International Journal of Mass Spectrometry 278 (2008) 122–128

Contents lists available at ScienceDirect

International Journal of Mass Spectrometry

journal homepage: www.elsevier.com/locate/ijms

Influence of salt bridge interactions on the gas-phase stability of DNA/peptide complexes

Sandra Alves[a], Amina Woods[b], Alice Delvolvé[a], Jean Claude Tabet[a,*]

[a] Laboratoire de Chimie Structurale Organique et Biologique, UMR 7613? BP45, Université Pierre et Marie Curie, 4 Place Jussieu, Paris 75252, France
[b] NIDA IRP, NIH, 333 Cassell Drive, Baltimore, MD 21224, USA

ARTICLE INFO

Article history:
Received 19 January 2008
Received in revised form 25 April 2008
Accepted 28 April 2008
Available online 3 May 2008

Keywords:
Noncovalent DNA–Peptide complex
Salt bridge interaction
ESI mass spectrometry
Orbitrap

ABSTRACT

Negative ion mode electrospray ionization mass spectrometry was used to study DNA duplexes–peptide interaction. In the present study, we show that peptides that contain two adjacent basic residues interact noncovalently with DNA single strand or duplex. Fragmentation of the complexes between peptides containing basic residues and DNA were studied under collisions and showed unexpected dissociation pathways, as previously reported for peptide–peptide interactions. The binary complexes are dissociated either along fragmentation of the covalent bonds of the peptide backbone and/or along the single DNA strand backbone cleavage without disruption of noncovalent interaction, which demonstrates the strong binding of peptide to the DNA strand. Sequential MS/MS and MS^n were further performed on ternary complexes formed between duplexes and peptides to investigate the nature of interaction. The CID spectra showed as major pathway the disruption of the noncovalent interactions and the formation of binary complexes and single-strand ions, directed by the nucleic acid gas-phase acidity. Indeed, a preferential formation of complexes with thymidine containing single strands is observed. An alternative pathway is also detected, in which complexes are dissociated along the covalent bond of the peptide and/or DNA according to the basicity. Our experimental data suggest the presence of strong salt bridge interactions between DNA and peptides containing basic residues.

1. Introduction

DNA's functions depend on interactions with proteins [1]. Crucial biological processes such as DNA replication, restoration, compaction, transcription, and degradation are all regulated by proteins capable of recognizing, and thereby complexing with the DNA double helix. These protein interactions can be non-specific, or the protein can bind specifically to a single DNA sequence. The most intensively studied of these are the various classes of transcription factor proteins, which regulate transcription. To study such noncovalent macromolecules requires characterization of the biochemical and structural knowledge of each partner, and hence the use of appropriate experiments. Spectroscopic techniques commonly used are UV/vis absorbance, fluorescence [2], circular dichroism [3] as well as biological assays such as DNAse I digestion or various activity tests [4]

Mass spectrometric methodology was developed for the study of noncovalent complexes [5,6] using mostly Electrospray ionization (ESI) [7]. Indeed, the soft desorption/ionization feature of electrospray allows the weakly bound noncovalent complexes formed in solution to be transferred into gas phase after soft desolvation conditions and detected by mass spectrometry. Few examples demonstrated that MALDI [8] ionization is indeed an alternative technique within optimized experimental conditions [9,10] as the use of a less acidic matrix. ATT as matrix was used for the detection of specific DNA–peptide complexes [11–14] ESI–MS approach allows probing the molecular weight and consequently complex stoechiometry [11–16] Analytical approaches were also developed to determine binding constants [17] taking into account the response factors of ESI. By comparing the results obtained by ESI–MS and other spectroscopic methods, we ensure that the complexes detected correspond with those present in solution [18]. Some examples showed affinity constant relative values in good agreement with conventional solution-phase methods [17,19]. In addition, tandem mass spectrometry (MS/MS), a widely available technique, can be used for the characterization of noncovalent interactions; the method can provide reproducible relative gas-phase stabilities. The binding mode and details on other aspects of binding can be provided by investigating the product-ion spectra of the **multideprotonated noncovalent complexes in the gas phase [20–22].

ESI–MS/MS of DNA double helices is expected to separate into single strands, with collision energy proportional to their stability. In fact, this fragmentation channel is only dominant with high-energy collisions [20]. Under low-energy collision conditions [23]

* Corresponding author. Tel.: +33 1 44 27 32 63; fax: +33 1 44 27 38 43.
E-mail address: tabet@ccr.jussieu.fr (J.C. Tabet).

1387-3806/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijms.2008.04.023

(in an ion trap), covalent fragmentation are detected, involving loss of neutral (or charged) base and eventually, by oligonucleotide backbone cleavage (e.g., $(a_i - B)$ and w_i product ions). Furthermore, the extent of covalent cleavage is dependent upon the activation voltage and resonant excitation duration [20,23] and also on both the duplex size and charge state [23] Nevertheless, reliable gas-phase experiments have been correlated to solution behavior, leading authors to assume a preservation (at least partially) of helix conformation [20,21,24,25].

Similarly, product-ion spectra were used to characterize complexes with drugs. Drug molecules typically bind DNA either via minor groove or between base pairs (intercalation). Both covalent and noncovalent dissociation pathways have been reported for various DNA duplex/drug complexes, and general correlations have been drawn between the predominant fragmentation pathway and the mode of drug binding to the duplex [16,22,23,26]. However, the dissociation pathways exhibited by both the duplexes and the drug/duplex complexes were found to be markedly sensitive to charge state [23] and the excitation conditions [20,27,28]. Brodbelt and coworkers [23] suggested that dissociation pathways are probably more reflective of specific drug–DNA interactions for the lower charge state complexes. At higher charge state, the major pathway is the strand separation, which is explained by high coulombic repulsion [27] and/or partial unzipping process during ionization event.

In our study, we show that ESI–MS allows the observation of noncovalent complexes between basic peptide and nonself complementary duplex. Sequential MS^n measurements with the LTQ-Orbitrap instrument were used to explore the noncovalent interactions involved in DNA complex formation. Different types of interactions are able to stabilize DNA complexes in solution [18,29] including hydrogen bonds, van der Waals, hydrophobic and electrostatic interactions. Non-specific DNA–protein interactions in solution such as histone–DNA complexes are formed through basic residues making ionic bonds with the acidic sugar-phosphate backbone of the DNA [1,18,29]. There is no consensus on the quantitative contribution of these interactions in the gas phase. Some gas-phase studies of peptide–DNA or duplex interactions have been undertaken [11–14,30–32]. It is generally assumed during ESI processes that proton transfer leading to neutralization of negatively charged phosphate group [27,31]. Vertes and coworkers [30] first suggested ionic interactions in DNA–peptide complexes. Similarly, Woods et al. highlighted the role of electrostatic interactions between peptides containing several basic residues (R or K) and peptides containing acidic [33,34] or phosphorylated residues [34,35–38] and also with the phosphate groups in DNA strands [11,12] Our group previously reported the detection of noncovalent complexes between highly acidic oligonucleotides and highly basic PNA molecules using ESI mass spectrometry and assumed the existence of zwitterion (ZW) [39]. In the previous study [32], the dissociation pathways of single-stranded DNA–peptide complexes were investigated and suggested the presence of strong salt bridge interactions between DNA and peptide containing adjacent basic residues. The presence of salt bridge interaction implies the existence of zwitterionic forms of DNA–peptide complex. In the present work, sequential MS^n experiments were done on different DNA–peptide systems to investigate the existence of salt bridge bonds in noncovalent systems involving highly basic residues [30–32,36,37].

2. Experimental

2.1. Peptide and DNA sample preparation

Peptides such as PPGFSPFRR and PPGFSPFKK (noted as -**RR** and -**KK**) were purchased from Bachem and dissolved in milli-Q water to a stock concentration of 1 mM (stock solution at 1 mg/mL). Single-stranded oligodeoxynucleotides dodecamers were purchased from DNAtechnology (Denmark). It consists of nonself-complementary homo-oligonucleotides A_{12}, T_{12} (as ds duplex) and hetero oligonucleotides AAA AAC CAA AAA with TTT TTG GTT TTT (as dsB duplex). Oligonucleotide solutions were prepared in 1 M CH_3COONH_4 without further purification, and annealed at 80 °C and slowly cooled to room temperature to allow the duplex formation. Duplex solutions were mixed in equimolar proportions with peptides and then diluted to obtain a final concentration of 5 μM in 50/50 (v/v) CH_3OH/100 mM CH_3COONH_4 for mass spectrometry.

2.2. Mass spectrometric experiments

Experiments were performed using an electrospray source in negative ion mode combined with a LTQ-Orbitrap hybrid instrument (ThermoFisher Corporation, San Jose, USA). The proof-of-principle of the Orbitrap was first described by Makarov and coworkers [40] and is not discussed here. Recently the LTQ-Orbitrap hybrid instrument was developed by ThermoFisher Corporation [41] Briefly; the front part is a Linear Ion Trap [42] (LTQ) capable of detecting mass spectra or MS^n product-ion spectra with low resolution. Ions can be further released into a curved C-trap (an RF only quadrupole), which accumulates and stores the ions and they are consequently transferred and analyzed into the Orbitrap analyzer, an electrostatic ion trap, which demonstrated a high resolving power and mass accuracy measurements [43].

General ionization conditions were as follows: an accelerating voltage of 3.4 kV, a sheath gas flow of 25 (ua), no auxiliary gas, an ion transfer tube temperature 250 °C and the tube lens voltage of 180 V. Mass spectra were acquired in the Orbitrap analyser with a resolution of $R = 60{,}000$ (at 400 Th), after accumulation to a target value of 10^5 and a mass range from m/z 300 Th to m/z 2000 Th. For the CID spectra, the target complex ions were isolated for fragmentation in the linear ion trap and activated using collisionally induced dissociation under the following conditions: a variable normalized collision energy [44] (in %), a default activation $q = 0.250$ and a fixed activation time of 1.5 ms. The resulting fragment ions were recorded in the linear Ion Trap (after radial ejection) or eventually in the Orbitrap. In the latter case, the product ions were recorded with high resolution and mass accuracy, so that the isotopic ion profile makes it easy to identify the charge state. All data were acquired using external calibration with a mixture of caffeine, MRFA peptide and Ultramark 1600 dissolved in 50/50 (V:V) water/acetonitrile solution.

3. Results

Choice of PPGFSPFRR and PPGFSPFKK peptides (i.e., -**RR** and -**KK**) was motivated by previous results, which show a strong interaction between peptides containing adjacent basic residues and single-stranded DNA [30–32] or with peptide containing acidic residues or phosphorylated groups [33–38]. Our previous experiments [32] were performed on small 8-mer homo-oligonucleotide with bradykinin and peptide derivates (as -**RR** and -**KK**) and showed a peptidic backbone cleavage due to the proline effect (PP motif loss) from DNA–peptide complexes. In order to explore the possibility of observing noncovalent interactions between duplexes and basic peptides, mixtures of equal amounts of A_{12}/T_{12} duplex and -**RR** peptide were analyzed by ESI mass spectrometry in negative ion mode (Fig. 1). In Fig. 1 the negative ion mass spectrum shows the formation of the following ions, peptide [-**RR**–H]$^-$ (m/z 1058.5) and the major multideprotonated single-strand T_{12} and A_{12}. Formation of multideprotonated binary complexes consisting

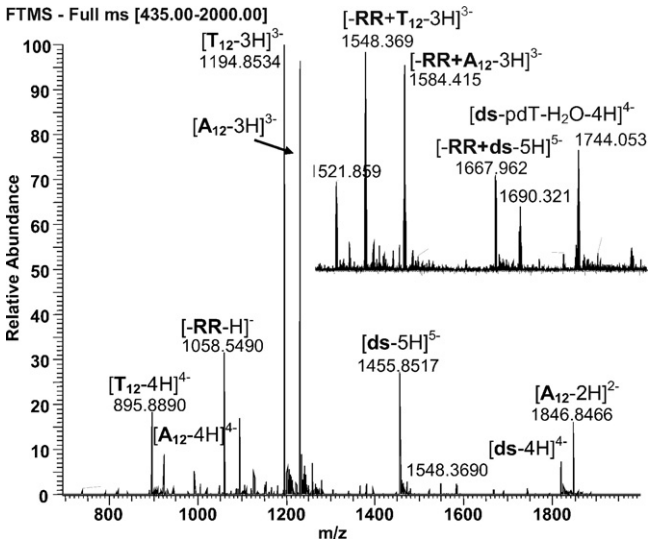


Fig. 1. Negative ESI mass spectrum of A_{12}/T_{12} duplex and -**RR** peptide equimolar mixture in CH_3COONH_4 aqueous buffer (100 mM) with 50% of methanol (using the FT-Orbitrap mode detection).

of single strand and peptide as [-**RR** + T_{12}–3H]$^{3-}$ (*m*/*z* 1548.4), [-**RR** + A_{12}–3H]$^{3-}$ (*m*/*z* 1584.4) and duplex anions [**ds**–5H]$^{5-}$, [**ds**–4H]$^{4-}$ species at *m*/*z* 1455.4 and 1820.1, respectively were also observed. We detected the formation of A_{12}/T_{12} (as **ds**) duplex complex with the -**RR** peptide at *m*/*z* 1667.9 with a preferential 1:1 stoechiometry (i.e., [-**RR** + **ds**–5H]$^{5-}$ ions) and another species at *m*/*z* 1744.5 corresponding to a fragment of quadrupled deprotonated [**ds**–4H]$^{4-}$ complexes produced by the release of thymidine molecules. Similarly, the formation of DNA–peptide complexes were also investigated for the PPGFSPFKK (noted as -**KK**) peptide with A_{12}/T_{12} duplex and for the $T_5G_2T_5/A_5C_2A_5$ duplex with both the -**RR** and -**KK** peptides. In all experiments, ESI mass spectra show the formation of binary complexes of single strand with peptide and complexes of duplex with peptide (with a stoechiometry of 1:1) (data not reported herein).

The behavior of the DNA–peptide complexes is further studied using tandem mass spectrometry, to investigate the gas-phase stability of binary and ternary complexes. The study of their respective dissociations induced by low-energy collisions was performed using hybride LTQ-Orbitrap instrument. First, CID spectra of triply deprotonated binary complexes of the -**RR** peptide with T_{12}, A_{12}, $T_5G_2T_5$ and A_5C_2A5 single-stranded DNAs are reported in Fig. 2. All triply deprotonated complexes dissociate by peptidic covalent bond cleavage through the loss of PP motif from the N-terminus [32]. Product ions from oligonucleotide backbone cleavage [31,32] without disruption of the noncovalent interaction are also observed according to the DNA sequence (peaks of noncovalent product ions labelled by an open circle ○ in Fig. 2). For example, CID spectra of the **ssDNA**/-**RR** complexes composed of A_{12}, $T_5G_2T_5$ and $A_5C_2A_5$ display noncovalent product ions in spite of adenine (as Ade) or guanine (as Gua) loss (i.e., at *m*/*z* 1539, *m*/*z* 1514.3 and *m*/*z* 1523.2, in Fig. 2b–d, respectively). Moreover, noncovalent bond cleavage yielding single strand and peptide ions are not observed. This behavior demonstrates the strong interaction between these single strands and peptide containing adjacent Arg residues.

In Fig. 2 the influence of the DNA sequence is demonstrated by the presence of various noncovalent product ions: the Ade loss for the $A_5C_2A_5$ hetero oligonucleotide is specifically detected, similarly to the A_{12} strand and the Gua loss for $T_5G_2T_5$. This trend is attributed to the relatively higher basicity of guanine and adenine nucleic base. [45–48] the base nature influence on DNA single-strand cleavage pathways was previously demonstrated [21]. However, previous studies [49,21] showed that the base loss pathway is not just related to the nucleic base nature but also to its position in the sequence. DNA covalent bond cleavage of the binary [-**RR** + $T_5G_2T_5$–3H]$^{3-}$ complexes (*m*/*z* 1563.7) is directed by the basic guanine: all single-stranded and noncovalent product ions originate from the Gua loss. Indeed, the complementary (a_6-gua)/w_6 and (a_7-gua)/w_5 product-ion pairs are observed. For example, [-**RR** + (a_7-Gua)–2H]$^{2-}$ (*m*/*z* 1502.9) and [-**RR** + w_6–2H]$^{2-}$ (*m*/*z* 1462.3) are produced with the

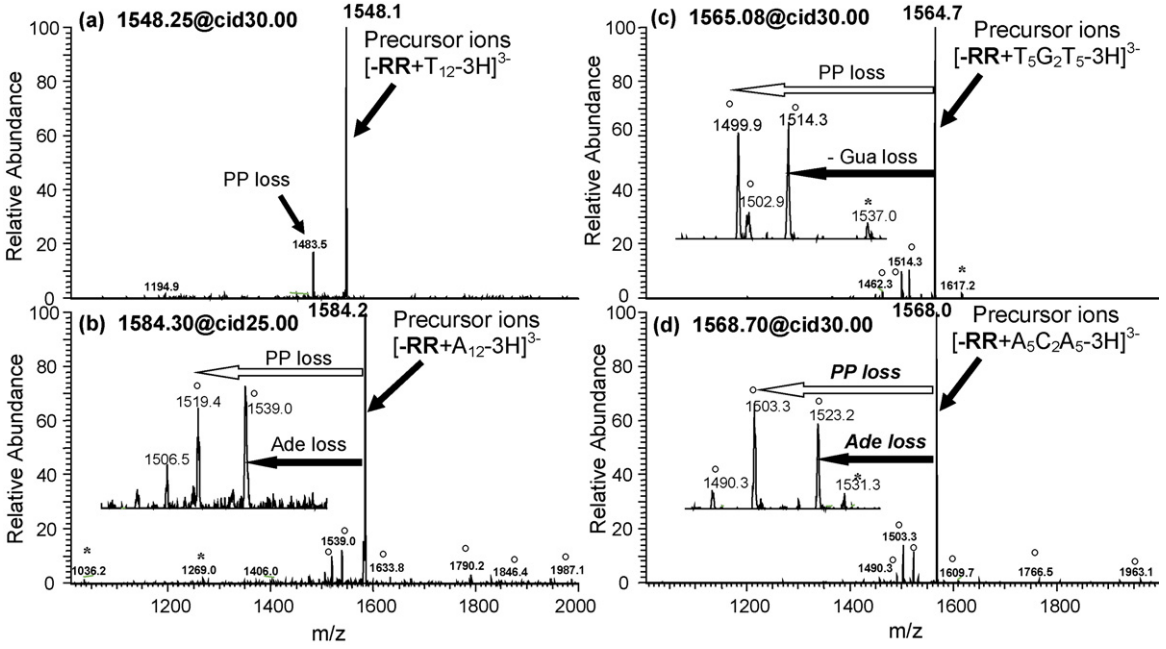


Fig. 2. CID spectra of various deprotonated binary complexes: (a) [-**RR** + T_{12}–3H]$^{3-}$ (*m*/*z* 1548.2), (b) [-**RR** + A_{12}–3H]$^{3-}$ (*m*/*z* 1584.3), (c) (-**RR** + $T_5G_2T_5$–3H)$^{3-}$ (*m*/*z* 1565.1), and (d) [-**RR** + $A_5C_2A_5$–3H]$^{3-}$ (*m*/*z* 1580.7) activated and analyzed with the LTQ analyser with 30% of normalized collision energy (excepted for (b)), a precursor ion isolation window of 2 Th (the labelled peaks noted as * and ○ correspond to single-stranded and noncovalent product ions, respectively).

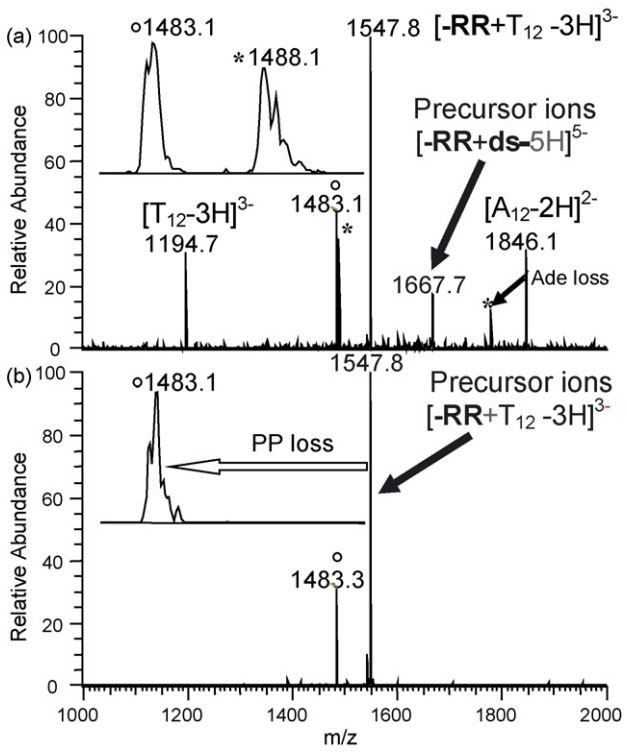


Fig. 3. (a) CID spectrum of the [-**RR** + **ds**–3H]$^{5-}$ (*m*/*z* 1667.7) duplex-peptide complex, and (b) sequential MS^3 experiment of the binary [-**RR** + T_{12}–3H]$^{3-}$ complex (*m*/*z* 1548.3) activated and analyzed in the LTQ analyser with: (a) 25% of normalized collision energy, a precursor ion isolation window of 2 Th, and (b) 25% of normalized collision energy for both the precursor ions (the labelled peaks noted as * and ○ correspond to the single-strand oligonucleotide and noncovalent product ions, respectively).

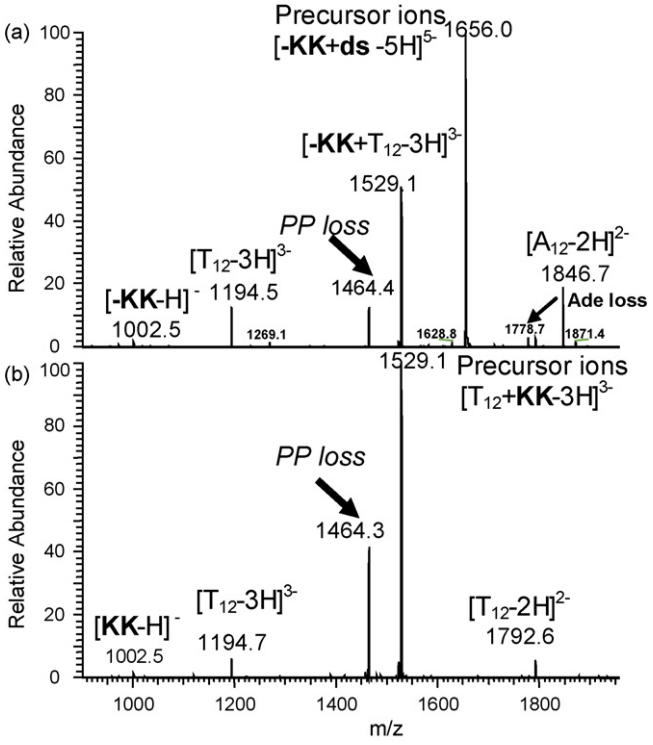


Fig. 4. (a) CID spectrum of [-**KK** + **ds**–3H]$^{5-}$ (*m*/*z* 1656.0) duplex-peptide complex and (b) sequential MS^3 experiment of the binary [-**KK** + T_{12}–3H]$^{3-}$ (*m*/*z* 1529.1) complex activated and analyzed in the LTQ analyser with: (a) 25% of normalized collision energy, a precursor ion isolation window of 2 Th and (b) 25% of normalized collision energy for both the precursor ions (the labelled peaks noted as * and ○ correspond to the single-strand oligonucleotide and noncovalent product ions, respectively).

complementary $(w_5–H)^-/(a_6\text{-}B)^-$ (*m*/*z* 1537.0 and *m*/*z* 1617.2, Fig. 2c). We also observed at higher excitation conditions, noncovalent product ions formed between DNA and the peptide $\mathbf{y_7}$ product ions (*via* PP loss) such as *m*/*z* 1365.7 and *m*/*z* 1405.3 corresponding to $[(a_7\text{-Gua}) + \mathbf{y_7}–2H]^{2-}$ and $[w_{6+} + \mathbf{y_7}–2H]^{2-}$, respectively.

In another approach, gas-phase stability of ternary complexes consisting of DNA duplex and basic peptide is investigated. From mixture of the A_{12}/T_{12} duplex (noted as **ds**) and the -**RR** peptide, CID spectrum of the quintuply deprotonated (-**RR** + **ds**–5H)$^{5-}$ duplex-peptide complex (*m*/*z* 1667.7) is performed by sequential MS/MS and MS^3 experiments (Fig. 3). Under the same CID conditions, the complex dissociates mainly through disruption of the noncovalent interaction generating the binary (-**RR** + T_{12}–3H)$^{3-}$ complex as base peak and the single strand $(A_{12}–2H)^{2-}$ and $(T_{12}–3H)^{3-}$ anions, whereas the binary (-**RR** + A_{12}–*n*H)$^{n-}$ complex product ions are not detected. Furthermore, two additional product ions were detected at *m*/*z* 1483.1 and *m*/*z* 1488.1. The former is a triply deprotonated specie produced by covalent peptidic bond cleavage of the binary [-**RR** + T_{12}–3H]$^{3-}$ complex by loss of a 194 u neutral (PP peptide loss as $(b_2–H)$ neutral) without disrupting the noncovalent interaction, yielding to the production of the $[\mathbf{y_7} + T_{12}–3H]^{3-}$ noncovalent product ions. The latter *m*/*z* 1488.1 product ion is a doubly charged species, which should correspond to unexpected abundant single-strand DNA y_{10}^{2-} product ions from the T_{12} backbone cleavage. Weak abundant T_{12} and A_{12} single-strand product ions obtained by DNA backbone consecutive dissociations are also observed such as the *m*/*z* 1776.7 product ion due to the Ade loss from the single strand $(A_{12}–2H)^{2-}$ (corresponding peaks labelled by * in Fig. 3).

In Fig. 3, the preferential observation of binary [-**RR** + T_{12}–3H]$^{3-}$ complex product ions as base peak and the concomitant production of the complementary single-strand $(A_{12}–2H)^{2-}$ product species originate from noncovalent disruption of the selected complex (Fig. 3a). However, the origin of $[T_{12}–3H]^{3-}$ (*m*/*z* 1194.7) is unclear as it can be produced either from the [-**RR** + **ds**–5H]$^{5-}$ complex by production of complementary not detected [-**RR** + A_{12}–2H]$^{2-}$ product ions or from consecutive dissociations of binary [-**RR** + T_{12}–3H]$^{3-}$ complex anions by loss of neutral -**RR** peptide or consecutive noncovalent bond disruption from A_{12}/T_{12} duplexes [38], which are not detected in the MS/MS experiments. To gain a better understanding of fragmentation pathways of the ternary complex, the multideprotonated [-**RR** + T_{12}–3H]$^{3-}$ binary complex was further fragmented in sequential MS^3 experiments (Fig. 3b) and shows as the only pathway, the peptide covalent bond cleavage yielding the PP peptide loss (*m*/*z* 1483.1). Above all, single-strand T_{12} and/or -**RR** product ions were not observed, which highlights the strong binding mode existing between the basic peptide to the T_{12} single strand.

Similar CID experiments were done on the A_{12}/T_{12} (noted as **ds**) complexes with the -**KK** peptide and are reported in Fig. 4. CID spectrum of [-**KK** + **ds**–5H]$^{5-}$ displays a similar fragmentation pathways to the previously studied complexes, as we detected the preferential formation of the binary [-**KK** + T_{12}–3H]$^{3-}$ complex and the single-stranded $[T_{12}–3H]^{3-}$ (*m*/*z* 1194.5) and $[A_{12}–2H]^{2-}$ (*m*/*z* 1846.7) product ions through noncovalent bond disruption process. The sequential MS^3 experiments of [-**KK** + T_{12}–3H]$^{3-}$ (*m*/*z* 1528.1) still show a major covalent bond fragmentation pathway (through PP loss from peptide backbone) but also to a lesser extent noncovalent interaction disruption and the formation of T_{12} anions by loss of deprotonated or neutral peptide. The observation to a greater

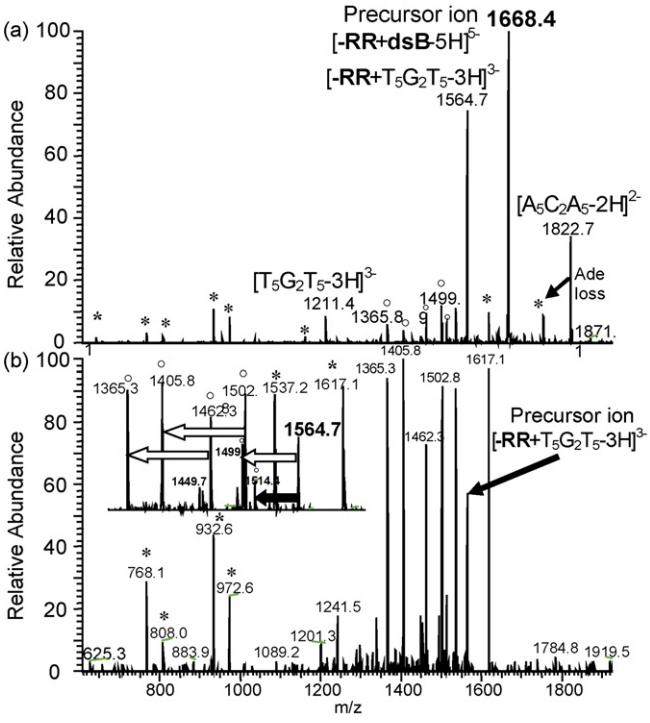


Fig. 5. (a) CID spectrum of [-**RR** + **dsB**–3H]$^{5-}$ (*m*/*z* 1668.4) duplex-peptide complex, and (b) sequential MS^3 experiment of the binary [-**RR** + $T_5G_2T_5$–3H]$^{3-}$ (*m*/*z* 1564.7) complex activated and analyzed in the LTQ analyser with: (a) 25% of normalized collision energy, a precursor ion isolation window of 2 Th, and (b) 30% and 35% of normalized collision energy for the *m*/*z* 1668.4 and *m*/*z* 1564.9 precursor ions, respectively (the labelled peaks noted as * and ○ correspond to the single-strand oligonucleotide and noncovalent product ions, respectively).

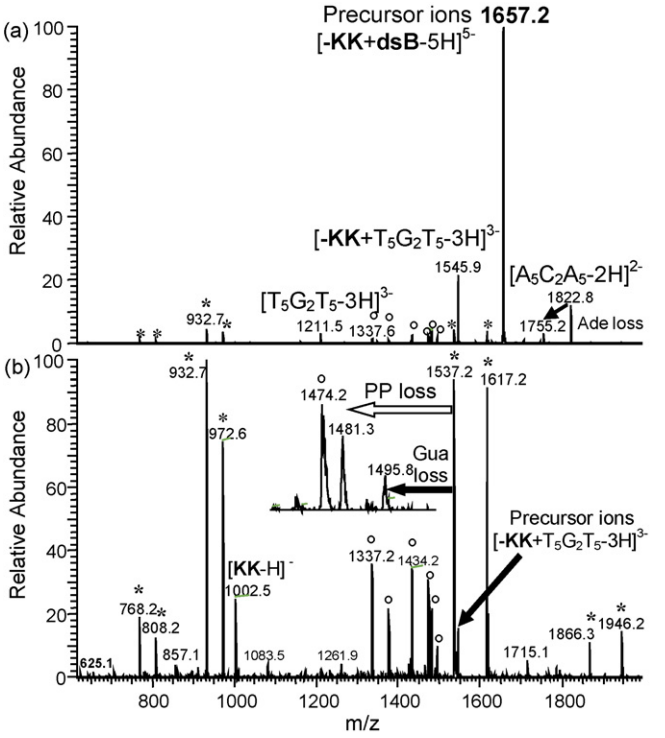


Fig. 6. (a) CID spectrum of [-**KK** + **dsB**–3H]$^{5-}$ (*m*/*z* 1657.2) duplex-peptide complex, and (b) sequential MS^3 experiment of the binary [-**KK** + $T_5G_2T_5$–3H]$^{3-}$ (*m*/*z* 1564.7) complex activated and analyzed in the LTQ instrument with: (a) 25% of normalized collision energy, a precursor ion isolation window of 2 Th and (b) 30% and 35%of normalized collision energy for the *m*/*z* 1657.2 and *m*/*z* 1564.9 precursor ions, respectively (the labelled peaks noted as * and ○ correspond to the single-strand oligonucleotide and noncovalent product ions, respectively).

extent of noncovalent cleavage with -**KK** peptide in comparison with the DNA/-**RR** peptide complexes suggests a weaker DNA/-**KK** peptide interaction. These results make sense since the side chain of Arg ends in a guanidinium group with a triple resonance stabilization [18,35], while Lys has only an amine (NH_2) group. However, the interaction was stable enough to observe preferential covalent bond cleavage [32].

In Fig. 5, another duplex consisting of TTT TTG GTT TTT and AAA AAC CAA AAA single strands (noted as **dsB**) was also investigated using tandem mass spectrometry. The introduction of G/C base pairs into the duplex is expected to strengthen its stability [21]. Moreover, the introduction of guanosine unit, which displays an amphoteric character (highly basic nucleic base and highly acidity of guanosine nucleotide) should greatly influence the ternary complex fragmentation pathways [45–48].

The CID spectrum of [-**RR** + **dsB**–5H]$^{5-}$ (Fig. 5a) displays the noncovalent disruption with production of preferential binary [-**RR** + $T_5G_2T_5$–3H]$^{3-}$ complexes and both the single strand [$T_5G_2T_5$–3H]$^{3-}$ and [$A_5C_2A_5$–2H]$^{2-}$ product ions. In addition, noncovalent product ions [e.g., (w_i + -**RR**) and (($a_i - B$) + -**RR**) ions] and single strand w_i and ($a_i - B$) product-ion pairs were detected (peaks labelled by ◦ and *, respectively in Fig. 5a and b). Most of the product ions came from consecutive dissociations of the binary complexes as demonstrated from the [-**RR** + $T_5G_2T_5$–3H]$^{3-}$ sequential MS^3 experiments. The provided CID spectrum displays noncovalent product ions, which correspond to the peptide and/or DNA bond cleavages at *m*/*z* 1499.9 (due to PP peptide loss) and *m*/*z* 1514.2 (related to Gua loss), respectively. They are followed by DNA backbone dissociation yielding [-**RR** + w_6–2H]$^{2-}$ and [-**RR** + (a_7-Gua)–2H]$^{2-}$ and complementary single-stranded (a_6-Gua)$^-$ and w_6^- product ions.

In Fig. 6, the complexes formed between dsB duplex and -KK peptide were also studied to investigate the influence of the nature of the peptidic residue. The sequential MS/MS and MS^3 experiments display the same reactivity as previously established (see Fig. 6). Nevertheless, a greater amount of single-stranded product ions was observed, demonstrating again a weaker K residue-DNA interaction relatively to that of R residue with DNA (Fig. 6).

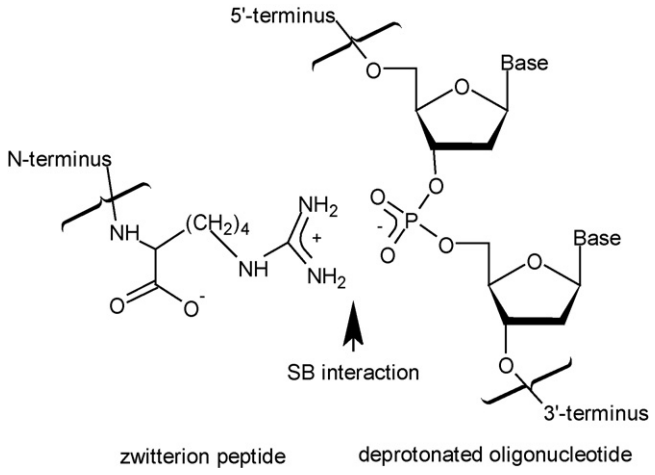
4. Discussion

The CID spectra of DNA-basic peptides complexes (**dsT_xA_y**) displayed, as expected, a major fragmentation pathway by noncovalent bond cleavage according to the following relation:

$$[\boldsymbol{ds}\mathbf{T_xA_y} + \text{peptide} - 5\text{H}]^{5-} \rightarrow [\mathbf{ssT_x} + \text{peptide} - 3\text{H}]^{3-} + \mathbf{ssA_y^{2-}}$$

However, a particular orientation of the fragmentation is observed as preferential multideprotonated binary complexes with thymidine containing single strands[21] (noted as **ssT_x**) and the release of adenosine single-strand ions are detected. In addition, the binary complex between single-strand **ssT_x** and peptide dissociates mainly by covalent bond cleavage (e.g., by loss of PP peptide motif), which demonstrates the strong binding of both the -**RR** or -**KK** peptide to DNA single strand.

To explain the noncovalent interaction of peptide to preferential T_{12}, single strand, electrostatic and/or hydrogen bonding are suggested. Their stability should be determined by the gas-phase relative acidity of nucleotide and amino acid [45,50] (e.g., higher acidity of thymine nucleotide relative to that of adenosine). Nevertheless, the duplex ion formation by noncovalent bond dissociation and complementary loss of deprotonated or neutral peptide is not



Scheme 1. Cartoon showing the salt bridge interactions between the guanidinium group of arginine and the phosphate group of the oligonucleotide strand.

observed, although the multiple hydrogen bonds and eventually, weak base stacking interaction which are generally assumed to be involved in the DNA helix [20,24,27] An alternative model of DNA helix structure has been proposed from studies of DNA complexes with PNA [39] or drug molecules [51] based on the formation of DNA zwitterionic forms allowing stabilization of the DNA helix and their complexes by the presence of stronger ion dipole interactions as compared to the weaker strength characterizing the dipole-dipole interactions.

Nevertheless, the ternary DNA–peptide complex is strongly stabilized through the noncovalent interactions between DNA and peptide. A reasonable explanation is that the noncovalent interaction detected by ESI–MS is an ionic interaction between the negatively charged sugar-phosphate backbone of DNA and the positively charged side chains of R or K rich peptides (see Scheme 1) [12,30,32]. Such ionic interactions are involved in the formation of DNA–peptide complexes in solutions. It is found in both non-specific binding and specific DNA complexes, where ordered structures that lead to selective recognition and binding of specific DNA sequences. In these specific systems, interactions can be base specific when mediated by H-bonds with the amide side chain of the asparagine or glutamine residues, but are sequence independent where ionic interactions bridges the DNA phosphate backbone and the cationic side chains of Arg, Lys and His residues.

Existence of gas-phase salt bridge interactions and consequently of zwitterionic forms has been assumed to be at the base of the stability of various noncovalent macromolecules [39,51], in particular those involving peptides [36,52–56]. Most of the studies investigated the increased stability of amino acid zwitterions by an external factor such as the addition of solvent molecules [57] and/or presence of counterions [58,59]. ZW forms are also favoured by cluster formation [60,61]. Within the assumption of salt bridge interactions involving deprotontated phosphate group of DNA and protonated amino acid side chain, as has occurred for the following systems

$$\{\mathbf{R}-\mathbf{PO_3}-, {}^{+}\mathbf{H_3N}-\mathbf{R}\} \text{ and } \{\mathbf{R}-\mathbf{PO_3}-, {}^{+}\mathbf{H_2N(NH_2)C}-\mathbf{NH}-\mathbf{R}\}$$

for the -**KK** and -**RR** peptides, respectively, the dependence of fragmentation pathways upon the DNA and peptide gas-phase acidity and proton affinity can be explained. The stabilization of salt bridged structures is dependent on the gas-phase basicity of the proton donating group and the gas-phase acidity of the proton acceptor groups involved [62–64] The lower proton affinity of the K residue [47] compared to the R residue explains the weaker DNA–peptide interaction in -**KK** containing complexes and the larger extent of noncovalent bond cleavage (see Fig. 3 versus Fig. 4). Additionally, the nucleic base loss reaction and consecutive DNA backbone fragmentation in competition with the peptidic bond cleavage (e.g., from the binary $[\text{-}\mathbf{RR}+\mathbf{T_5G_2T_5}-3\text{H}]^{3-}$ complex able to release a guanine unit in Figs. 5 and 6) reflect a competition for protonation and then a competition for zwitterion location between the basic Gua and Ade [46] and basic R or K side chain [47].

5. Conclusion

Interactions between nucleic acids and proteins play an important role in numerous biochemical processes, including DNA replication, recombination and repair. Basic residues are significant determinants of the nature of these biomolecular associations, notably by noncovalently interacting with a variety of other amino acids or by intermolecularly mediating complexation with phosphate groups. In our study, sequential MS/MS and MS^n experiments were done on different DNA–peptide systems to investigate the existence of salt bridge formation in noncovalent systems involving highly basic residues. The CID spectra showed that the major pathway involves the disruption of the noncovalent interactions and the formation of binary complexes and single-strand ions, directed by the nucleic base gas-phase acidity. An alternative pathway in which the binary complex is dissociated along the covalent bond was also detected. All experimental observations suggest that the phosphate–basic residue interactions remained ionic in the gas phase.

References

[1] N.M. Luscombe, S.E. Austin, H.M. Berman, J.M. Thorton, Genome Biol. 1 (2001) 1.
[2] A.A. Jain, M.R. Rajeswari, Biochim. Biophys. Acta 1622 (2003) 73.
[3] K. Namoto, J. Gardiner, T. Kimmerlin, D. Seebach, Helv. Chim. Acta 89 (2006) 3087.
[4] E.B. Dizhe, I.A. Ignatovich, S.V. Burov, A.V. Pohvoscheva, B.N. Akifiev, A.M. Efremov, A.P. Perevozchikov, S.V. Orlov, Biochemistry 71 (2006) 1350.
[5] S.A. Hofstadier, R.H. Griffey, Chem. Rev. 101 (2001) 377.
[6] F. Rusconi, F. Guillonneau, D. Praseuth, Mass Spectrom. Rev. 21 (2002) 305.
[7] C.M. Whitehouse, R.N. Dreyer, M. Yamashita, J.B. Fenn, Anal. Chem. 57 (1985) 675.
[8] M. Karas, F. Hillenkamp, Anal. Chem. 60 (1988) 2301.
[9] A.S. Woods, J.C. Buchabaum, T.A. Worr ll, J.M. Berg, R.J. Cotter, Anal. Chem. 67 (1995) 4462.
[10] P. Lecchi, L.K. Pannell, J. Am. Soc. Mass Spectrom. 6 (1995) 972.
[11] S. Lin, R.J. Cotter, A.S. Woods, Proteins Struct., Funct. Genet. 2 (1998) 12.
[12] S. Lin, S. Long, S.M. Ramirez, R.J. Cotter, A.S. Woods, Anal. Chem. 72 (2000) 2635.
[13] S.Z. Luo, Y.M. Li, W. Quiang, Y.F. Zhao, H. Abe, T. Nemoto, X.R. Qin, H. Nakanishi, J. Am. Soc. Mass Spectrom. 15 (2004) 28.
[14] L.J. Deterding, J. Kast, M. Przybylski, K.B. Tomer, Bioconjugate Chem. 11 (2000) 335.
[15] L. Oehlers, C.L. Mazzitelli, J.S. Brodbelt, M. Rodriguez, S. Kerwin, J. Am. Soc. Mass Spectrom. 15 (2004) 1593.
[16] C.L. Mazzitelli, Y. Chu, J.J. Reczek, B.L. Iverson, J.S. Brodbelt, J. Am. Soc. Mass Spectrom. 18 (2007) 11.
[17] V. Gabelica, N. Galic, F. Rosu, C. Houssier, E. De Pauw, J. Mass Spectrom. 38 (5) (2003) 491.
[18] K.A. Schug, W. Lindner, Chem. Rev. 105 (2005) 67.
[19] F. Rosu, V. Gabelica, C. Houssier, E. De Pauw, Nucleic Acids Res. 30 (16) (2002) e82.
[20] V. Gabelica, E. De Pauw, J. Am. Soc. Mass Spectrom. 13 (2002) 91.
[21] V. Gabelica, E. De Pauw, Int. J. Mass Spectrom. 219 (2002) 151.
[22] K.X. Wan, M.L. Gross, T. Shibue, J. Am. Soc. Mass Spectrom. 11 (2000) 450.
[23] K.M. Keller, J. Zhang, L. Oehlers, J.S. Brodbelt, J. Mass Spectrom. 40 (2005) 1362.
[24] S. Pan, X. Sun, J.K. Lee, J. Am. Soc. Mass Spectrom. 17 (2006) 1383.
[25] J. Gidden, A. Ferzoco, E.S. Baker, M.T. Bowers, J. Am. Chem. Soc. 126 (2004) 15132.
[26] F. Rosu, C.-H. Nguyen, E. De Pauw, V. Gabelica, J. Am. Soc. Mass Spectrom. 18 (2007) 1052.
[27] F. Rosu, S. Pirotte, E. De Pauw, V. Gabelica, Int. J. Mass Spectrom. 253 (2006) 156.
[28] J.J. Wilson, J.S. Brodbelt, Anal. Chem. 79 (2007) 2067.
[29] M.E. Vasquez, A.M. Caamano, J.L. Mascarenas, Chem. Soc. Rev. 32 (2003) 338.
[30] X. Tang, J.H. Callahan, P. Zhou, A. Vertes, Anal. Chem. 67 (1995) 4542.

[31] P. Terrier, J. Tortajada, W. Buchmann, J. Am. Soc. Mass Spectrom. 18 (2007) 346.
[32] S. Alves, A.S. Woods, J.-C. Tabet, J. Mass. Spectrom. 42 (2007) 1613.
[33] A.S. Woods, M.A. Huestis, J. Am. Soc. Mass Spectrom. 12 (2001) 88.
[34] F. Ciruela, J. Burgueno, V. Casado, M. Canals, D. Marcellino, S.R. Goldberg, M. Bader, K. Fuxe, L.F. Agnati, C. Lluis, R. Franco, S. Ferre, A.S. Woods, Anal. Chem. 76 (2004) 5354.
[35] A.S. Woods, J. Proteome Res. 3 (2004) 478.
[36] S.N. Jackson, H.-Y.J. Wang, A.S. Woods, J. Proteome Res. 4 (2005) 2360.
[37] A.S. Woods, S. Ferré, J. Proteome Res. 4 (2005) 1397.
[38] S.N. Jackson, H.-Y.J. Wang, A. Yergey, A.S. Woods, J. Proteome Res. 5 (2006) 122.
[39] A. Devolvé, J.-C. Tabet, S. Bregant, C. Afonso, F. Burlina, F. Fournier, J. Mass Spectrom. 41 (2006) 1498.
[40] M. Hardman, A.A. Makarov, Anal. Chem. 75 (2003) 1699.
[41] J.V. Olsen, L.M.F. de Godoy, G. Li, B. Macek, P. Mortensen, R. Peach, A. Makarov, O. Lange, S. Horning, M. Mann, Mol. Cell. Proteomics 4.12 (2005) 2010.
[42] D.J. Douglas, A.J. Frank, D. Mao, Mass Spectrom. Rev. 24 (2005) 1.
[43] Q. Hu, R.J Noll, H. Li, A.A. Makarov, M. Hardman, R.G. Cooks, 40 (2005) 430.
[44] V. Gabelica, M. Karas, E. De Pauw, Anal. Chem. 75 (2003) 5152.
[45] J.E. Chipuk, J.S. Brodbelt, J. Am. Soc. Mass Spectrom. 18 (2007) 724.
[46] F. Greco, A. Liguori, G. Sindona, N. Uccella, J. Am. Chem. Soc. 112 (25) (1990) 9092.
[47] E.P. Hunter, S.G. Lias, J. Phys. Chem. 27 (3) (1998) 413 (Ref. data).
[48] S. Mezzache, Thesis of Université Pierre et Marie Curie.
[49] R. Daneshfar, J.S. Klassen, J. Am. Soc. Mass Spectrom. 15 (2004) 55.
[50] C.M. Jones, M. Bernier, E. Carson, K.E. Colyer, R. Metz, A. Pawlow, E.D. Wischow, I. Webb, E.J. Andriole, J.C. Poutsma, Int. J. Mass Spectrom. 267 (2007) 54.
[51] Y. Xu, C. Afonso, R. Wen, J.-C. Tabet, J. Mass Spectrom., in press.
[52] P.D. Schnier, W.D. Price, R.A. Jockusch, A.S. Lemoff, E.R. Williams, J. Am. Chem. Soc. 118 (1996) 7178.
[53] M.A. Freitas, A.G. Marshall, Int. J. Mass Spectrom. 182/183 (1999) 221.
[54] R. Antoine, M. Broyer, P. Dugourd, G. Breaux, F.C. Hagemeister, D. Pippen, R.R. Hudgins, M.F. Jarrold, J. Am. Chem. Soc. 125 (2003) 8996.
[55] C. Lifshitz, Int. J. Mass Spectrom. 234 (2004) 63.
[56] F. Kjeldesen, O.A. Silivra, R. Zubarev, J. Am. Chem. Eur. J. 12 (2006) 7920.
[57] J.H. Jensen, M.S. Gordon, J. Am. Chem. Soc. 117 (1995) 8159.
[58] C. Kapota, J. Lemaire, P. Maître, G. Ohanessian, J. Am. Chem. Soc. 126 (2004) 1836.
[59] M. Boutin, C. Bich, C. Afonso, F. Burlina, F. Fournier, J.-C. Tabet, J. Mass Spectrom. 41 (2007) 25.
[60] R.R. Julian, J.L. Beauchamp, W.A. Goddard III, J. Phys. Chem. A 106 (2002) 32.
[61] P. Nemes, G. Schlosser, K. Vékey, J. Mass Spectrom. 40 (2005) 43.
[62] E.F. Strittmatter, E.R. Williams, Int. J. Mass Spectrom. 212 (2001) 287.
[63] T. Wyttenbach, M. Witt, M.T. Bowers, J. Am. Chem. Soc. 122 (2000) 3458.
[64] A.S. Lemoff, M.F. Bush, E.R. Williams, J. Am. Chem. Soc. 125 (2003) 13576.