^+$ ions are formed by desolvation of peptide–matrix–metal ion complexes in the gas phase.[26] The decompositions of $[M + Cat]^+$, however, appear to be insensitive to the manner by which they are formed. For example, cationized peptides produced by laser desorption in a Fourier transform mass spectrometer[27] decompose similarly to those desorbed by FAB, as will be discussed later.

There are, however, large differences in the CA mass spectra of peptide $[M + Cat]^+$ ions when different monovalent metals serve as the cation (see Table I). The loss of the C-terminal residue to give a $[B_2 + Cat + OH]^+$ (see Scheme I) is a dominant fragmentation for complexes of Li^+, Na^+, Ag^+, and Cu^+, but not for complexes with K^+; these latter complexes preferentially release K^+ upon CA. The preferential release of the larger alkali metal ions was observed for collisionally activated $[M + Cat]^+$ of fatty alcohols[3a] and peptides,[9a] and is due to the decreasing bond energies as one descends the series (Li, Na, K, etc.). The much larger losses of H_2O and CO_2 for peptides cationized with Ag^+ and Cu^+ point to intrinsic differences in the interaction of transition metals with peptides and is the subject of continuing research.

CA of $[M + H]^+$ versus $[M + Li]^+$ Ions. The CA mass spectra of $[M + H]^+$ and $[M + Li]^+$ of methionine–enkephalin, Figure 1, a and b, respectively, illustrate the complementary information obtained by activating the two different ions. The spectrum of $[M + H]^+$ gives complete sequence information: fragment ions resulting from retention of the C-terminus in the ion (X- and Y-type fragment ions) and of the N-terminus (A- and B-type fragment ions) are produced. The spectrum of the $[M + Li]^+$ ion is much simpler. The most abundant fragment ion of m/z 449 results from loss of the C-terminal methionine residue (Scheme I). High-resolution mass analysis of this ion produced in the FAB source indicates an atomic composition of $C_{22}H_{26}N_4O_6Li$ which corresponds within 4 ppm to a structure of $[B_4 + Li + OH]^+$ as shown in Scheme I. This ion is a unique product of $[M + Cat]^+$ and has no analogies in the decomposition of $[M + H]^+$ ions.

On the other hand, the m/z 403 ion from $[M + Li]^+$ corresponds to the m/z 397 ion from $[M + H]^+$. This ion is designated as $[A_4 + Li - H]^+$ (**1**) in which a lithium ion has been substituted



for a proton. Other abundant fragment ions from $[M + Li]^+$ arise by losses of the side chains of tyrosine and methionine and water to give ions of m/z 472, 518, and 562, respectively. The predominance of $[B_{n-1} + Li + OH]^+$ and A-type fragments is a general trend in the CA decompositions of lithiated peptides.

Elimination of C-Terminus. The novel fragmentation that produces m/z 449 from methionine–enkephalin (Figure 1b) was also observed in the metastable and collisionally induced fragmentation of the TyrAlaGlyPheLeu $[M + Li]^+$ ions, providing further evidence for the generality of the process (see Figure 2, a and b). The ion of m/z 463 results from the loss of the leucine residue. Furthermore, the metastable decompositions of peptide $[M + Na]^+$ ions also give this type of product, and a mechanism for its formation was recently proposed.[10,12] Prior to that work, the ion corresponding to this fragmentation had appeared in the literature; however, it had been wrongly assigned as a C-type fragment ion.[9,28]

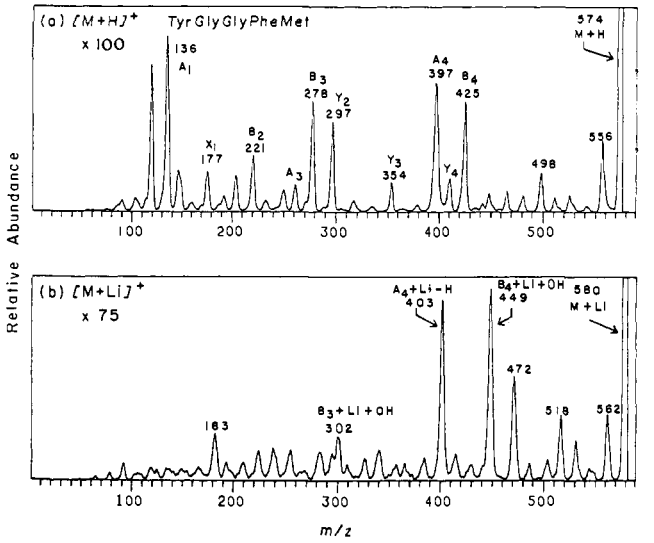


Figure 1. CA mass spectra of methionine–enkephalin: (a) $[M + H]^+$, m/z 574; (b) $[M + Li]^+$, m/z 580.

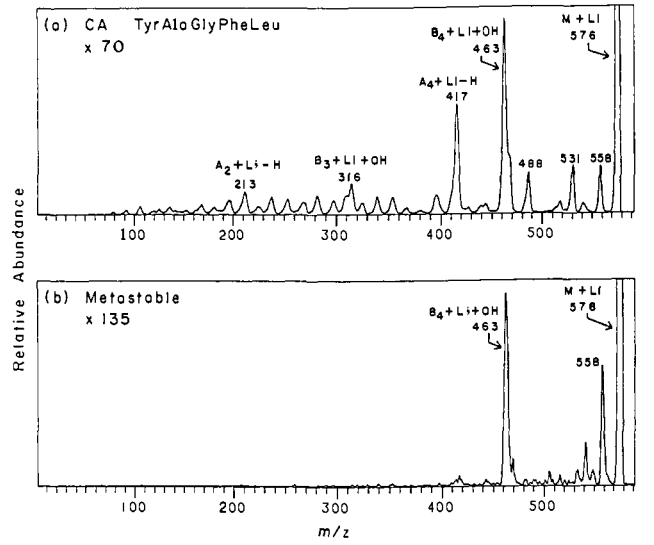


Figure 2. (a) CA mass spectrum and (b) metastable ion mass spectrum of TyrAlaGlyPheLeu $[M + Li]^+$, m/z 576.

We have substantial evidence that supports the mechanism for migration of an oxygen to produce the peptide shortened by one peptide (Scheme I). This fragmentation is general; we observed it to occur for 60 di- to nonapeptides having all common amino acids at the C terminus. The evidence is as follows.

The fragment ion, a putative smaller peptide, decomposes in a manner identical with the original metal–peptide complex. The CA spectrum of the m/z 449 product ion produced uniquely by loss of the leucine residue from collisional activation of the $[M + Li]^+$ of leucine–enkephalin (a so-called MS/MS/MS experiment)[29] shows that the most abundant second generation ion arises also by loss of the C-terminal residue (phenylalanine) to give an ion of m/z 302 (Figure 3).

To confirm that the fragment ion is not a C-type fragment, isotopic labeling experiments were conducted. Collisional acti-

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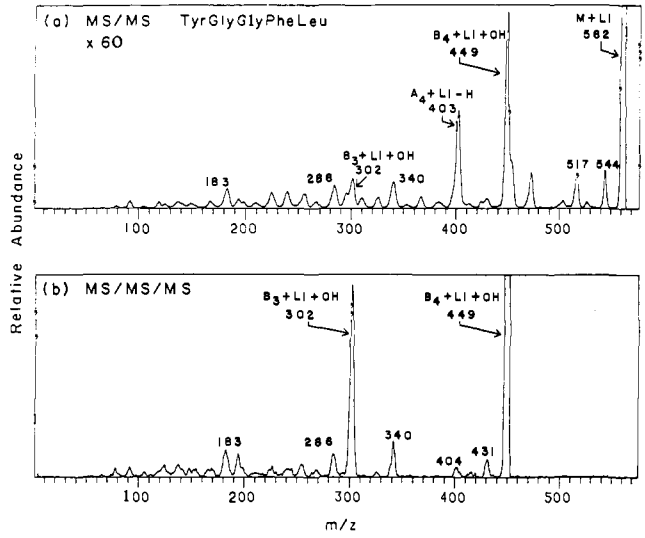
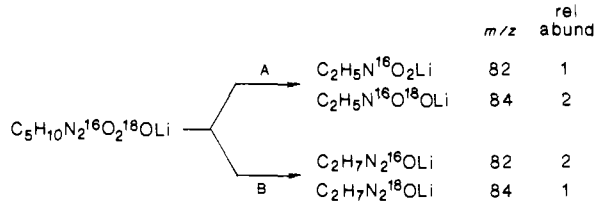


Figure 3. CA mass spectra of leucine-enkephalin: (a) MS/MS of [M + Li]$^+$, m/z 562; (b) MS/MS/MS of daughter ion of m/z 449.

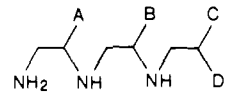
Scheme II



vation of ^{15}N- and ^{18}O-containing peptides gives neutral losses consistent with the loss of CO and an imine. For example, high-resolution mass analysis of the [M + H]$^+$ ion of carbobenzoxyglycine[^{15}N]alanine confirms that greater than 99% of the sample contains the ^{15}N label. Collisional activation of the labeled [M + Li]$^+$ ion shows that the nitrogen atom is being lost. CBZ-glycine[^{15}N]alanine [M + Li]$^+$ (m/z 288) decomposes upon CA to give a daughter ion of m/z 216 consistent with the loss of CO and ^{15}NH=CHCH$_3$. The metastable decompositions also produce an ion of m/z 216, confirming that the mass assignments are accurate and not lowered because of energy shifts.[30]

Collisional activation of ions containing natural abundance of heavy isotopes (so-called "A + 1" and "A + 2" ions)[31] provides the opportunity to conduct labeling experiments without synthesizing isotopically enriched materials.[32] The [A + 2] ion of GlyAla [M + Li]$^+$ of m/z 155 contains, in part, one ^{18}O atom, located with equal probability, at one of three sites occupied by oxygen atoms in the molecule. If this ion fragments as per Scheme I, producing [B + Li + OH]$^+$, then one-third of the fragment ions will contain two ^{16}O atoms (m/z 82) and two-thirds will contain one ^{16}O atom and one ^{18}O atom (m/z 84). This would produce an abundance ratio of m/z 82:84 ions of 1:2 (Scheme II, A). However, if the [M + Li]$^+$ fragments to yield [C + Li + 2H]$^+$, as was previously proposed,[9,28] the predicted abundance ratio of m/z 82:84 is 2:1 (Scheme II, B). The abundances of the components that comprise the [A + 2] peak (m/z 155) of the [M + Li]$^+$ isotopic cluster region are calculated to be 78% containing one ^{18}O, 16% containing two ^{13}C atoms, and 5% containing one ^{13}C and one ^{15}N. A resolution of 70 000 is required to separate the ^{18}O isotope-containing ion from the species containing two ^{13}C atoms. Because this resolution seriously lowers the sensitivity, a resolution of 10 000 was used and the CA spectra was corrected for the contribution of the species containing two ^{13}C atoms. To increase the S/N, the CA signal was acquired over a narrow mass range (m/z 81 to 85), and the chemical noise from the matrix was reduced by using the continuous flow FAB technique (CF-FAB).[33]

Table II. "Glycine-Glycine-Glycine" Model Compounds



compd	A	B	C	D	loss of C-terminus from [M + Li]$^+$?
I	O	O	O	OH	yes
II	H_2	H_2	H_2	OH	no
III	H_2	H_2	O	OH	no
IV	O	H_2	O	OH	no
V	O	O	H_2	CH_3	no
VI	O	O	O	OCH_3	no
VII	O	O	O	NH_2	no

Collisional activation of GlyAla [M + Li]$^+$ [A + 2] gives fragment ions of m/z 82 and 84 with relative abundances of approximately 1:2. This agrees with the predicted ratio for losses of C^{18}O:C^{16}O and NH=CHCH$_3$ (Scheme II, A) and supports the Renner-Spiteller suggestion.[10]

Further evidence for the mechanism and its structural requirements comes from a study of analogous compounds (see Table II). Collisional activation of tripeptide analogues reveals that a free C-terminal acid and a carbonyl on the residue adjacent to the C-terminus are necessary for this fragmentation. Moreover, modification of a peptide to give an amide or ester at the C-terminus prevents this fragmentation. The CA spectrum of the methyl ester of TyrAlaGlyPheLeu shows no detectable evidence for product ions arising from transfer of an oxygen atom or a methoxide ion.

The mechanism we give for the loss of the C-terminus (see Scheme I) differs from that proposed by Tang et al.[12] in which the metal ion is chelated between two carbonyl oxygens and the attacking nucleophile is an hydroxide ion rather than an oxygen atom. If the Tang et al. mechanism is correct, it is not unreasonable to expect that migration of methoxide ion would occur for peptide methyl esters. This does not occur, however, suggesting that the C-terminal carboxylate anion is necessary for this fragmentation. Furthermore, the formation of the [M + Li]$^+$ complex as a zwitterion is attractive because it is the most probable form of the peptide in solution. This fragmentation also occurs for N-terminally blocked peptides (e.g., *N*-dansyl, *N*-CBZ, and *N*-naphthoyl peptides). This is readily accomodated by the mechanism in Scheme I; protonation at some site other than the N-terminus is required. In addition, lithiated dipeptides decompose in a similar manner, which may suggest that the metal ion does not require chelation between two carbonyl oxygens.[34]

The loss of the C-terminus (Scheme I) is similar to metal ion catalyzed hydrolysis of peptides in solution.[35] Metalation as well as protonation are known to occur at the most basic site in the peptide; for neutral amides, this is the carbonyl oxygen.[36] The metal ion polarizes the carbonyl, which increases the partial positive charge on the carbon and makes it susceptible to nucleophilic attack by H_2O or hydroxide. The metalloenzyme carboxypeptidase may catalyze the removal of the C-terminal amino acid from peptides and proteins by an analogous mechanism.[37]

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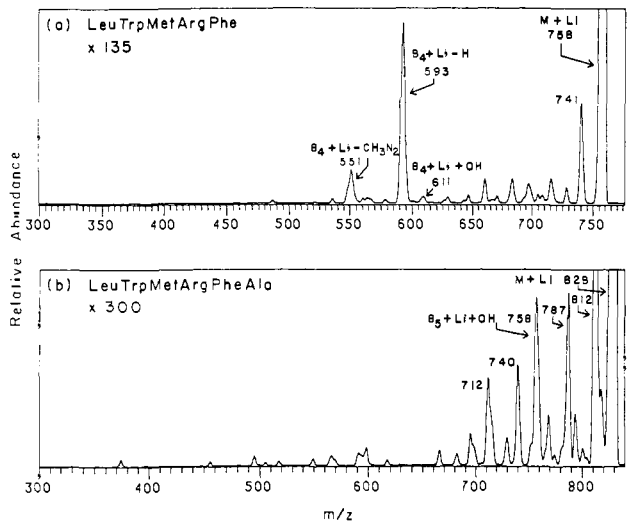
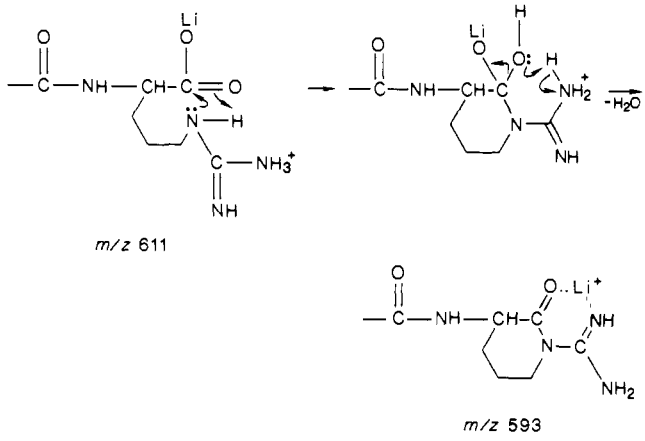


Figure 4. Metastable ion mass spectra of $[M + Li]^+$ of (a) LeuTrpMetArgPhe of m/z 758 and (b) LeuTrpMetArgPheAla of m/z 829.

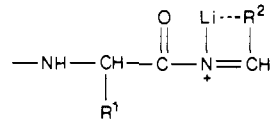
Scheme III



Specific Interactions with Arginine Residues. The most abundant fragment ion formed in the metastable decompositions of most peptides is indeed the $[B_{n-1} + Li + OH]^+$ ion. An exception to this general trend occurs, however, when an arginine residue adjoins the C-terminus. The metastable decompositions of $[M + Li]^+$ of LeuTrpMetArgPhe (Figure 4a) produce as most abundant an ion of m/z 593 $[B_4 + Li - H]^+$. The ion of m/z 611, which is formed by loss of the C-terminus residue, is of very low abundance. A revealing contrast is the metastable ion spectrum of LeuTrpMetArgPheAla $[M + Li]^+$ (Figure 4b), a peptide in which the arginine residue no longer adjoins the C-terminus. Although the most abundant ion is loss of NH_3, the $[B_5 + Li + OH]^+$ ion at m/z 758 is now a major ion, and the $[B_4 + Li - H]^+$ ion is now of low abundance.

The ion of m/z 593 is proposed to be produced by the facile loss of water from the ion of m/z 611 by the mechanism of Scheme III. Protonation at the arginine side chain is consistent with the abundant loss of ammonia seen in the decompositions of other peptides containing arginine residues. A similar process occurs in the metastable and collisionally induced decompositions of GlyHisArgPro $[M + Li]^+$ (see Table III).

Deprotonation Reactions: Interaction with Histidine. Although metalation of neutral amides in solution occurs at the carbonyl oxygen, a much stronger peptide–metal ion interaction is possible if deprotonation of the amide nitrogen occurs.[13a] The deprotonated nitrogen becomes the most basic site, and metallation occurs there. The predominant sequence ion in the CA spectra of cationized peptides, besides that from loss of the C-terminal residue, is an A-type ion in which the metal ion is substituted for a hydrogen, structure **2**. These fragment ions are especially abundant when



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cleavage occurs at certain amino acid residues that provide a side chain (R^2) capable of forming a six-member chelate ring with the metal ion (Table III). These fragment ions dominate the CA spectra, but they are of low abundance in the metastable ion spectra. The relative abundances of these ions, designated as $[A_n + Li - H]^+$, for different amino acid residues are qualitatively similar to the cation binding affinities in solution. For example, the aromatic amino acids stabilize the binding of transition metal ions to deprotonated amides by donation of π electrons.[38] The most abundant ions produced from collisional activation of ProPheGlyLys and AlaGlyPheLeu $[M + Li]^+$ ions, besides those from

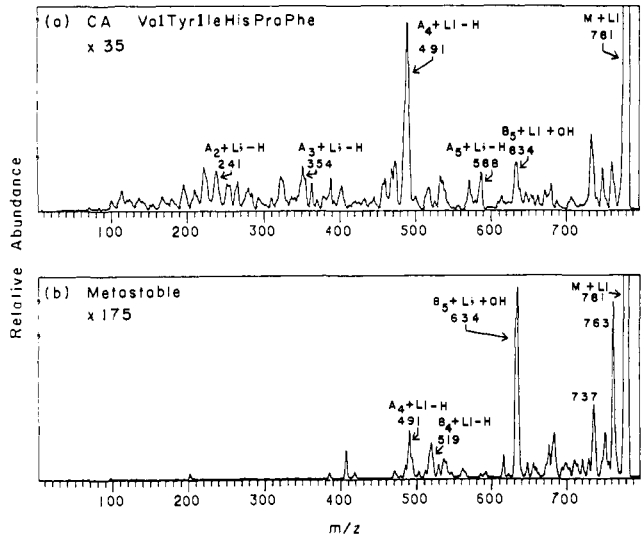

Figure 5. (a) CA mass spectrum and (b) metastable ion mass spectrum of ValTyrIleHisProPhe $[M + Li]^+$, m/z 781.

Scheme IV

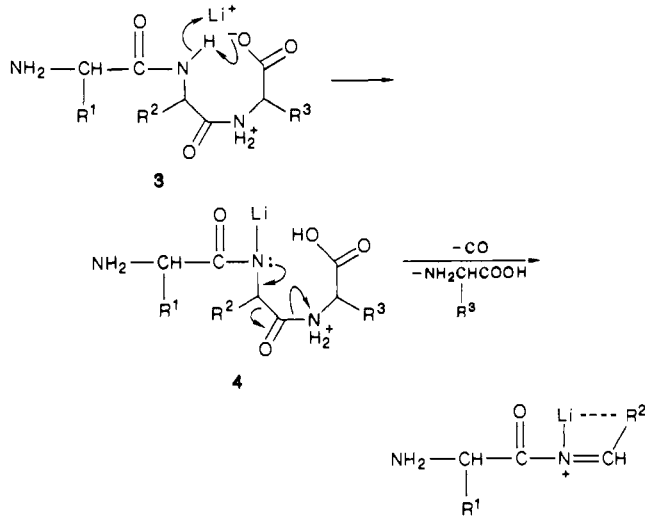

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Table III. Relative Abundance of $[A_n + Li - H]^+$ Ions from CA of $[M + Li]^+$ of Various Peptides

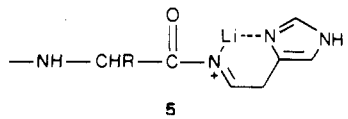
peptides[a]	m/z of	M + Li	A_1	A_2	A_3	A_4	A_5	A_6	A_7	A_8	B′[b]	B″[c]	Other abundant ions
GLY		353		31								100	
GPA		250		41								100	
LWM		455	1	48								100	
GGG		196	6	34								100	
ALG		266	7	62								100	M + Li − H_2O (72)
GLA		266		62								100	
HipHL		436		40							41	100	
GGL		252	6	41								100	
RFA		399	20	51							48	57	M + Li − NH_3 (100)
WGG		325	40	14								57	
PFGK		454		44	8							100	
AGFL		413	1	12	61							100	
VIHN		488		10	63						37	46	M + Li − NH_3 (100)
GHRP		472		36	38						100	50	
VGDE		425		2	7							32	M + Li − H_2O (100)
YGGFL		562			10	51						100	
YAGFL		576		12		55						100	
YGGFM		580	5		15	95						100	
LWMRF		758		9	21	13					99	40	M + Li − NH_3 (95)
RKDVY		686		4	5	15						14	M + Li − NH_3 (100)
YIHPF		682		26	100	16						29	
GGGGGG		367	3	27	27	36	28					100	
KCTCCA		634		16	35	36	26					81	M + Li − H_2S (100)
SGAGAG		425	5	20	58	30	100					61	
HLGLAR		672	7	17		39	37					86	M + Li − NH_3 (100)
VYIHPF		781		21	27	100	22					25	
LWMRFA		829		8	8	23	55					54	M + Li − NH_3 (100)
RVYVHPF		923		12	21	24	36	19				9	M + Li − NH_3 (100)
MEHFRWG		968			34	55	16	50			99	34	M + Li − NH_3 (100)
PRVFVHPY		1038			4	7	7	17	11			5	M + Li − NH_3 (100)
SarRVYIHPF		1008			3	10	12	20	10			10	M + Li − NH_3 (100)
RPPGFSPFR		1066					46			16		48	M + Li − CH_2N_2 (100)

[a] Amino acid single letter codes are used. [b] B′ = B_{n-1} + Li − H. [c] B″ = B_{n-1} + Li + OH.

the losses of the C-terminus, are A-type fragments at the phenylalanine residues.

An extreme example of this interaction is found by comparing the CA and metastable spectra of the $[M + Li]^+$ ion (m/z 781) of ValTyrIleHisProPhe (Figure 5, a and b, respectively). The CA spectrum of fragments is dominated by the $[A_4 + Li - H]^+$ ion of m/z 491 with minor contributions from other A fragments and from the product ion formed by the loss of the C-terminal phenylalanine (to give the m/z 634 ion). In contrast, the loss of the C-terminal phenylalanine is the most facile decomposition of the metastable species, and now $[A_4 + Li - H]^+$ is produced as a minor species.

We propose the mechanism of Scheme IV to account for formation of the $[A_n + Li - H]^+$ ions. In solution, the most stable complexes involve interaction of the metal ion with a deprotonated amide nitrogen as part of a six-membered ring that also incorporates the amino acid side chain. Those A fragments containing aromatic side chains as part of R^2 in Scheme IV produce the most abundant $[A_n + Li - H]^+$ ions, presumably because the aromatic ring participates in the formation of stable chelate rings by donation of their π electrons (see Table III). Because histidine forms a very stable chelate ring in solution,[39] it is expected that the most abundant $[A_n + Li - H]^+$ product ions arise from Li^+ attachment at the amide bond involving the histidine residue. The probable structure of the resulting ion, **5**, has a stable six-member ring



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including the carbonyl oxygen, the lithium metal ion, and the pyridine nitrogen of the imidazole ring.

The special metal ion interaction leading to **5** underscores the potential for utilizing gas-phase chemistry to establish solution analogies. The pyridine nitrogen of the imidazole ring of histidine is known to be a principal binding site for transition metals in metalloproteins.[17] Furthermore, proteins containing as few as a single histidyl residue have strong affinity for immobilized-metal affinity columns.[40]

The differences between the metastable ion and CA spectra (especially in Figure 5) are quite remarkable. One explanation is that an isomerization (**3** → **4**) occurs upon collisional activation. Another is that both structures **3** and **4** are produced in the source, and the spectra reflect differences in the relative stabilities of the two molecular ions. Based on the following observations, we propose that the collision-induced isomerization mechanism is preferred.

The metastable and CA spectra, especially for a peptide containing a histidine (Figure 5), are qualitatively very different. The loss of the C-terminal amino acid residue is the most abundant product ion produced metastably, whereas the most abundant ion produced by collisional activation is the A-type fragment at the amide bond involving the histidine residue. Although not as dramatic, similar differences are present in the metastable and collisional activation decompositions of peptides that do not contain histidine (Figure 2). Upon close inspection, the abundances of the $[B_{n-1} + Li + OH]^+$ ions relative to the parent $[M + Li]^+$ ion do not increase appreciably upon collisional activation. The abundances of A-type fragments, however, increase substantially with collisional activation. For example, the relative abundance of the A_4 ion (m/z 417) from TyrAlaGlyPheLeu $[M + Li]^+$ (Figure 2) increases by a factor of 30 upon collisional activation; the $[B_4 + Li + OH]^+$ ion, however, increases by a factor of 2. Similarly, the A_4 ion (m/z 491) from ValTyrIleHisProPhe $[M + Li]^+$ (Figure 5) increases by 20, whereas the ion of m/z 634 from loss of C-terminal phenylalanine increases by a factor less than 2. If these two fragment ions arise from decompositions of molecular ions having two structures, their relative abundances by collisional activation would be expected to be approximately the same as those by metastable decompositions. The differences between the metastable decompositions presented by Tang et al.[12] and the collision-induced decompositions presented by Russell[9]

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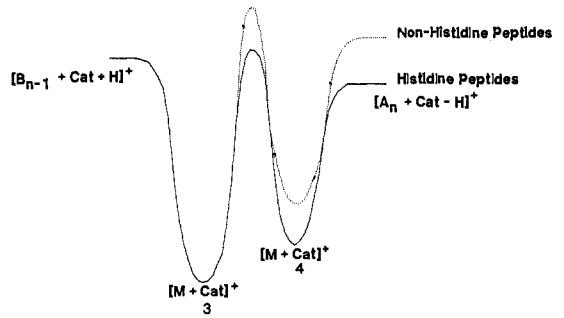

Figure 6. Potential energy surface for decomposition of peptidal $[M + Cat]^+$ ions.

and co-workers are also consistent with our observations.

The results may be explained in terms of a schematic potential energy surface (see Figure 6). The parent ion is primarily the zwitterionic species **3**. The energy needed to cause isomerization to the deprotonated amide species **4** is more than that needed to cause the metastable decomposition to $[B_{n-1} + Cat + OH]^+$. Consequently, in the absence of collision gas, the dissociation to the latter ion is preferred. Isomerization, however, also occurs metastably, as indicated by low abundance $[A_n + Cat - H]^+$ ions. Therefore, the barrier for isomerization must be only slightly larger than the barrier for dissociation.

Upon collisional activation, more ions have sufficient energy for isomerization, a process that is kinetically favored for higher energy ions because the principal requirement is a simple metal ion migration. The rearranged ions subsequently decompose, producing $[A_n + Cat - H]^+$ ions. This is especially true for peptides with histidine residues. The losses of CO and an amino acid (or smaller peptide) from the C-terminus of the isomerized species is expected to be facile; thus, the rate-determining step is the isomerization. The more favorable interaction with histidine lowers the isomerization barrier and results in a predominance of A fragments at histidine residues. If this mechanism is correct, then the relative abundances of histidine fragments should decrease as the histidine residue is moved further from the C-terminus. Collisional activation of MetGluHisPheArgTrpGly $[M + Li]^+$ (Table III) suggests that this is true, although further investigation of peptides containing histidine residues at varying positions is needed and is continuing in our laboratory.

This mechanism is consistent with energy requirements of metal ion–peptide binding in solution. The input of energy, such as raising the pH of the solution, to give deprotonation of the amide bond, also leads to metal–peptide coordination at sites other than the C-terminus.

Utility for Structural Determinations. The structural utility of the decompositions of $[M + Cat]^+$ peptides is illustrated for the octapeptide SarArgValTyrHisProPhe, which gives an $[M + Li]^+$ at m/z 1008. The C-terminal amino acids is immediately apparent from the metastable ion spectrum (Figure 7a); the ion of m/z 861 arises from loss of the phenylalanine residue. With the knowledge that this peptide has a C-terminal phenylalanine, the CA spectrum (Figure 7b) is inspected for an A-type fragment m/z 815. After locating an m/z 815 ion, the partial sequence of the peptide can be determined by looking for other A-type ions and from a general knowledge of which amino acid residues should produce abundant $[A_n + Li - H]^+$ ions. Although complete sequence determination is possible from the CA spectrum of the $[M + H]^+$ ion, the decompositions, both metastable and collisionally induced, of $[M + Li]^+$ ions provide complementary information.

Conclusions

The decompositions of metal ion–peptide complexes in the gas phase are in accord with known solution chemistry concepts and reflect the nature of the interactions of the metal ion with the peptide. Two interactions have been deciphered in this report.

In solution, coordination of alkali metal ions with peptides occurs at the carbonyl oxygens. This interaction is known to catalyze hydrolysis of the amide bond by promoting nucleophilic attack

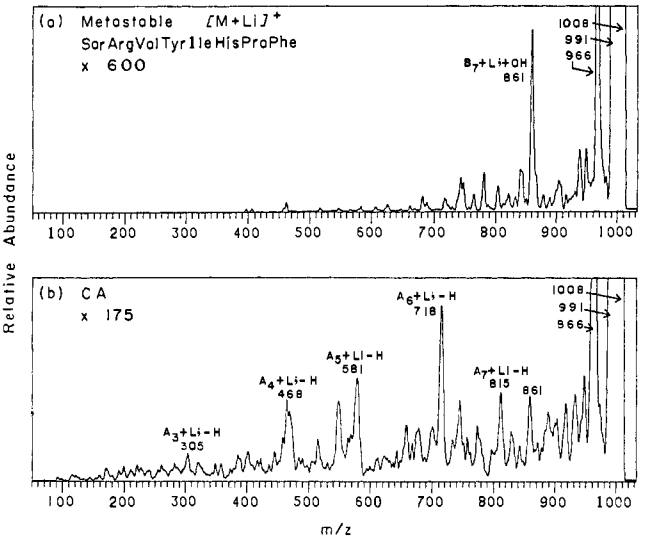

Figure 7. Metastable ion mass spectrum and (b) CA mass spectrum of $[M + Li]^+$ of SarArgValTyrIleHisProPhe, m/z 1008.

by either H_2O or hydroxide ion at the carbonyl carbon. Similar reactions occur in the gas phase, and are consistent with metal ion binding at the carbonyl oxygens of the C-terminus and the adjoining amino acid residue. The attacking nucleophile is the carboxylate anion of the C-terminus, and a product ion is produced by subsequent expulsion of the C-terminal amino acid residue. This fragment ion is dominant in both the metastable and CA mass spectra and is structurally useful for determining the C-terminal amino acid. The process is general and, in some ways, mechanistically similar to the C-terminal amino acid cleavage by the enzyme carboxypeptidase.

Transition metal ions bind strongly to peptides in solution by replacing a nitrogen-bound amide proton. This interaction is even more favorable when a six-member chelate ring is possible with a side chain donor atom. Predominant–$CONHCHR^1CON^+$-(Met)=CHR^2 ions are formed by collision-induced isomerization and subsequent decomposition of the $[M + Li]^+$ ion. A series of these ions results by interaction of the metal ion at different amino acid residues; their relative abundances are consistent with metal ion affinities of specific amino acids in solution and in the gas phase. We find no evidence for alkali metal ion interaction with the N-terminus.

Research will be extended to the interactions of other metal ions such as alkaline earths and transition metals and specially designed peptides.

Experimental Section

Reagents and Procedures. The peptides were obtained from either Sigma Chemical Co. (St. Louis, MO) or Chemical Dynamics Corp. (South Plainfield, NJ) and were used as received. Glycerol, thioglycerol, and the alkali iodides were obtained from Aldrich Chemical Co. (Milwaukee, WI). The glycerol/thioglycerol/alkali iodide matrix was prepared by mixing equal weights of glycerol and thioglycerol and saturating with the alkali iodide. The DT matrix was prepared by mixing 25 g of dithiothreitol with 5 g of dithioerythritol (>97%, Aldrich) and heating at 40 °C.

Carbobenzoxyglycine[^{15}N]alanine was prepared from carbobenzoxyglycine nitrophenyl ester (Sigma) and [^{15}N]alanine (Sigma, 99% ^{15}N) by using a previously described method.[41]

For FAB-MS/MS experiments, approximately 1 μg of peptide was mixed on a copper or gold probe tip with either DT, for production of $[M + H]^+$ ions, or glycerol/thioglycerol/alkali iodide for production of $[M + Cat]^+$ ions.

Instrumentation. CA and metastable ion mass spectra were obtained by using a Kratos (Manchester, UK) MS-50 triple analyzer mass spectrometer of EB/E design, which was previously described.[42] MS-I is a standard high-resolution Kratos MS-50 (ESA and magnet). MS-II is

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a second electrostatic analyzer (ESA-II). The field-free regions (FFR), located between the source and ESA-I (1st FFR), and between the magnet and ESA-II (3rd FFR), are equipped with standard collision cells. An Ion Tech saddle-field atom gun (Ion Tech, Middlesex, England) was used for producing 7-to 8-keV Ar atoms for FAB desorption in a commercially available Kratos FAB source.

For FAB-MS/MS experiments, an ion of interest was selected by using MS-I at a mass resolution of approximately 1000 (width at 10% height). Mass selected ion kinetic energy spectra (MIKES) were obtained by scanning MS-II. Twenty scans were averaged using software written in this laboratory. CA experiments were done by activating the mass selected ion in the third FFR by using a helium pressure that gave a 50% main beam suppression.

For MS/MS/MS experiments, source-produced ions were activated in the first field-free region. The fragment ion of interest was then transmitted to the third FFR by setting both the first ESA and the magnet at the appropriate values. The selected daughter ion was collisionally activated and the resultant second generation fragment ions were analyzed by scanning the final electric sector.[29]

Acknowledgment. Preliminary results were presented at the 35th and 36th ASMS Conferences on Mass Spectrometry and Allied Topics; Denver, CO, 1987; and San Francisco, CA, 1988. We are grateful to Dr. K. B. Tomer for assistance in the early stages of this research, and to Professor K. G. Standing and Professor D. H. Russell for preprints of recently published work. This work was supported by the Midwest Center for Mass Spectrometry, a National Science Foundation Regional Instrumentation Facility (Grant No. CHE-8620177).

An Algorithm for the Design of Propagating Acidity Fronts

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Abstract: On the basis of elementary chemical considerations, it is shown that proton-producing redox reactions of oxyanions are expected to be autocatalytic and to manifest propagating acidity fronts. The conditions of proton production in redox reactions are established and verified experimentally by the reactions of $S_2O_3^{2-}$, $S_4O_6^{2-}$, $S_2O_4^{2-}$, SO_3^{2-}, $S_2O_6^{2-}$, and $N_2H_5^+$ with BrO_3^-, IO_3^-, ClO_3^-, ClO_2^-, and $S_2O_8^{2-}$. Twenty new propagating acidity front reactions have been discovered among the possible combinations of these reactions.

Propagating reaction fronts in autocatalytic reactions were discovered by Luther[1] in 1906. His discovery, however, seems to have been forgotten until recently.[2] Interest in the study of propagating acidity fronts and other forms of chemical waves was renewed after the discovery[3] and interpretation[4] of the fascinating Belousov–Zhabotinsky (BZ) reaction.

The number of reaction systems exhibiting chemical-wave behavior, apart from the various modifications of the original BZ system[5-11] is less than 20.[12-21] Three of them, the hydrolysis of alkyl sulfates[1] and the iodate–arsenite[21] and the chlorite–thiosulfate[17,22] systems are characterized by propagating acidity fronts; i.e., the diffusion of the proton produced in the reaction is coupled to its catalytic effect.

The considerations below show that propagating acidity fronts are a generally expected phenomenon in proton-producing redox reactions of oxyions.

Chemical Considerations

It is well-known that the structure of most oxyanions in deprotonated form is symmetrical; the bond order of the central atom–oxygen bonds is higher than 1. Breaking strong bonds in a symmetrical structure requires a high activation energy. Thus the oxyanions are expected to be kinetically stable against redox transformation in alkaline medium. In protonated form, however, the original symmetry is lost, the order of one of the central atom–oxygen bonds decreases to 1. In other words, protonation is expected to decrease the kinetic stability of the oxyanion, making redox transformations more facile.

It follows from these qualitative considerations that proton-producing redox reactions of oxyanions may be expected to be autocatalytic and to manifest a propagating acidity front, if the reactants are mixed in alkaline solution and a drop of acid is used to initiate the reaction in an originally homogeneous unstirred solution.

Proton production is easy to anticipate from the stoichiometry of the reaction. Oxidizing agents usually consume protons and electrons, while reducing agents liberate both species. Therefore,

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