Fragmentation of protonated oligopeptides XLDVLQ (X=L, H, K or R) by surface induced dissociation: additional evidence for the 'mobile proton' model

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Abstract

The fragmentation of a series of singly protonated peptides, X-Leu-Asp-Val-Leu-Gln (XLDVLQ, X = Leu(L), His(H), Lys(K), or Arg(R)), was investigated by surface induced dissociation (SID) in a tandem quadrupole mass spectrometer. The SID collision energies required for the fragmentation were found to increase with increasing gas-phase basicity of the 'X' amino acid residue. The results are consistent with previous observations reported for other series of peptides and can be explained based on the 'mobile proton' model. Enhanced cleavage at the C(O)–N bond located C-terminal to the Asp residue (Asp-Xxx) was observed only in the presence of Arg, the most basic common amino acid residue. The results suggest that the acidic hydrogen of the Asp side chain becomes significant as an alternative source of proton to promote the 'charge-directed' cleavage of the amide linkage of Asp-Xxx (e.g., via a cyclic intramolecular hydrogen bond), when the 'ionizing' proton is 'sequestered' by the Arg residue. Lower abundances of side chain cleavage d ions by SID were observed relative to those previously detected by high energy CID in sector and sector-hybrid instruments. ©1999 Elsevier Science B.V. All rights reserved.

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1. Introduction

Over the last decade, mass spectrometry (MS) has played an increasingly important role in the characterization of peptides and proteins [1,2]. This is largely attributable to the development of ionization techniques applicable to nonvolatile molecules, in particular, electrospray ionization (ESI) [3], and matrix assisted laser desorption ionization (MALDI) [4]. The fragmentation of protonated peptides by tandem mass spectrometry (MS/MS) provides a means for elucidation of the primary structure of peptides [5–13]. In MS/MS, a particular ion of interest (e.g., a protonated peptide) is first selected and then activated, most commonly by collision with a gaseous target (collision induced dissociation, CID [14]) or by collision with a surface (surface induced dissociation, SID [15,16]). Finally, the unimolecular dissociation product ions formed from the activated ion are analyzed. The CID technique is available commercially but SID is not yet available

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on commercial instruments. Cleavages at the amide linkages and other positions can occur when protonated or multiply protonated peptides are activated in the gas phase. MS/MS sequencing based on the dissociation patterns has several advantages over conventional Edman peptide sequencing. MS/MS is an attractive approach for dealing with mixtures of peptides, N-terminal blocked peptides, identification of post-translational modification (e.g., phosphorylation sites of proteins) [12], and for rapid identification of proteins by database matching of a few peptides in the protein digests [17]. Even in Edman sequencing communities, mass spectrometry is now increasingly used for sample purity assessment, to estimate the number of Edman cycles needed, and to eliminate Edman ambiguities [18].The author of a recent review on interpretation of MS/MS spectra of peptides stated that interpretation of MS/MS data remains "the limiting factor in greater dissemination of this method of analysis" [13]. Improving our knowledge of peptide fragmentation mechanisms could thus substantially increase the utility of sequencing by MS/MS. Much progress has been made recently in understanding the mechanisms of peptide fragmentation. For example, general models such as the 'mobile proton' model [19] and, equivalently, the 'heterogeneous proton distribution' [20,21] have been proposed for the low-energy pathway to form b_n and y_n ions by charge-directed cleavage of amide linkages. A charge-remote process has been suggested for the formation of side chain cleavage ions, e.g., d_n ions from $[a_n + 1]$ ions [22]. Understanding he selective/enhanced cleavage at a specific amide linkage is of interest because it is directly related to the successful application of MS/MS in the determination of primary structure of peptides. For example, the selective/enhanced cleavage of the amide linkage at the C(O)–N bond C-terminal to an aspartic acid residue (i.e., at Asp-Xxx) [23–26], or at the C(O)–N bond N-terminal to a proline residue (i.e., Xxx-Pro) [10] has been previously noticed and investigated.

In conjunction with many other approaches, including linked scan measurements [22,27], derivatization [19,23], isotopic labeling [28,29], multistage mass spectrometry (e.g., MS/MS/MS) [30], neutral fragment re-ionization [30,31], and quantum chemical calculations [11,26], the investigation of relative energetics of peptide fragmentation [19,32] can provide useful mechanistic insights. Surface induced dissociation (SID), developed originally by Cooks and coworkers [15], is a suitable method for studying relative fragmentation energetics because (i) the distribution of internal energy deposited into the projectile ions by SID is relatively narrow (compared to that in CID) and (ii) the average internal energy is easily varied [15,33]. As a consequence, when SID is used in conjunction with ESI, an ionization method which produces 'cold' ions, a sharp increase in percent fragmentation of protonated peptides can be achieved by gradually increasing the SID collision energy. In fact, the correlation observed in ESI/SID between the energies required for peptide fragmentation and peptide composition (e.g., gas phase basicities of amino acid residues) has provided one important piece of experimental evidence for the development of the 'mobile proton' model [19].

In the present study, the ESI/SID fragmentation of a series of synthetic peptides X-Leu-Asp-Val-Leu-Gln (XLDVLQ; X = Leu(L), His(H), Lys(K), or Arg(R)) was investigated. These peptides differ only in the N-terminal amino acid residues. All of the three common amino acid residues that have a basic side chain, His, Lys, and Arg, are included. The peptides chosen are good candidates for the further examination of the influence of the gas phase basicity of amino acid residues on peptide fragmentation. They were also used to probe the likelihood of enhanced cleavage at the aspartic acid residue in the presence of different basic amino acid residues. Another aspect of this work is a comparison of the occurrence of side chain cleavage d_n ions in SID spectra to those previously observed for these peptides by high energy (keV) CID [34]. Finally, the relative dissociation onset for His and Lys containing peptides was investigated by comparing dissociation data for HLDVLQ/KLDVLQ with data for HAAAA/KAAAA, Nα-Ac-HAAAA/Nα-Ac-KAAAA, and AAHAA/AAKAA.

2. Experimental section

2.1. Electrospray ionization/surface induced dissociation (ESI/SID)

ESI/ SID experiments were carried out in a custom tandem mass spectrometer that was described previ-

ously [16,19,35]. It is composed of two Extrel 4000 unit quadrupoles positioned at $90°$. The target surface is placed between two quadrupoles. Incident angles of the precursor ion beam are $45–50°$ to the surface normal. Protonated peptide ions were generated by an electrospray ionization (ESI) source which is a modified version of the designs by Chowdhury et al. [36] and Papac et al. [37]. The precursor ions of interest were mass selected by the first quadrupole. The product ions produced by SID were analyzed by the second quadrupole. The collision energy of the incident ion (all singly charged) was determined by the potential difference between the skimmer cone of the ESI source and the surface. The surface used in this study is a self-assembled monolayer (SAM) on gold prepared from octadecanethiol (i.e., $CH_3(CH_2)_{17}S$-Au, referred to as C18 SAM), or 2-(perfluorooctyl)-ethanethiol (i.e., $CF_3(CF_2)_7CH_2CH_2S$-Au, referred to as FC10 SAM) [11,38].

Several precautions were taken in the investigation of relative energetics for the fragmentation of the protonated peptides. For example, data points at various relative collision energies for peptide 'pairs' containing lysine versus histidine are acquired under the same instrumental operating conditions by introducing one sample after another. If a set of experiments emphasizes the relative energetics, the ESI source is operated under conditions that are reasonable compromises between two objectives: (i) to minimize the additional internal energy deposition in the region between the capillary and skimmer cone, and (ii) to maintain a reasonably high intensity of ion beams entering the mass analyzer. For all the samples, the electrostatic potential applied to the capillary of the ESI source is kept 30 V higher than that on the skimmer cone. No tubular electrostatic lens was used in the capillary-skimmer region due to the excessive internal energy deposition observed when it is in use, although we have found that installation of a tubular lens with adjustable electrostatic potential in that region may enhance ion intensities produced from the ESI source. The acquisitions of the ESI/SID spectra to obtain relative energetics results shown are not fully optimized in terms of signal to noise ratio of the spectra, because some of the precursor ion intensity was sacrificed to avoid excessive 'energization' of the ion population prior to surface collision.

2.2. Nα-monoacetylation of HAAAA and KAAAA

Fmoc-Lys(Boc)-Ala-Ala-Ala-Ala and Fmoc-His(Trt)-Ala-Ala-Ala-Ala attached to Wang Resin were prepared using solid-phase synthesis protocols outlined by Atherton and Sheppard [39]. The Fmoc (9-fluoroenylmethoxycarbonyl) protecting group at the N-terminus of Lys or His was then removed by piperidine in DMF (*N,N*-dimethylformamide). The free N-terminal peptides attached to the resins were subsequently acetylated using acetic-anhydride in DMF while the side chains of Lys or His were protected by *t*-butyloxycarbonyl (Boc) or trityl (Trt, triphenylmethyl) group, respectively. Finally, Nα-Ac-Lys(Boc)-Ala-Ala-Ala-Ala and Nα-Ac-His(Trt)-Ala-Ala-Ala-Ala were cleaved from the resin and the Lys or His side chains were deprotected using TFA(trifluoroacetic acid)/H_2O/EDT(1,2-ethanedithiol) (95 : 2.5 : 2.5). The Fmoc derivatives of the amino acids required for the synthesis were obtained from Novabiochem (CA, USA) or Advanced Chemtech (KY, USA). The C-terminal Ala residue for the synthesis was purchased already attached to Wang Resin from Novabiochem (CA, USA). Once a Nα-monoacetylated peptide was precipitated in diethyl ether, its identity and the purity were confirmed by ESI mass spectrometry.

3. Results and discussion

3.1. Relative fragmentation efficiency

SID fragmentation efficiency values are plotted in Fig. 1 as a function of laboratory collision energy (eV) for electrospray generated $[M+H]^+$ ions of XLDVLQ (X=L,H,K, or R). The fragmentation efficiency is defined as the ratio

$$\frac{\sum \text{fragment ion peak area}}{\text{parent ion peak area} + \sum \text{fragment ion peak area}}$$

It is seen in Fig. 1 that more energy is required for fragmentation of peptides containing a basic residue (H, K or R) than for that with no basic residue (LLDVLQ). Arginine, with a highly basic guanidino side chain, has the highest gas phase basicity among the 20 common amino acid residues. Accordingly, the highest onset energy is observed for protonated RLDVLQ.
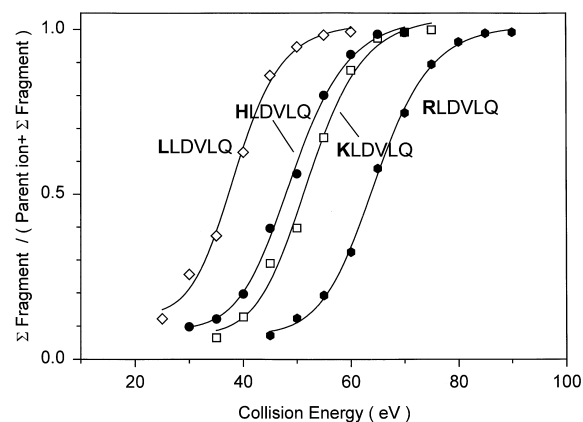
Fig. 1. ESI/SID fragmentation efficiency curves for singly pro-tonated peptides of XLDVLQ (X = L, H, K or R). The collision target surface used is a self-assembled monolayer (SAM) of octa-decanethiolate on gold (i.e., $CH_3(CH_2)_{17}S$-Au, C18 SAM).

Table 1

Gas phase basicities of amino acids and some molecules related to lysine or histidine

Molecules	GB (kcal mol^{-1})	
	[44]	[43]
Leucine	210.5	209.6
Lysine	227.3	221.8
Histidine	227.1	223.7
Arginine	240.6	237.0
1,5-diaminopentane[a]	226.1	
Butylamine	211.9	
Histamine[b]	229.9	
4(or 5)-methylimidazole	220.1	

[a] For $H_2N(CH_2)_4CH(NH_2)$-X: X = COOH–lysine: X = H–1,5-diaminopentane.

[b] For imidazol-4(or 5)-yl-$CH_2CH(NH_2)$-X: X = COOH–histidine; X = H–histamine.

The general trend in SID collision energy required for fragmentation of these peptides on the timescale of the instrument (μs) is consistent with previous results obtained in our research group using other series of peptides [19]. Based on the 'mobile proton' model [19], the trend in onset energies of the ESI/SID fragmentation efficiency curves shown in Fig. 1 can be explained as follows. The population of different protonated forms of a peptide depends on the internal energy content of the peptide and the relative gas-phase basicities of the different protonation sites of the peptide. In the electrospray ionization process mainly the thermodynamically most stable protonated forms are generated. These forms may be highly solvated and 'ball-like' involving significant intramolecular solva-tion of the protonation site [40,41]. Ion activation in MS/MS alters the population of different protonated forms (mobilizes the proton via rapid intramolecular proton transfer). Quantum chemical bond order calcu-lations clearly indicate that the strength of the amide bond is significantly different in the different proto-nated forms. It has been shown that the amide bond is significantly weaker in those forms in which the amide nitrogen is involved in the protonation (either entirely or partially, e.g., via H-bonds) [11,42]. These amide-N protonated forms can, therefore, be consid-ered as 'fragmenting structures'. This means that by transferring the proton from a more basic site (but not a fragmenting structure, e.g., from a basic side chain,

a N-terminal amino nitrogen, or a oxygen carbonyl) to the less basic amide nitrogen atoms, the cleavage of the amide C(O)–N bond can be promoted by a 'charge-directed' pathway. It is more difficult to mo-bilize a 'strongly held' or 'sequestered' proton (e.g., from the guanidino basic site of the Arg side chain) than a less strongly bound proton (e.g., from the rel-atively less basic N-terminal α-amino group). Thus, higher SID collision energies are required to fragment protonated peptides containing basic residues than that is required for a non-basic peptide.

Another interesting aspect of the fragmentation ef-ficiency curves in Fig. 1 is the relative fragmentation energetics of Lys- versus His-containing peptides. The gas phase basicities (GBs) of the 20 common amino acids have been previously measured by dif-ferent methods in several research groups. These studies have been recently reviewed and evaluated by Harrison [43] and by Hunter and Lias [44]. Un-certainties of the measured GBs and limitations of different methods are noted [43]. For example, the relative GBs of histidine(H) versus lysine(K) can be found in the literature as H = K [45,46], H < K [47], or H > K (see [43] and the references therein). For the purpose of the following discussion, Table 1 lists the GB values according to the reviews by Harrison [43] and Hunter and Lias [44]. The relatively large gap observed in the dissociation energy requirement between R- and H/K-peptides, or that between L and H/K-peptides, corresponds to the relatively large difference in GBs of arginine versus histidine/lysine

residues ($>10\,\text{kcal mol}^{-1}$, see Table 1), or GBs of his-tidine/lysine versus leucine residues ($>10\,\text{kcal mol}^{-1}$, see Table 1). The GB of lysine is evaluated to be almost the same as, or slightly lower than that of histidine (Table 1). Accordingly, the curve positions for the peptides containing lysine or histidine residue are relatively close to each other (Fig. 1). However, contrary to the order of GB(lysine) ≤ GB(histidine) as evaluated by Harrison or by Hunter and Lias (Table 1), the position of the fragmentation efficiency curve for protonated KLDVLQ is observed at slightly higher collision energies than that for protonated HLDVLQ (Fig. 1). In order to eliminate the influence of side chains other than those of lysine and histidine for possible discrepancies in the positions of the KLDVLQ versus HLDVLQ curves, additional model peptides were investigated: HAAAA/KAAAA (Fig. 2a). A higher onset energy is still observed for fragmentation of KAAAA versus HAAAA, similar to that of HLDVLQ versus KLDVLQ (Fig. 2a versus Fig. 1).

A cyclic hydrogen bond between the α-amino terminus and the protonated side chain amine of lysine, which is similar to that which exists in protonated 1,5-diaminopentane, is believed to contribute to the high GB of lysine [43,48] (see GBs of lysine, 1,5-diaminopentane, and butylamine in Table 1). Some authors have suggested that this cyclic intramolecular proton bridging between the α-amino nitrogen and the ε-amino nitrogen would make lysine more basic than histidine in the gas phase since the more rigid histidine side chain does not favor such an interaction [48]. Similarly, Harrison [43] pointed out that the higher GB of arginine relative to guanidine or methyl substituted guanidine can be explained by an additional stabilization of intramolecular hydrogen bonding with the α-amino group in protonated arginine. While this is plausible for the amino acid, different additional stabilization of the charge center in an Arg containing *peptide* has been suggested from the investigation of the gas phase conformation of bradykinin (Arg-Pro-Pro-Gly-Ser-Pro-Phe-Arg) by the Bowers group and the Williams group [41,49]. For the lowest-energy conformations of singly protonated bradykinin, the charge center on the guanidino group of Arg is stabilized by the coordination of several of the backbone carbonyl groups and the C-terminus. No involvement of the α-amino group was noted in the proposed gas phase structures of bradykinin
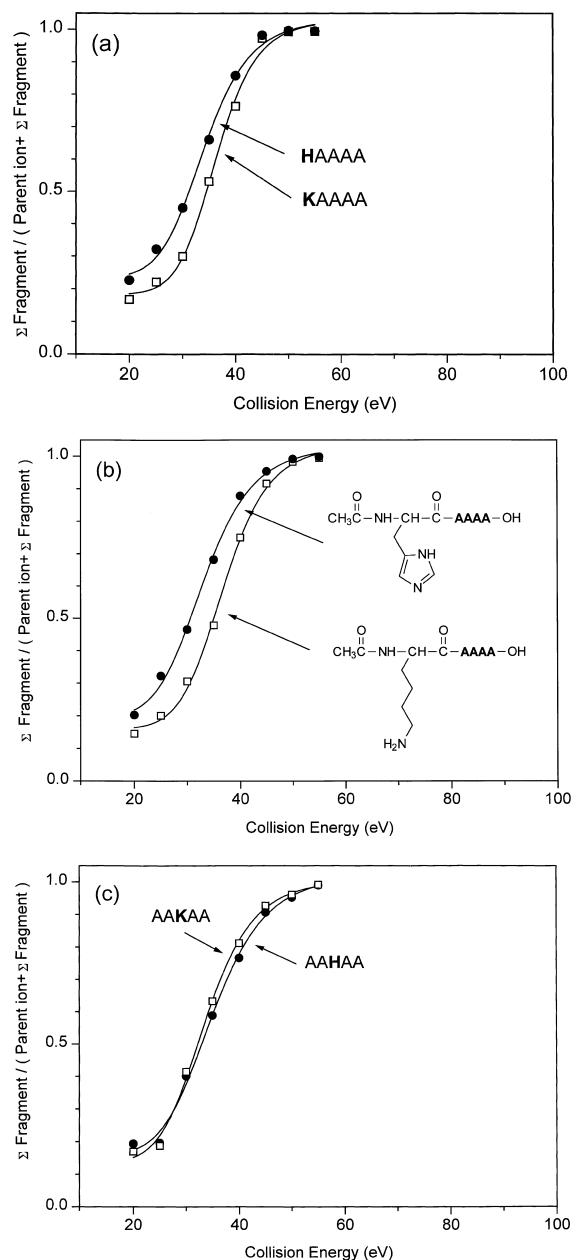

Fig. 2. ESI/SID Fragmentation efficiency curves for singly protonated peptides of (a) (H/K)AAAA; (b) Nα-(mono)acetylated (H/K)AAAA; and (c) AA(H/K)AA. The same SAM surface, C18 SAM, as that in Fig. 1 is used.

[41,49].In order to investigate whether coordination to the N-terminal α-amino group stabilizes the protonation site at the side chain, and to determine whether this contributes to the relative energetics of dissocia-

tion of K- versus H-containing peptides, monoacetylation only at the N-terminal α-amine nitrogen of KAAAA and HAAAA was performed (see, experimental section). The fragmentation efficiency curves of the monoacetylated peptides are given in Fig. 2b. Virtually no change was observed for the positions of the fragmentation efficiency curves after monoacetylation at the N-terminal nitrogen (Fig. 2b versus 2a). This is in contrast to the shift of fragmentation efficiency curves to low energies after acetylation at the free N-terminal α-amine nitrogen of non-basic peptides (where the charge center of $[M+H]^+$ generated from the ESI source is believed to be at the N-terminal nitrogen) [50].

There are two possible explanations for the results of Fig. 2b versus 2a. One is that the N-teminal α-amino group of lysine and that of histidine have similar contributions in stabilizing the charge center at their basic side chain. More plausibly, the lack of significant involvement of the α-amino group in stabilization of the protonation site at the side chain could account for the fact that no change occurs in the relative energetics of protonated peptide fragmentation after Nα-monoacetylation of KAAAA versus HAAAA.

Another set of experiments have been performed for the peptides AAKAA and AAHAA, i.e., with Lys and His 'moved' to the middle of the sequence. The fragmentation efficiency curves for these two peptides are almost on top of each other, i.e., dissociation energy requirements are virtually the same (Fig. 2c). These results show that the position of the amino acids has an effect on the relative ease of fragmentation. At this point, it is difficult to conclusively explain this dependence on the position of the amino acids. After all, ESI/SID fragmentation efficiency curves result from unimolecular dissociations of activated ions (i.e., they are 'kinetically' controlled), so they are not direct measures of relative GBs of different individual amino acid residues in a series of peptides. It is not clear whether a small difference in the GBs can be resolved by the positions of the curves, even though the instrumental operating conditions are carefully controlled. The relative energetics do not exclusively depend on the relative GBs of *individual* amino acid residues but they may also be dependent on conformations of peptides. Unfortunately, the low-energy conformational structures of these protonated peptides in the gas phase are unknown. However, it is rea-

sonable to assume that some conformational changes may play a significant role in fragmentation when the difference in the GBs of the amino acid residues is small. The conformations may affect the apparent GB of a basic site in a peptide by additional stabilization, and/or may influence the intramolecular proton transfers. For example, the flexible lysine side chain versus the more rigid side chain of histidine might allow easier stabilization of the protonation charge center at the lysine side chain by the peptide backbone carbonyl groups and/or the C-terminus when Lys versus His is located at the N-terminus. The additional flexibility of the lysine side chain may be less important when K and H are at the center of a pentapeptide. It is worth noting that the GBs for the lysine versus histine containing tripeptides, reported by Carr and Cassady, have the following order: GB (KGG or GGK) > GB (HGG, or GGH) when K or H is located at the terminus, but GB(GKG) = GB(GHG) when K or H is in the middle of the sequence[45]. This reported dependence of relative GBs on the position of K versus H in the sequence of tripeptides is in agreement with our measured dissociation onsets for the fragmentation of K versus H containing pentapeptides (Fig. 2a and 2c).

3.2. Asp-Xxx cleavage

Enhanced cleavage of the amide linkage C-terminal to the aspartic acid residue has been previously noticed and investigated [23–26]. The involvement of the acidic carboxylic hydrogen at the aspartic acid side chain was confirmed by esterification that blocks the enhanced cleavage at Asp-Xxx [23]. The fact that the long time window of trapping instruments (quadrupole ion trap or FT-ICR) favors the selective cleavage at Asp-Xxx suggests the cleavage to be a low enthalpy but entropically disfavored process[24]. Martin and coworkers [23] proposed a mechanism that involves the proton transfer from the Asp side chain to the adjacent amide nitrogen C-terminal to Asp. Beauchamp and coworkers [26] proposed a salt bridge model to explain the selective cleavage at Asp-Xxx in sodiated peptides. In a collaborative study with the Futrell group and the Gaskell group, Wysocki and coworkers recently suggested that the difference in observed enhanced cleavages at an aspartic amide linkage versus glutamic amide linkage (i.e., Asp-Xxx
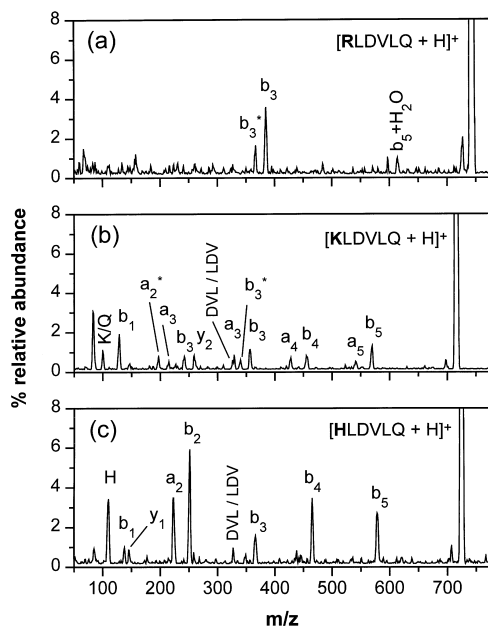
Fig. 3. SID spectra for ESI generated $[M+H]^+$ ions of (a) RLD-VLQ at 50 eV collision; (b) KLDVLQ at 40 eV collision; and (c) HLDVLQ at 40 eV collision with a C18 SAM surface. Note that only with the presence of arginine, the most basic common amino acid residue, the selective cleavage at the C(O)–N bond C-terminal to the Asp residue is observed (b_3 and b_3^* ions). The peaks labeled with an asterisk are b_n-17/a_n-17, presumably due to the NH_3 loss from side chains of Arg or Lys residues.

versus Glu-Xxx) can be explained by a kinetic effect due to the different ring size of the cyclic H-bond between the Asp/Glu side chain acidic hydrogen and the oxygen or nitrogen of its amide group C(O)–N. It was also suggested, based on that collaborative work, that the requirement for the occurrence of selective cleavage at Asp-Xxx in peptides containing Arg is that the number of ionizing protons is no more than the number of arginines present in protonated peptides [51]. In other words, it is the lack of excess 'ionizing' proton that allows involvement of the Asp acidic hydrogen. Whether other basic residues could 'sequester' the charge allowing involvement of the Asp side chain in initiating the cleavage is addressed in this paper as follows.

The ESI/SID spectra of singly protonated RLD-VLQ, KLDVLQ and HLDVLQ are shown in Fig. 3. The labeling of peaks in this figure follows the nomenclature defined by Roepstorff and Fohlman [52] and

modified by Biemann [22]. For example, b_n or y_n designates the N-terminal or the C-terminal charged fragment, respectively, when the cleavage at the nth (from the N- or C-terminus, respectively) amide linkage occurs. The ions that formally correspond to a loss of 28 amu (CO) from $b_n$ions are called a_n ions. For comparison of enhanced cleavage at the C(O)-N bond C-terminal to the Asp residue (i.e., at the linkage of Asp-Val in this case) in the presence of R, K, or H, the spectra in Fig. 3 represent relatively low collision energies such that the percent fragmentation in SID for each of the oligopeptides is low (refer to Fig. 1). Selective cleavage at Asp-Val, represented by ions b_3 and b_3-17, is only observed in the presence of arginine (Fig. 3a), but does not occur when arginine is replaced with the other common amino acid residues that have a basic side chain (lysine or histidine, Fig. 3b and 3c). As discussed above, the measured GB of arginine is much higher than that of histidine or lysine (Table 1). The ionizing proton is likely to be sequestered by the guanidino side chain of arginine, quite possibly solvated by carbonyl groups of the peptide. When a proton is 'sequestered' at such a basic side chain, the acidic hydrogen of the Asp residue becomes significant as an alternative source of proton to initiate low energy charge-directed cleavage, i.e., the involvement of the side chain acidic hydrogen is allowed when the 'ionizing' protons are tightly bound elsewhere in the ion. This is particularly the situation at relatively low collision energies when the activation is insufficient to promote intramolecular transfer of the 'ionizing' proton *with a reasonable rate*, to less basic sites in the molecule (e.g., to amide nitrogen to promote charge-directed cleavage of amide linkages).

The 80 eV SID spectrum of ESI generated singly protonated RLDVLQ is also provided here for comparison (Fig. 4). It is clear that the cleavages at Asp-Val (b_3 and b_3-17) are less dominant at 80 eV than at 50 eV (Fig. 4 versus Fig. 3a). The preferential fragmentation at Asp-Xxx occurs at relatively low SID collision energies, whereas 'fragment rich' spectra can be obtained at relatively higher collision energies. As a practical implication, SID might provide more structural information by opening more fragmentation channels compared to these reported preferential low-energy cleavages (e.g., at Asp-Xxx) produced by CID in trapping instruments (quadrupole ion trap and FT-ICR). Note

Table 2
Comparison of d_n ion (a cleavage at the side chain of amino acid residues) occurrence between SID and high energy CID

	ESI/SID[a]	LSIMS/SID[a]	MALDI/CID[b] 800 eV, Xe	LSIMS/CID[b] 4 keV, He
LLDVLQ	not found	not found	no data	extremely weak, if any d_n
HLDVLQ	not found	not found	d_2, d_3	d_2, d_3, d_4, d_5
KLDVLQ	not found	not found	d_2, d_4	d_2, d_3, d_4, d_5
RLDVLQ	d_3	d_3	d_2, d_3, d_4, d_5	d_2, d_3, d_4, d_5

[a] Both C18 and FC10 SAM surfaces were used in SID to investigate the occurrence of the d ions.
[b] The instrumental conditions for the acquisition of the CID spectra were previously described in [34].
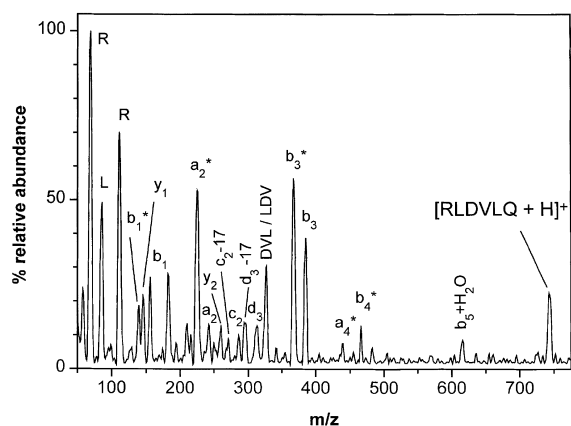
Fig. 4. 80 eV SID spectra for ESI generated $[M+H]^+$ ion of RLDVLQ collision with a C18 SAM surface.

however that preferential fragmentation could still be investigated at relatively low collision energies in SID.

3.3. d ion formation

Ions generated by the side chain cleavages such as d, w, or v ions [22] are useful for the differentiation of residues with the same mass but different side chain structures (e.g., leucine versus isoleucine). These types of cleavages are usually observed under high energy (keV) gas-phase collision conditions compatible with sector or sector-hybrid tandem mass spectrometers. Some occurrence of d, w, or v ions of peptides in SID has been previously observed [11]. For protonated peptides XLDVLQ (X = H, K or R), a series of d ions with relatively high intensities were previously detected in high energy (keV) CID [34]. It is of interest to further investigate the d ion occurrence in SID (at eV collisions), in particular, in comparison with those observed in high energy CID (at keV collisions). The d ions detected in SID along with those previously

observed in high energy CID are tabulated in Table 2. In the SID spectra of the ESI generated $[M+H]^+$ ion of RLDVLQ at collision energies ≥ 70 eV with a C18 SAM surface, a d_3 ion and a d_3-17 ion are observed (e.g., Fig. 4). However, no d_n ions were detected for other XLDVLQ peptides (X = L, H, and K). It is worth noting that, in order to search for d ions in SID, a fluorinated SAM surface (which provides higher internal energy deposition than C18 SAMs [38,53]) was also utilized. SID was also investigated for $[M+H]^+$ ions generated by bombardment with Cs^+ ions (i.e., liquid secondary ion mass spectrometry, LSIMS, producing 'hotter ions than ESI generated $[M+H]^+$ ions [16]). The only d ions detected were still the d_3 and d_3-17 ions from singly protonated RLDVLQ. Therefore, it is reasonable to conclude that the different abundances of d ions between SID and keV CID such as shown in Table 2 are caused by differences between these two activation methods.

4. Conclusions

The ESI/SID results reported in this paper for XLDVLQ (X = L, H, K and R) provide additional evidence that the energy required for fragmentation of peptides increases with increasing basicity of amino acid residues. It has also been demonstrated that when the gas phase basicities are similar, the position of the amino acid residues in the sequence can result in slight changes in the positions of the fragmentation efficiency curves (such as those for H and K in KAAAA/HAAAA, AAKAA/AAHAA). This may be related to conformational effects that can affect the apparent GB of a basic site in peptides by different additional stabilization, and/or alter the relative energy requirements for proton mobilization.

A selective cleavage at Asp-Xxx (at DV in this study) was observed only for the peptide containing the most basic common amino acid residue, arginine, (i.e., only for RLDVLQ). This preferential cleavage at Asp-Xxx was not as obvious in previously acquired high energy CID spectra. However, examination of the high energy CID spectra of RLDVLQ reveals that the backbone cleavages occur mainly as a_n ion series, except for a b_3 ion and a b_3-17(presumably NH_3) ion at the Asp-Xxx position. This is consistent with recent results from our research group for other R- and D-containing peptides. In contrast to the detection of several d ions for the peptides XLDVLQ in high energy CID spectra, the only d ions detected in the SID spectra were d_3 and d_3-17 ions in the spectrum of RLDVLQ. This could be attributed to the differences in energy distributions and/or energy deposition mechanisms of SID and high energy CID. It is desirable to use different activation methods for both mechanistic studies and analytical applications of peptide fragmentation.

Acknowledgements

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