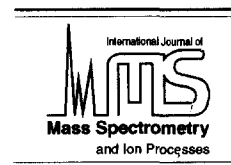




ELSEVIER

International Journal of Mass Spectrometry and Ion Processes 174 (1998) 95–100



Are peptides without basic residues protonated primarily at the amino terminus?

Hari Nair, Vicki H. Wysocki*

Department of Chemistry, University of Arizona, Tucson, AZ 85721-0041, USA

Abstract

The question of site of protonation in peptides that do not contain basic residues is addressed. The additional basicity of the peptide conferred by the amino terminus is removed by N-acetylation. Comparison of the surface-induced dissociation energy requirements for the fragmentation of the peptides with and without the N-terminal amine indicates that the N-terminal amine sequesters the proton. © 1998 Elsevier Science B.V.

Keywords: Site of protonation; Peptides; N-Terminal amino group; Fragmentation; Surface-induced dissociation

1. Introduction

Knowledge of the structures of gas-phase protonated peptides is important because the structure influences the dissociation [1–5] and, thus, the sequence determination of the peptides. Sequence determination will be most effective for total unknowns when it is clearly understood how the various structural parameters affect dissociation pathways. Furthermore, determination of differences and similarities between gas phase and solution structures will help clarify the role of solvent in determining the structure and function of peptides and proteins, and will further define the role that gas phase experimental studies can play in structure-function investigations. By using methods which generate limited excess internal energy, a homogeneous population of protonated peptides can be produced in which the proton is sequestered at the basic side

chain [2,3,5,6]. Computational and experimental investigations have suggested the involvement of additional stabilizing intramolecular solvation by other heteroatoms such as the amide oxygens [6–12]. It has not been clearly established whether peptides with no basic residues are protonated predominantly at the amino terminus (with intramolecular solvation to other sites) or whether other structures, such as those in which multiple amide oxygens solvate the proton without the involvement of the amino terminus, might account for a significant percentage of the total ion population. Results from a variety of computational and experimental studies such as bracketing techniques for small oligoalanines and oligoglycines suggest that in the peptides composed only of nonbasic residues, the proton may be solvated completely by the various amide oxygens [7–12]. This work aims to establish whether a gas-phase population of a protonated nonbasic peptide can exist predominantly as the amino-protonated form.

* Corresponding author.

2. Experimental

Experiments have been performed in a dual quadrupole mass spectrometer which is described elsewhere [13,14]. The ions are produced by electrospray ionization with a heated capillary design which is a modified version of the electrospray design described in Ref. [15]. N-Acetylation of peptides was performed by the addition of acetic anhydride (1:2) followed by continuous stirring for 10 min. The singly protonated peptides produced by electrospray ionization were mass selected and subjected to surface-induced dissociation (SID) over a range of collision energies. Self-assembled monolayer (SAM) surfaces prepared by the spontaneous assembly of octadecanethiolate (C18) on gold were used as collision targets [14]. For the smaller, nonbasic peptides of the type used in this study, the C18 surface provides access to lower internal energy deposition than would a fluorinated surface [14].

A series of SID spectra were obtained by applying collision energies in 2.5–5.0 eV increments over an energy range of 10–45 eV and the fragmentation efficiencies ($[\text{fragment ions}]/[\text{fragment ions} + \text{unfragmented precursor ions}]$) were calculated.

3. Results

Fig. 1 shows the comparison of fragmentation efficiency curves for AAAAA and N-acetyl AAAAA. The fragmentation efficiency curve for the peptide with an intact amino terminus is shifted to higher collision energy than the peptide with acetylated N-terminus. Similar experiments were performed for a series of oligoalanines $(\text{Ala})_n$, where $n = 2-6$, and the results are plotted in Fig. 2. For comparison, results for $(\text{Gly})_n$, where $n = 2-5$ and N-acetyl GGGGG are included. The collision energy values corresponding to

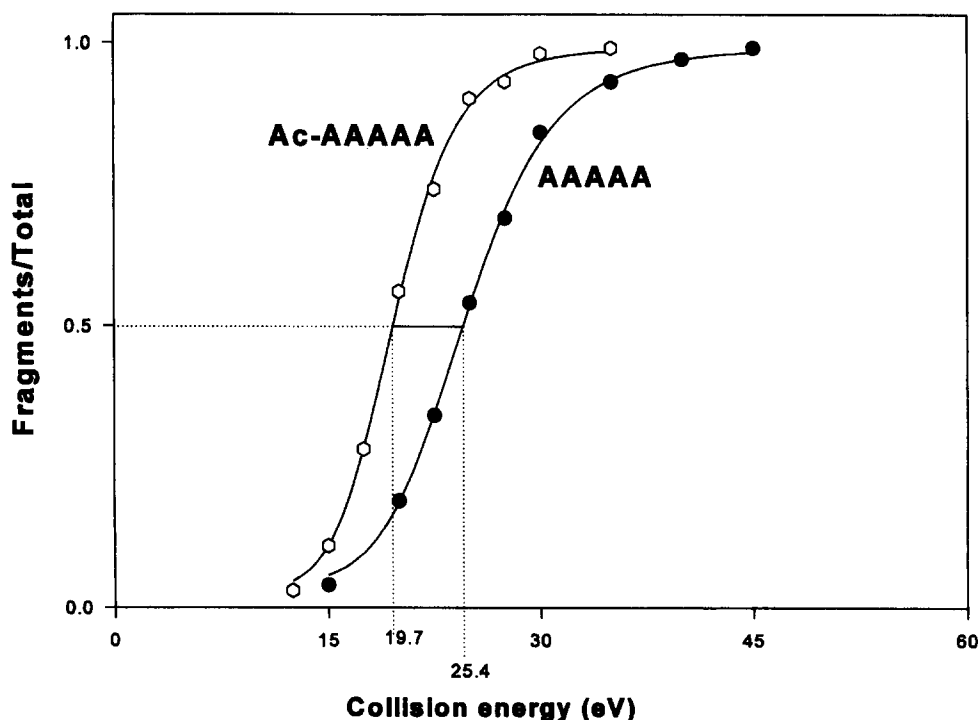


Fig. 1. Fragmentation efficiency curves of $[\text{M} + \text{H}]^+$ ions for AAAAA (●) and N-Ac AAAAA (○) obtained upon collision with an octadecanethiolate SAM surface.

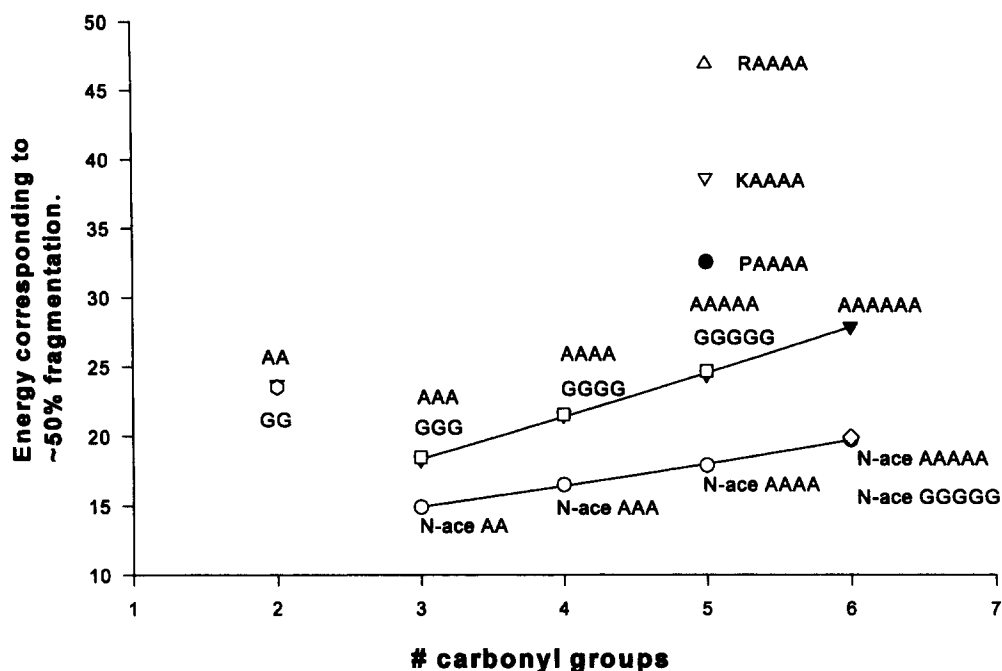


Fig. 2. Plot of collision energy corresponding to ~50% fragmentation of oligopeptides vs. the number of carbonyl oxygens.

~50% fragmentation measured from the type of curves shown in Fig. 1 are presented in Table 1. The results for N-acetyl AAAAA extend previous results for XAAAA, where X = A, P, K and R; the values of the collision energy corresponding to ~50% fragmentation for these peptides are included in Fig. 2 and Table 1 for

comparison. The energies required for ~50% fragmentation of the precursors shown in Table 1 show clearly that for all of the peptides investigated, those with N-acetylated amino terminus require lower energies for fragmentation compared to the peptides with intact amino terminus.

Table 1

Collision energy required to achieve ~50% fragmentation for the peptides examined. Collision energy values for peptides containing basic residues are provided for comparison

Peptide ^a	Energy corresponding to ~50% fragmentation (eV)	
	Intact N-terminus	Acetylated N-terminus
AA	23.7	14.9
AAA	18.3	16.5
AAAA	21.5	17.9
AAAAA	25.4	19.7
GG	23.5	—
GGG	18.4	—
GGGG	21.6	—
GGGGG	25.6	19.9
PAAAA	32.8	—
KAAAA	38.8	—
RAAAA	46.8	—

^a The GB (in kcal/mol) of the amino acids are A (212.4), G (202.4), P (221.9), K (226.0), R (> 233.8) [20].

4. Discussion

For all peptides, it is clear that prior knowledge of the likely location of charge and the mobility of that charge would assist in interpretation of dissociation spectra produced upon activation of the protonated peptide and in the development of a general predictive model for peptide dissociation. It is generally accepted that nonbasic peptides fragment more easily than basic peptides and that rich fragmentation patterns result because charge can be located heterogeneously with only limited energy input, i.e. the fragmentation of peptides is charge-directed, requiring proton localization at the cleavage site [1–5]. This heterogeneous population can be more easily achieved (requires less energy input) for nonbasic peptides, where gas-phase basicity (GB) differences between the most basic (amino terminus) and second most basic sites (amide oxygen) are relatively small, and less easily achieved (greater energy input) if more basic residues are present. One model for the fragmentation of peptides in MS/MS experiments is that the activation process facilitates proton transfer from more basic sites (e.g. basic side chains) to less basic sites (e.g. amide oxygen and nitrogen) and initiates cleavage. *Ab initio* and MNDO bond order calculations [16] have indicated that the amide N-protonated form can cleave readily; such a form is hypothesized as a fragmenting structure and can play an important role in dissociation even if it is not a dominant member of the original ion population prior to activation. The data of Table 1 are consistent with this general model. Although the spectra are not shown, the derivatization mainly influences the energetics of fragmentation and not the type of product ion formed and therefore it is reasonable to assume that the mechanisms of formation of the product ions in both N-acetylated and the intact N-terminal peptides are similar. In both cases, the spectra consist almost entirely of **b**, **a** and **y** type ions. The fact that the SID fragmentation efficiency curves shift

to higher energy with an increase in the basicity of the most basic residue suggests that the basic residue controls some aspect of the fragmentation. It is not a priori obvious that there should be a dependence of fragmentation efficiency on GB because fragmentation is a *kinetic* process and the GB is a *thermodynamic* property. A straightforward explanation for such a relationship, coupled with the fact that the fragmentation patterns are quite similar (for the N-terminal derivatized peptides and those with an intact amino terminus), is that the basic residue sequesters the proton and fragmentation requires intramolecular proton transfer which is dependent on the relative GBs of the various basic sites in the molecule. The fragmentation of peptides with and without an intact amino terminus clearly requires different energy input (Fig. 2).

For diglycine, the difference in the energies between the amino N-protonated form and the O-protonated form (carbonyl oxygen of first amino acid) calculated at the 6-31G* SCF and MP2 6-31G**/6-31G* SCF level is 5.5 kcal/mol (Somogyi et al., unpublished data). This is in general agreement with the number published by Cassady and co-workers for triglycine (5.08 kcal/mol 6-31G**/3-21G) [10]. In the unmodified peptide, protonation can occur in at least two possible ways such as illustrated in Fig. 3(a,b). A proton can either be solvated by the different basic sites such as the amino terminus and the neighboring amide oxygens, or be completely solvated by the amide oxygens of the peptide backbone away from the amino terminus. If the proton is completely solvated by the amide oxygens, then the N-terminal amine would have no significant influence on the protonation and the subsequent fragmentation. N-Acetylation removes the additional GB conferred by the amino terminus by converting the N-terminal amine to an amide bond. This results in a peptide backbone with a number of sites of comparable, lower proton affinities. Fig. 3(c) shows the expected protonation for the N-terminal derivatized peptide in which the proton is

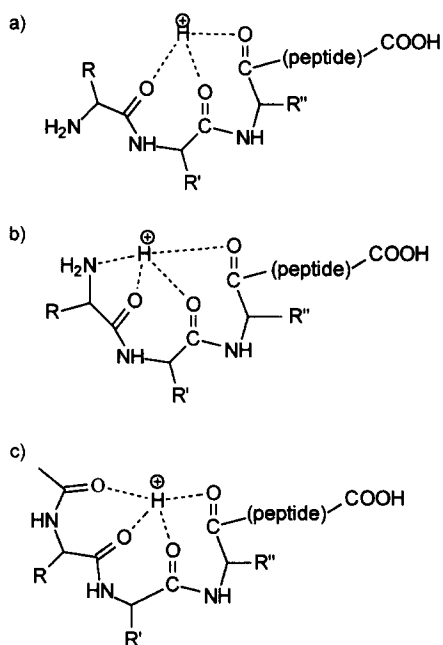


Fig. 3. Cartoon depicting the plausible protonation behaviors for (a) and (b) peptide with intact N-terminus and (c) peptide with derivatized N-terminus.

expected to be solvated by the various amide oxygens.

The N-terminal amine can be involved in the protonation (Fig. 3(b)), either by participating in solvation or by being protonated predominantly at that site. In either case, subsequent charge directed cleavage involving proton transfer would require energy in excess of that required for similar processes in the derivatized peptides that do not contain a N-terminal amine. However, in the cases shown in Fig. 3(a,c), the proton transfer should occur with comparable ease as the N-terminal amine is not expected to be involved in protonation or solvation of the proton. The experimental results clearly show that a difference in the energy requirements exists for the fragmentation of peptides with and without the N-terminal amine. This suggests that the N-terminal amine is involved in the protonation. Thus the protonated structures depicted in Fig. 3(b,c) are consistent with the experimental results while

that shown by Fig. 3(a) is not. A population of protonated peptides in which proton is sequestered at the amino terminus *can* be achieved, with additional intramolecular solvation possible as suggested by Bursey and co-workers [12], and the energy required to fragment these peptides reflects this sequestration.

4.1. Dipeptides

Another notable feature of the data in Fig. 2 is the deviation in the fragmentation behavior of the dipeptides compared to that of the higher members in both the oligoalanine and the oligoglycine series of peptides. As shown in Table 1 and Fig. 2, 50% fragmentation of AA requires higher energy than 50% fragmentation of AAA. (Similar behavior is also shown by GG in comparison with the higher members of the series.) Upon acetylation, AA behaves linearly with the higher members of the series, AAA, AAAA and AAAAA. This anomaly in behavior shown by the dipeptides might be attributed to a strong interaction between the N- and the C-terminus that may be unique to dipeptides. Investigation of GB of a series of dipeptides by Gorman and Amster [17] and Casady and co-workers [18] using bracketing technique has indicated the possibility of strong H-bonding interactions between the amino terminus and the amide oxygen. Many research groups have included dipeptides as models for understanding theoretical and experimental aspects of peptide structure and fragmentation in the gas phase [7–12,16–19]. Whatever the origin of the anomaly in the behavior of dipeptides compared to the peptides with more amino acid residues, our observation indicates that dipeptides may not be good models for studying the structure-fragmentation relationship of larger peptides. For the (Ala)_n and (Gly)_n series of peptides, the dependence of fragmentation on the size of the peptide is the subject of an investigation in progress.

5. Summary

The above results show that additional energy is required for fragmentation of peptides that contain an N-terminal amino group compared with those that do not contain the N-terminal amino group. For nonbasic peptides, the N-terminal amine is involved in sequestering the ionizing proton.

Acknowledgements

This work was funded by a grant from NIH (grant No. RO1GM51387).

References

- [1] (a) D.F. Hunt, J.R. Yates III, J. Shabanowitz, S. Winston, C.R. Hauer, *Proc. Natl. Acad. Sci. USA* 86 (1986) 6233; (b) R.S. Johnson, S.A. Martin, K. Biemann, *Int. J. Mass Spectrom. Ion Proc.* 86 (1988) 137.
- [2] K.D. Ballard, S.J. Gaskell, *Int. J. Mass Spectrom. Ion Proc.* 111 (1991) 173.
- [3] X.-J. Tang, P. Thibault, R.K. Boyd, *Anal. Chem.* 65 (1993) 2824.
- [4] D. Fabris, M. Kelly, C. Murphy, Z. Wu, C. Fenselau, *J. Am. Soc. Mass Spectrom.* 4 (1993) 652.
- [5] (a) J.L. Jones, A.R. Dongré, Á. Somogyi, V.H. Wysocki, *J. Am. Chem. Soc.* 116 (1994) 8368; (b) A.R. Dongré, J.L. Jones, Á. Somogyi, V.H. Wysocki, *J. Am. Chem. Soc.* 118 (1996) 8365.
- [6] T. Wytenbach, G.V. Heldon, M.T. Bowers, *J. Am. Chem. Soc.* 118 (1996) 8355.
- [7] (a) J. Wu, C. Fenselau, *Int. J. Mass Spectrom. Ion Proc.* 111 (1991) 173. (b) Z. Wu, C. Fenselau, *Tetrahedron* 49 (1993) 9197.
- [8] J. Wu, E. Gard, J. Bregar, M.K. Green, C.B. Lebrilla, *J. Am. Chem. Soc.* 117 (1995) 9900.
- [9] K. Zang, D.M. Zimmerman, A. Chung-Philips, C.J. Cassady, *J. Am. Chem. Soc.* 115 (1993) 10812.
- [10] K. Zang, C.J. Cassady, A. Chung-Philips, *J. Am. Chem. Soc.* 116 (1994) 11512.
- [11] (a) S. Campbell, M.T. Rodgers, E.M. Marzluff, J.L. Beachamp, *J. Am. Chem. Soc.* 116 (1994) 9765; (b) S. Campbell, M.T. Rodgers, E.M. Marzluff, J.L. Beachamp, *J. Am. Chem. Soc.* 117 (1995) 12840.
- [12] (a) D.G. Morgan, M.M. Bursey, *Biol. Mass Spectrom.* 22 (1993) 502; (b) R.W. Yeh, J.M. Grimley, M.M. Bursey, *Biol. Mass Spectrom.* 20 (1991) 443.
- [13] V.H. Wysocki, J.-M. Ding, J.L. Jones, J.H. Callahan, F.L. King, *J. Am. Soc. Mass Spectrom.* 3 (1992) 27.
- [14] Á. Somogyi, T.E. Kane, J.-M. Ding, V.H. Wysocki, *J. Am. Chem. Soc.* 115 (1993) 5275.
- [15] S.K. Chowdhury, V. Katta, B.T. Chait, *Rapid Commun. Mass Spectrom.* 4 (1990) 81.
- [16] Á. Somogyi, V.H. Wysocki, I. Mayer, *J. Mass Spectrom.* 8 (1994) 704.
- [17] G.S. Gorman, I.J. Amster, *J. Am. Chem. Soc.* 115 (1993) 5729.
- [18] C.J. Cassady, S.R. Carr, K. Zhang, A.C. Philips, *J. Org. Chem.* 60 (1995) 1704.
- [19] M.M. Cordero, J.J. Houser, C. Wesdemiotis, *Anal. Chem.* 65 (1993) 1594.
- [20] G.S. Gorman, J.P. Spier, C.A. Turner, I.J. Amster, *J. Am. Chem. Soc.* 114 (1992) 3986.