Available online at www.sciencedirect.com
ScienceDirect
International Journal of Mass Spectrometry 261 (2007) 13–24
International Journal of Mass Spectrometry
www.elsevier.com/locate/ijms

Solution and gas phase hydrogen/deuterium exchange of oligoamide complexes by nanoelectrospray mass spectrometry

Honghai Jiang [a], Anthony R. Dolan [b], Minfeng Li [a], Marie A. Moy [b], Bing Gong [a], Troy D. Wood [a,c,*]

[a] *Department of Chemistry, Natural Sciences Complex, University at Buffalo, The State University of New York, Buffalo, NY 14260-3000, USA*
[b] *Nanogenesys Inc., Amherst, NY 14228, USA*
[c] *Department of Structural Biology, Natural Sciences Complex, University at Buffalo, The State University of New York, Buffalo, NY 14260-3000, USA*

Received 17 April 2006; received in revised form 21 July 2006; accepted 21 July 2006
Available online 22 August 2006

Abstract

Hydrogen bonding is crucial to biological systems and is intrinsic to non-covalent interactions. They are important in the formation of higher order structures of proteins as well as in the interactions of proteins with other biological molecules. Electrospray ionization (ESI) is an important tool in the study and characterization of molecular complexes and has proven to be extremely powerful and invaluable in the studying of biomolecular structures and non-covalent interactions. We have utilized solution and gas phase hydrogen/deuterium exchange (HDX) as a method to determine the specificity of supramolecular complex formation using monomers possessing sites containing hydrogen-bond donor and acceptor groups. By comparing the average number of exchanges for the monomer subunits to the average number of exchanges for the complex, we can distinguish if a specific complex is formed in solution, or whether it is the artifact of a gas phase process during ESI. In this paper we have investigated several non-covalent supramolecular complexes by nanoelectrospray (nanoESI) mass spectrometry (MS). By using the solution and gas phase HDX, we were able to identify several specific supramolecular complexes. Thus, solution and gas phase HDX combined with nanoESI-MS provides for a convenient method in ascertaining the origin and stability of non-covalent complexes.

Keywords: Hydrogen/deuterium exchange; Supramolecular complex; Nanoelectrospray

1. Introduction

Growing interest in understanding the interactions in living systems has made supramolecular chemistry an active field. Supramolecular chemistry has evolved from efforts to mimic the weak non-covalent interactions and the phenomenon of molecular recognition in biological systems [1–4]. Supramolecular chemistry, with the goal to gain control over the intermolecular non-covalent bond, is concerned with structure and function of entities formed by the association of two or more chemical species. The characterizing feature of supramolecular chemistry is that carefully designed synthetic structures recognize target molecules forming non-covalent complexes. Experimental methods for studying non-covalent complexes include spectroscopy (NMR, UV, mass spectrometry (MS), IR), electrochemistry (potentiometry), calorimetry, and X-ray crystallography [2]. Supramolecular chemistry and the quantification of non-covalent interaction strengths offer the basis for novel approaches in medicine, host–guest chemistry [5], chromatography [6], and biocatalysis [7].

Matrix-assisted laser desorption/ionization (MALDI) [8,9] and electrospray ionization (ESI) [10] have revolutionized the application of MS to the analysis of thermally fragile and high molecular weight biomolecules. ESI mass spectrometry has become an excellent tool for the characterization of a wide variety of non-covalent interactions including protein–ligand and protein–protein complexes, oligonucleotide complexes, and even pharmaceutical drugs complexed to proteins or oligonucleotides; several reviews provide excellent overviews on the

* Corresponding author. Tel.: +1 716 645 6800x2176; fax: +1 716 645 6963.
E-mail address: twood@acsu.buffalo.edu (T.D. Wood).

1387-3806/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijms.2006.07.014

breadth of non-covalent interactions which have been probed using ESI [11–16].

Solution and gas phase hydrogen/deuterium exchange (HDX) are promising methods in elucidating the solution or gas phase formation of non-covalent complexes. In this study, we apply HDX in elucidating the solution and gas phase formation of several molecular duplexes comprised of monomers containing hydrogen-bonding donor and acceptor groups. HDX is commonly used to provide insights into the roles of various non-covalent interactions in stabilizing the appropriate three-dimensional (3D) structural information of solution and gaseous ions, including information on protein conformation and has been applied successfully to obtain more structural information on various types of molecules [17–27], in particular to establish connections between the solution and gas phase conformations of molecules [27–31]. Generally, the HDX rate constants are effected by structural, steric and energetic factors, including the types and the accessibility of exchangeable protons, the presence of intramolecular and/or intermolecular hydrogen bonds, the nature of the isotopic exchange agent, and the number of collisions [32,33]. These features make the technique capable of providing insights into the roles of various non-covalent interactions in stabilizing the appropriate 3D structure of solution and gaseous ions, including protein conformation [17,22,34,35].

Presently, the application of nanoelectrospray (nanoESI) MS [36,37] for studying non-covalent complexes is highly desirable, although a notable recent report indicates desorption electrospray ionization (DESI) is even gentler than ESI at preserving protein conformation from solution into the gas phase and holds great promise for probing non-covalent complexation in solution [38]. In addition to the lower sample consumption and higher sensitivity offered by nanoESI as compared to conventional ESI, with its nanoliter per minute flow rates nanoESI may be softer than conventional ESI-MS during the transfer of complexes from solution into the gas phase (although the lower droplet size and higher charge density may preclude non-specific aggregation) [39,40]. Our method here is to use nanoESI-MS [41,42] with solution and gas phase HDX to explore the intermolecular hydrogen bonds of specifically designed hydrogen-bonded molecular duplexes [43–45]. In this method, which we developed for probing specificity of drug–drug complexes [29,30], a solution containing molecules which may form complex associations is injected with a deuterating reagent, and the solution is subsequently loaded for on-line ESI analysis (in the previous cases directly into a syringe for ESI, while here into a nanoESI emitter). HDX proceeds continually while mass spectra are collected; a comparison of the average number of H/D exchanges for the individual monomer units comprising the duplex with the number of H/D exchanges in the complex itself provides useful information about the existence of hydrogen bonds in the duplexes. If the total number of H/D atoms exchanged in a duplex is lower than the sum of H/D atoms exchanged in each of its components, this is strong evidence that the hydrogen bonds were formed in solution prior to ESI. If a hydrogen bond is formed during the ESI process (or the HDX is rapid relative to the process of hydrogen bonding), the average number of H/D atoms exchanged in a duplex should equal the sum of the number of H/D atoms exchanged in each of the components. A weakness of this approach is that the first several seconds of HDX cannot be probed until the sample is fully loaded and ready for ESI. Recently, Konermann and co-workers have developed an on-line pulsed HDX method to probe protein–protein complexes formed within tens of ms in solution [46,47]; in their method, two samples containing either aqueous or deuterated protein solution are infused via separate syringe pumps and then admixed in a tee immediately before entering the ESI needle. The HDX levels are then compared as in our method to distinguish whether a complex is formed in solution, or is an artifact of ESI. This HDX pulse labeling approach is advantageous because it enables determination of complex formation on the ms time scale, as opposed to the second time scale.

Here, the component monomers exhibit aggregates (homodimers and heterodimers) in nanoESI-MS; the hydrogen bonds of these duplexes (formed in solution or in the ESI process) were explored with solution and gas phase HDX to evaluate which hydrogen bonds of these duplex might originate in solution phase, or be formed during nanoESI. In some cases, HDX suggests that hydrogen bonds of the duplexes observed in the mass spectra are formed in solution. However, in other instances the evidence from nanoESI and HDX suggests that the duplexes are formed as an artifact of nanoESI; however, gas phase exchange suggests that once formed in nanoESI, such duplexes possess protection, indicating formation of hydrogen bonds during the spray process.

2. Experimental

2.1. Apparatus

All experiments were performed on a modified Thermo Electron LCQ Advantage quadrupole ion trap mass spectrometer (San Jose, CA). The ESI source was replaced by a home-built nanoESI source [26,48] designed and manufactured specifically for use with nanoESI instead of the standard ESI source of the LCQ. A schematic of the nanoESI source and the interface to the LCQ is shown in Fig. 1. This source contains within it an XYZ translatable emitter platform and mount through which voltage is applied. Methanol–*d*4 and deuterium oxide were used as the deuterating reagents in both solution and gas HDX experiments. For solution HDX, the sheath gas was not employed. Samples were introduced into the nanoESI source using polyaniline-coated emitters [41]. The emitters were positioned ∼4 cm from the inlet of the mass spectrometer and supplied with +4.0 kV to form positive ions. For gas phase HDX, nitrogen from sheath gas was bubbled through the methanol–*d*4 solution then evaporated into curtain gas stream, as shown in Fig. 1. Mass spectra were acquired for at least 3 min. Instrumental parameters and the operating conditions used are given in Table 1.

2.2. Reagents

Chloroform and acetic acid were HPLC grade and were obtained from Fisher Scientific Co. (Fair Lawn, NJ). All other

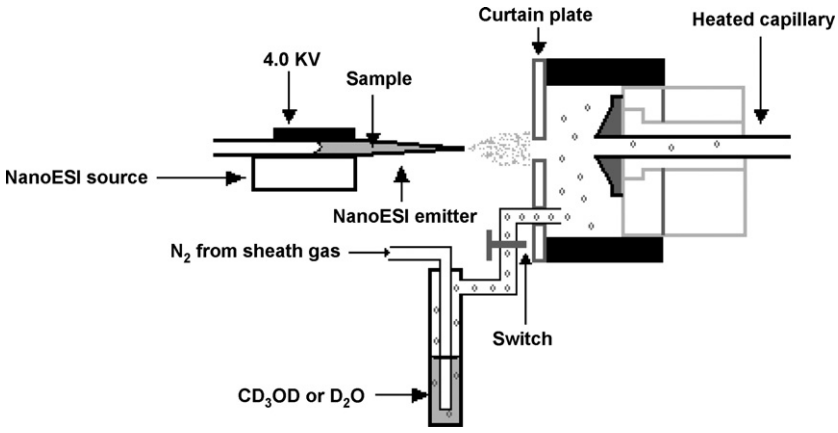

Fig. 1. Schematic diagram of the nanoESI source and the interface of the modified Thermo Electron LCQ mass spectrometer, which was employed to record solution (no sheath and curtain gas needed) and gas phase (with sheath and curtain gas) HDX mass spectral data.

organic solvents were purchased from Aldrich (Milwaukee, WI) and were of HPLC grade. Water was doubly distilled, deionized MilliQ water. Deuterium oxide and methanol–*d*4 were obtained from Cambridge Isotope Laboratories Inc. (Andover, MA) and Aldrich Chemical Company (Milwaukee, WI), respectively.

2.3. Compounds used to form non-covalent complexes

The three compounds used in this study (A, B, and C, Fig. 2) were designed and synthesized using previous methods [43–45]. The coupling reactions were carried out by standard peptide coupling methods, and the resulting oligoamides contain 1,3-disubstituted benzene rings linked by α-amino acid residues containing a variety of sequences using H-bonding donor (D_H) and acceptor (A_H) sites, i.e., amide O and H atoms. The exact masses of A, B, and C are 698.35 Da, 818.47 Da, 878.58 Da, respectively. Each of these compounds has four exchangeable H atoms. Samples dissolved in chloroform solutions were used in all experiments unless otherwise indicated.

2.4. Emitter fabrication for nanoESI

Fabrication of the nanoESI emitters used a protocol previously reported with a slight modification [42,48]. Uncoated borosilicate glass (i.d. = 0.69 mm, o.d. = 1.2 mm) was purchased from Sutter Instrument Co. (Novato, CA). Borosilicate nanoESI emitters with open emitter ends (orifice i.d. = 4 μm) were prepared by pulling heated glass capillaries with the Sutter Instrument Co. P-2000 laser-based capillary puller. Parameters were optimized to produce emitters with short tapers, relatively thick-walled orifices, and open emitter ends with orifices of 4 μm and a total emitter length of about 6.5 cm. The total pulling time was approximately 10 s for each glass tube. The uncoated emitters were then positioned onto a stage that was capable of mechanically lowering the emitters into a dispersion solution of polyaniline (Monsanto, St. Louis, MO). To avoid emitter clogging during the coating process, a stream of air was blown through the emitters during dipping. Evaporation of the PANI solution in air creates a coating layer of about 20–30 nm in thickness (as determined by SEM). After coating, the emitters were removed and stored until used. Borosilicate emitters used nanoESI with an average flow rate determined to be about 5 ± 2 nL/min using a recently described approach [49].

Table 1
Instrumental conditions used to record spectra in the LCQ mass spectrometers

	Solution phase HDX	Gas phase HDX
Spray voltage (kV)	4.0	4.0
Sheath gas flow rate (units)	0	10
Auxiliary gas pressure	0	0
Heated capillary temperature (°C)	210	210
Heated capillary voltage (V)	36	36
Tube lens offset/orifice voltage (V)	20	20
NanoESI emitter position (cm)	4	4

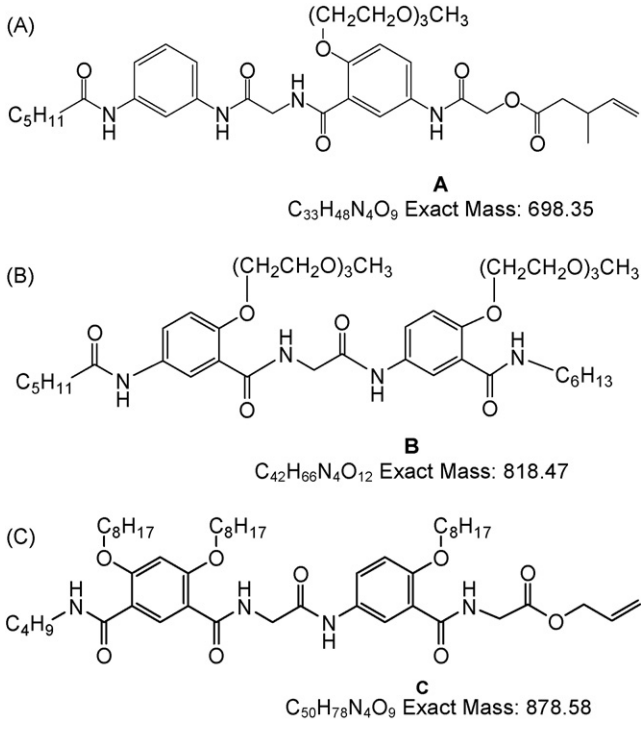

Fig. 2. Structures of the compounds A, B, and C studied in this paper.

2.5. Solution and gas phase HDX

In solution phase HDX, nanoESI-MS mass spectra were obtained for samples of: A (0.010 mM), B (0.010 mM), C (0.010 mM), and a mixture of A with C (both 0.010 mM) at room temperature. The HDX solvent mixture in these cases was always 90% chloroform/8% CD_3OD (or D_2O)/2% acetic acid (v/v/v) (positive mode). HDX was conducted and the data were acquired from 1 min after the exchange began during which the sample mixture was prepared and loaded into the nanoESI source. The gas phase HDX was conducted with the system described in Fig. 1. In gas phase HDX, the solvent mixture was 90% chloroform/8% CH_3OH (or H_2O)/2% acetic acid (v/v/v). Ions generated by nanoESI passed through an orifice in a curtain plate and the curtain gas (N_2 with CD_3OD or D_2O vapor) to enter the LCQ Advantage quadrupole ion trap mass spectrometer. Five replicate measurements of the same sample were acquired and the errors were calculated.

The non-deuterated and deuterated samples were analyzed under identical experimental conditions, and the resulting mass spectra were compared. At a certain exchange level, each of the initial isotopic peaks will produce its own isotopic distribution, due to multiple exchange levels. The average masses of the ions were calculated using Eq. (1):

$$M_{\mathrm{Avg}} = \left(\frac{\mathrm{RA}(M_1)}{\mathrm{TA}}\right) M_1 + \left(\frac{\mathrm{RA}(M_2)}{\mathrm{TA}}\right) M_2 + \cdots + \left(\frac{\mathrm{RA}(M_n)}{\mathrm{TA}}\right) M_n \quad (1)$$

where M_{Avg} is the average mass of the ion, RA the relative abundance of a given isotopic peak, TA the total relative abundance for all of the isotopic peaks in the distribution, and M_1, M_2, …, M_n are the masses for the first, second, and nth peaks in the isotopic distribution. The average masses for the non-deuterated species were subtracted from the average masses of the deuterated species, and the average number of H/D exchanges was determined by dividing this mass difference by the isotopic mass difference between D and H (1.006 Da). The reported errors represent the standard deviation from the mean determined from five replicate measurements on each individual sample. Summing acquisitions effectively averages out random errors associated with fluctuations in trapped ion populations.

3. Results and discussion

3.1. Aggregates of the compounds

As shown in Fig. 3, compound A (0.010 mM) in chloroform/methanol/acetic acid (90/8/2, v:v) yielded a mass spectrum of the singly protonated monomer (m/z 699.3), singly sodiated monomer (m/z 721.4), the singly protonated dimer (m/z 1397.0), and the singly sodiated dimer (m/z 1419.0). Compound B (0.010 mM) in chloroform/methanol/acetic acid (90/8/2, v:v) yielded a mass spectrum of the singly protonated monomer (m/z 819.5), singly sodiated monomer (m/z 841.6), singly potassiated monomer (m/z 857.5), the singly protonated dimer (m/z 1637.4), singly sodiated dimer (m/z 1659.4), and the singly potassiated dimer (m/z 1675.2). Compound C (0.010 mM) in chloroform/methanol/acetic acid (90/8/2 v:v) yielded a mass spectrum of the singly protonated monomer (m/z 879.5), singly sodiated monomer (m/z 901.7), singly potassiated monomer (m/z 917.5), the singly protonated dimer (m/z 1757.5), singly sodiated dimer (m/z 1780.6), and the singly potassiated dimer (m/z 1795.5). Since the nanoESI emitters were prepared by pulling heated borosilicate glass it is common for the mass spectra to have sodiated and potassiated adducts [29,41]. These emitters have shown very stable nanoESI and the long-term usability [37,41]. From Fig. 3, both monomers and dimers for all three compounds were observed in the nanoESI mass spectra. The only difference is that the dimer peak of compound B has high intensity in the mass spectra and almost has the same abundance as B monomer. Evidence that hydrogen bonding between the self-associated B molecules to form duplexes in solution was obtained previously from NMR experiments [43,45]. However, a question remained: are the homodimers of A or C observed here with nanoESI-MS really formed in solution from hydrogen bonding, or could they be artifacts of nanoESI? Solution phase HDX was employed to resolve this issue.

3.2. Kinetic study of solution HDX by nanoESI

Since the singly sodiated ions ($M + Na^+$) always have the highest relative intensity in these experiments, for comparison purposes for HDX under the same conditions this species is used throughout this paper. The HDX process of compound A in solution was monitored by nanoESI-MS. The mass spectra are shown in Fig. 4. Before HDX (0 min) the mass spectra indicate the presence of the singly sodiated monomer (left column) and singly sodiated dimer (right column) of A ions. The number of hydrogen atoms exchanged for deuteriums were calculated for various exchange times from the product of the mass shift determined using Eq. (1) divided by the mass difference between D and H. The average HDX levels of A monomer and A–A dimer with D_2O and CD_3OD versus the exchange time are plotted in Fig. 5. HDX is rapid in solution during the first 1 min, and after about 40 min, the HDX reaches a near kinetic completion. These mass spectra illustrate an important issue for the study of HDX of macromolecules: if the individual the isotopic envelope is relatively narrow before the reaction occurs, as HDX proceeds, the isotopic envelope first broadens. It then narrows as the reaction approaches completion. These trends also were observed in the mass spectra of Fig. 4. Sometimes the change of the envelope of the mass spectrometer may be used to obtain the protein structure information [50,51].

HDX rate constants are affected by the nature of the isotopic exchange reagent. Fig. 5 shows the different HDX rates with CD_3OD and D_2O. The initial exchange rate (up to 10 min) of both monomer and dimer of compound A are greater with D_2O than with CD_3OD [52]. However, when the HDX reaches completion (after 40 min of exchange), from student F- and t-tests, there is no significant difference between CD_3OD and D_2O HDX levels. After exposing the sample to exchange agents for 40 min, the average number of H/D exchanges for the A monomer by

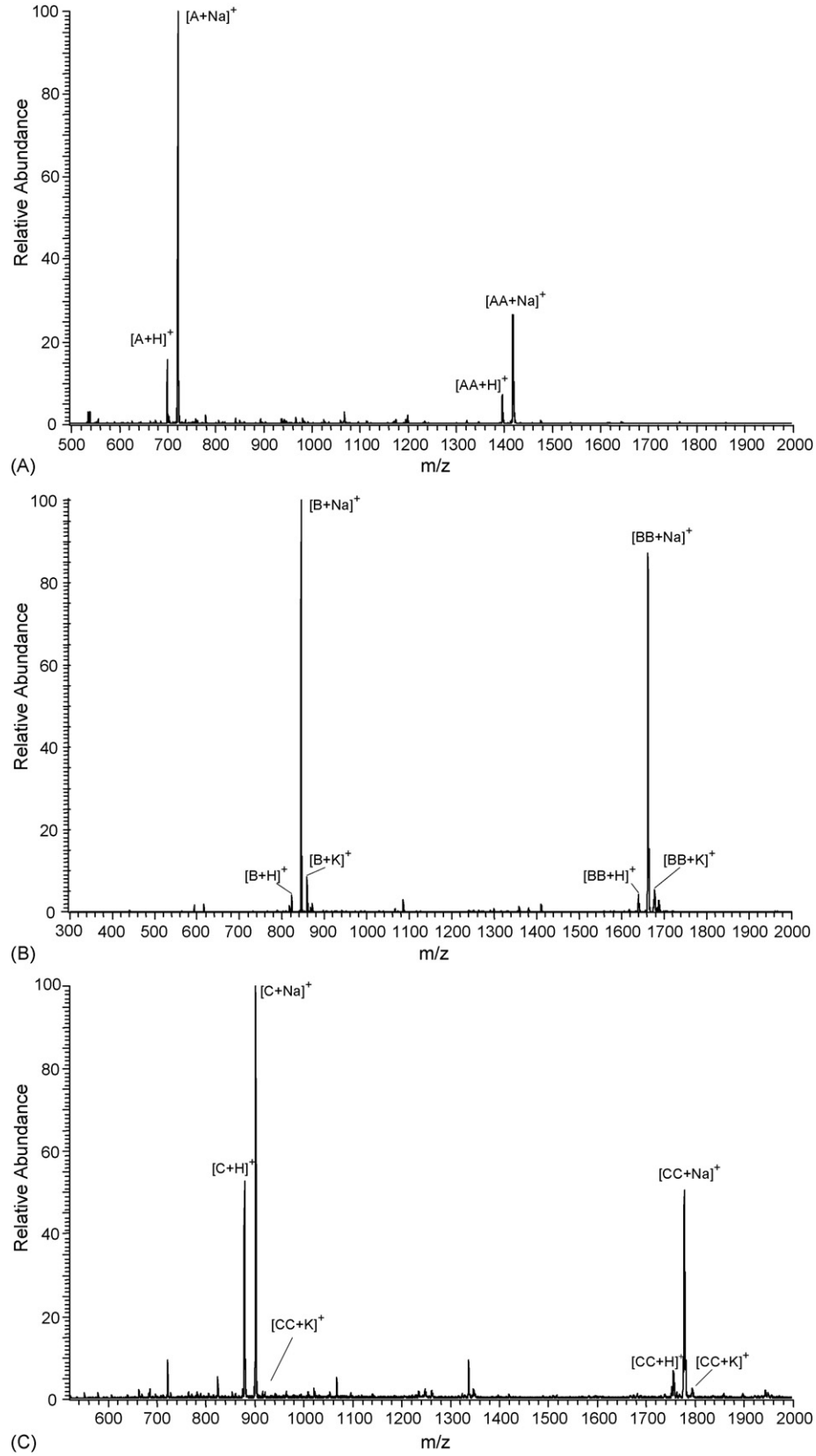

Fig. 3. Nanoelectrospray mass spectrum of 10 μM A, B, C, separately, in 90% chloroform with 8% methanol and 2% acetic acid.

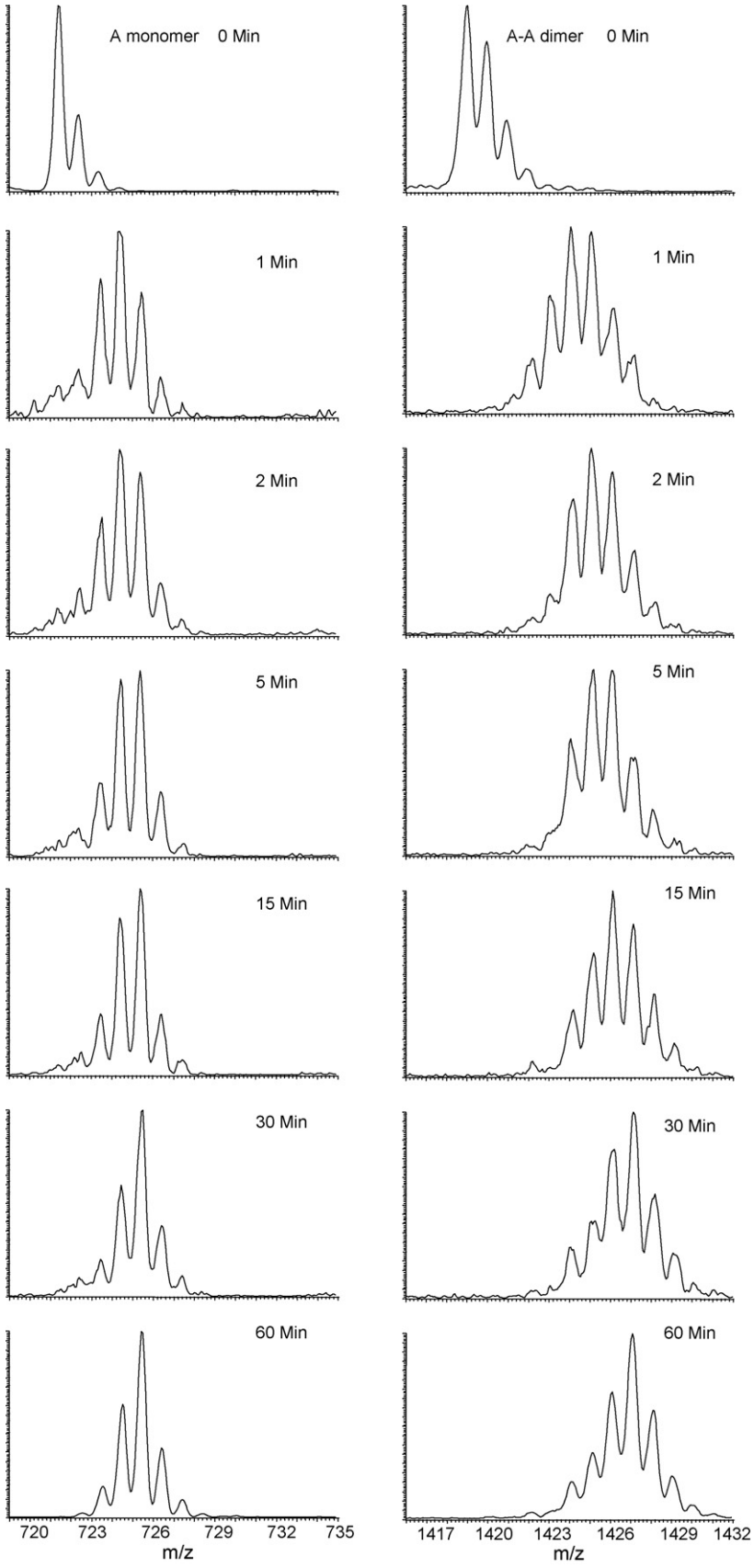

Fig. 4. Mass spectra of sodiated A monomer (left column) and sodiated A–A dimer (right column) at different solution HDX times as listed in the figure. The exchange agent is CD_3OD. All spectra were recorded under the same instrument condition.

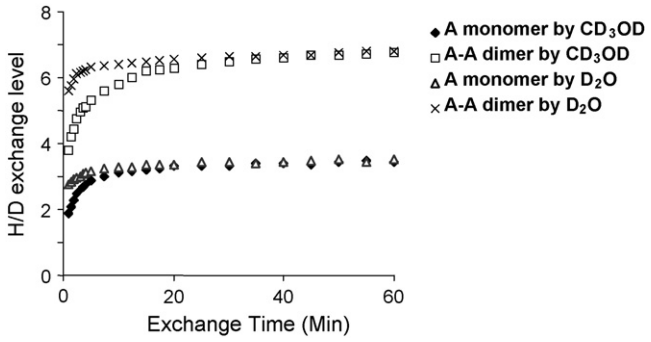

Fig. 5. Hydrogen exchange levels vs. exchange time for sodiated A monomer by CD_3OD (filled diamonds), the sodiated A–A dimer by CD_3OD (open squares), sodiated A monomer by D_2O (open triangles), and the sodiated A–A dimer by D_2O (cross).

CD_3OD is 3.42 ± 0.04 (Table 2), while the dimeric A complex has a level of H/D exchanges equal to 6.68 ± 0.06 (Table 2). By D_2O, the average numbers of H/D exchanges are 3.47 ± 0.05 (Table 2) for monomer and 6.74 ± 0.05 for dimer (Table 2). The exchange levels of dimer with both D_2O and CD_3OD are, within experimental error, about twice that of the monomer. There are only two possible interpretations of this result. One possibility is that the A dimer does not exist as a solution phase aggregate, but instead is a non-specific association formed during ESI. The other possibility is that HDX occurs in compound A kinetically fast relative to dimerization.

3.3. Solution phase HDX levels of compounds A, B, C, and mixture of A with C

Using the same method, solution phase HDX was performed for compounds A, B, and C separately in order to determine the origin of the dimer species. Mass spectra of isotopic distributions shown in Fig. 6 for A are described as follows. All distributions show the singly sodiated monomer before HDX (left top), after gas phase HDX (left middle), and after 60 min solution HDX (left bottom) as well as singly sodiated dimer before HDX (right top), after gas phase HDX (right middle), and after 60 min solution HDX (right bottom). We will discuss the gas phase HDX further below after discussing the solution phase HDX experiments. For solution phase HDX, the average HDX level of compounds A, B, and C in solution after exposing the samples to CD_3OD for 60 min of isotope exchange are calculated from the mass shift using Eq. (1) divided by the mass difference between D and H; these results are shown in Table 3. All standard deviations were calculated from five replicate measurements. The average number of HDX for the A monomer is 3.38 ± 0.07 (Fig. 6 and Table 3), while the dimeric A complex has a level of HDX equal to 6.73 ± 0.07 (Fig. 6 and Table 3). This number is 1.99 ± 0.05 times (Table 3) the number of HDX observed in the compound A monomer. Thus, the exchange level of the dimer is, within experimental error, twice that of the monomer. We can see here again the average number of exchanges for the A–A dimer is equivalent to the sum of the number of exchanges for the A monomer, suggesting either that this complex is formed during the nanoESI process or that HDX is kinetically fast relative to dimerization.

Table 2
Average HDX levels by CD_3OD and D_2O between 40 min and 60 min of compound A monomer and dimer calculated by Eq. (1)

Exchange agents	Monomer	Dimer	Dimer/monomer
CD_3OD	3.42 ± 0.04	6.68 ± 0.06	1.95 ± 0.03
D_2O	3.47 ± 0.05	6.74 ± 0.05	1.94 ± 0.03

Table 3
Average HDX level of compounds A, B, C as well as A–C dimer in solution by CD_3OD at 60 min

	Monomer	Dimer	Dimer/monomer
A	3.38 ± 0.07	6.73 ± 0.07	1.99 ± 0.05
B	3.15 ± 0.09	4.89 ± 0.09	1.55 ± 0.05
C	3.31 ± 0.08	6.60 ± 0.10	1.99 ± 0.06
A–C		5.11 ± 0.09	1.53[a] ± 0.06

Standard deviations were calculated from five times measurements.
[a] Value of dimer/(average of monomer A and C).

Similar results were obtained with B and C (Table 3, mass spectra not shown). Compound B has an average number of H/D exchanges equal to 3.15 ± 0.09 for the monomer. The dimeric B–B complex has a level of H/D exchanges at 4.89 ± 0.09 (Table 3), 1.55 ± 0.05 times the number of H/D exchanges observed in the B monomer. This is clear evidence that solution HDX in the B–B dimer is greatly restricted, implying that the B–B dimer complex must be formed by hydrogen bonds in solution *prior* to ESI and HDX. Compound C monomer shows 3.31 ± 0.08 (Table 3) H/D exchanges, while the average number of exchanges for the C–C dimer was 6.60 ± 0.10 (Table 3), 1.99 ± 0.05 times the number of H/D exchanges observed in the monomer. Thus, either the C–C dimer does not exist as a solution phase aggregate (but instead is a non-specific association formed during nanoESI) or HDX in compound C is kinetically fast relative to dimerization.

The nanoESI mass spectrum of the interaction of compound A with C (Fig. 7, 0.010 mM respectively, 90% chloroform/8% CH_3OH/2% acetic acid) reveals A (sodiated, m/z 721.5) and C monomers (protonated at *m/z* 879.5, sodium adduct at *m/z* 901.7), the A–A dimer (sodium adduct, *m/z* 1419.3) and C–C dimer (protonated at *m/z* 1758.7, sodiated at *m/z* 1780.7, potassiated at *m/z* 1796.6), and the sodiated A–C heteromeric complex (protonated at *m/z* 1577.5, sodiated at *m/z* 1599.5, potassiated at *m/z* 1615.5). This mixture of A and C is then monitored for HDX by nanoESI. As shown in Fig. 8, the A–C heteromeric complex indicates HDX proceeds in solution so that after 60 min, the average number of exchanges for the heteromeric complex A–C is 5.11 ± 0.09 (Fig. 8 and Table 3). The average number of H/D exchanges for the A monomer is 3.38 ± 0.07 (Table 3) and the C monomer is 3.31 ± 0.08 (Table 3). The average number of exchanges for the A–C complex is clearly less than the sum of the number of exchanges in its constituent monomers (5.11 versus 6.69). Therefore, it can be concluded that solution HDX in the A–C dimer is restricted and the A–C complex observed by ESI is the result of a solution phase interaction of hydrogen bonding. This result is consistent with previous NMR data (not

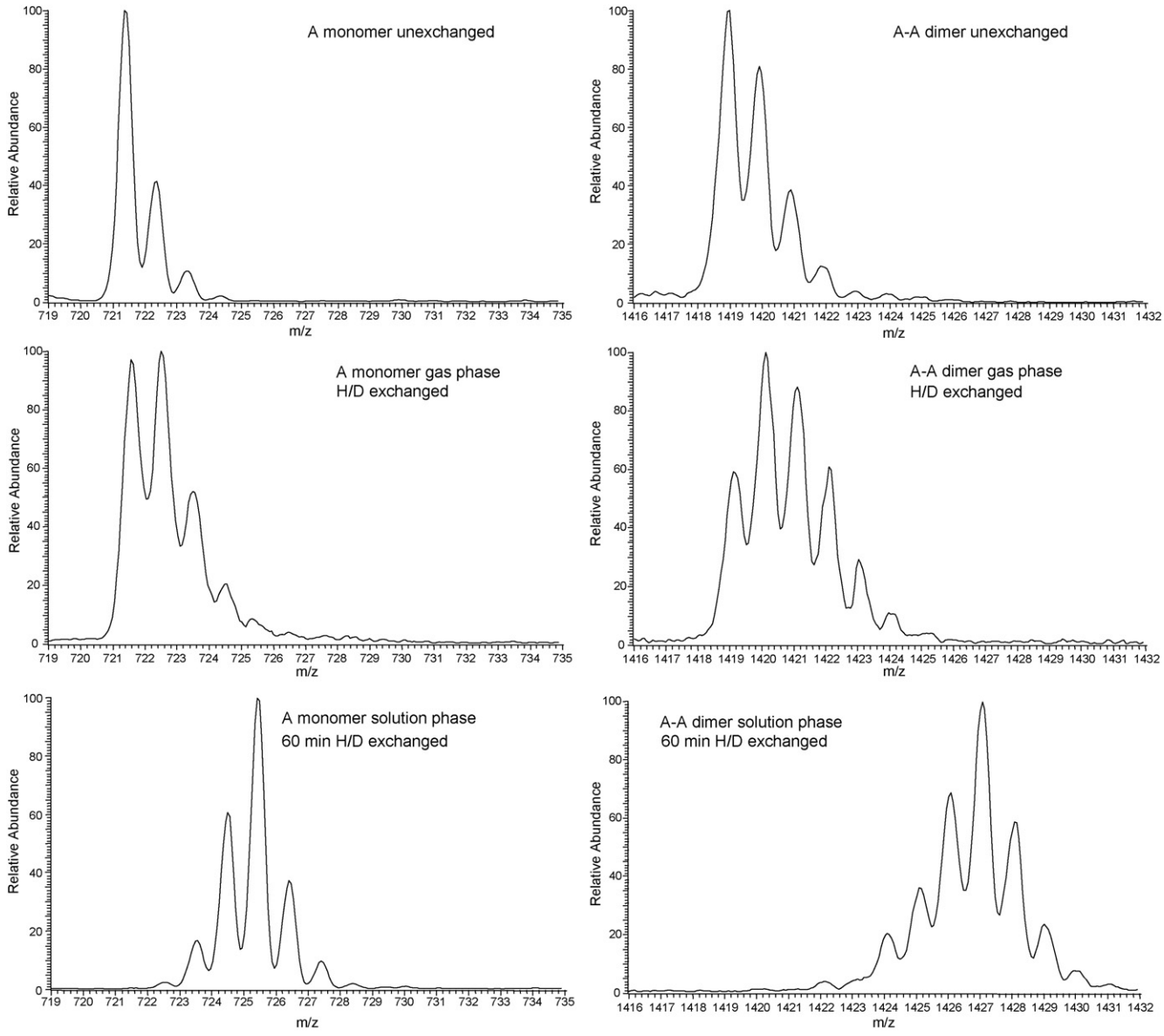

Fig. 6. Insets of isotopic distributions. Left column: sodiated monomer A before HDX (top), gas phase HDX (middle), and after 60 min solution HDX (bottom). Right column: sodiated A–A dimer before HDX (top), gas phase HDX (middle), and 60 min solution HDX (bottom).

shown) in our labs that suggest the A–C heteromeric complex is a solution phase dimer.

3.4. Solution phase HDX rates of compounds A, B, C, and mixture of A with C

Based on the kinetic study conducted previously, samples of compounds A, B, and C and the mixture of A with C in solution were exposed to CD_3OD for 1 min of isotope exchange individually. The average HDX rates (min^{-1}) for every sample in the first minute were calculated and shown in Table 4. During this first minute of exposure, the average HDX rate for the A monomer is $1.90 \pm 0.07\ min^{-1}$ (Table 4), while the A–A dimer has a HDX rate equal to $3.81 \pm 0.08\ min^{-1}$ (Table 4). The exchange rate of the dimer is, within experimental error, twice that of the monomer. Comparison of these two numbers suggests that the A–A complex is formed during nanoESI, and represents a non-specific interaction.

Compound B has an average HDX rate equal to $1.85 \pm 0.05\ min^{-1}$ for the monomer (Table 4), while the dimeric B complex has an HDX rate of at $3.05 \pm 0.06\ min^{-1}$ (Table 4);

Table 4
Average HDX rate (min^{-1}) of coumpound A, B, C as well as A–C dimer in solution by CD_3OD at the first minute

	Monomer	Dimer	Dimer/monomer
A	1.90 ± 0.07	3.81 ± 0.08	2.01 ± 0.09
B	1.85 ± 0.05	3.05 ± 0.06	1.65 ± 0.06
C	1.86 ± 0.08	3.70 ± 0.10	1.99 ± 0.10
A–C		3.15 ± 0.07	1.68[a] ± 0.10

Standard deviations were calculated from five times measurements.
[a] Value of dimer/(average of monomer A and C).

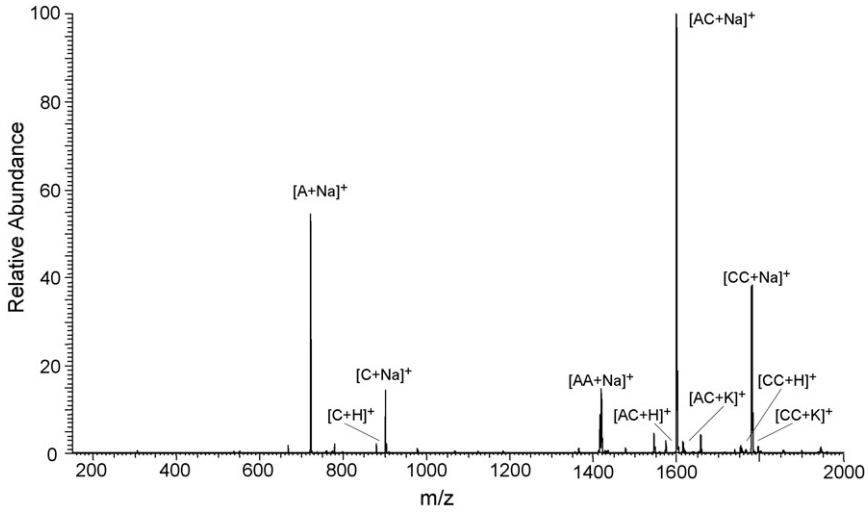

Fig. 7. Nanoelectrospray mass spectrum of a mixture of compounds A and C (both are 10 μM) in chloroform/methanol/acetic acid (90/8/2).

this number is 1.65 ± 0.06 times (Table 4) the rate of HDX rate observed in the B monomer. This is additional supporting evidence that solution HDX in the B–B dimer is restricted, implying that the B–B dimer complexes are formed in solution.

The average rate of HDX for the compound C was 1.86 ± 0.08 min^{-1} (Table 4), while the average exchange rate for the C–C dimer was 3.70 ± 0.10 min^{-1} (Table 4). The exchange rate of the C–C dimer was 1.99 ± 0.10 times (Table 4) the HDX rate observed in the C monomer, suggesting this complex is formed as a result of the nanoESI process.

The average HDX rate for complex A–C is 3.15 ± 0.07 min^{-1} (Table 4). The initial HDX rate for the A monomer is 1.90 ± 0.07 min^{-1} (Table 4) and the C monomer shows an initial HDX rate equal to 1.86 ± 0.08 min^{-1} (Table 4). Since the initial HDX rate for the A–C complex is less than the sum of the rates of exchanges in its constituent monomers, it can be concluded that the A–C complex observed by nanoESI is the result of a true solution phase interaction.

3.5. Gas phase HDX level of compounds A, B, C, and mixture of A with C

For gas phase HDX, the atmospheric interface of the nanoESI source was described in Fig. 1. Here, the HDX reaction was carried out by introducing the deuterating agent (CD_3OD or D_2O) into the N_2 sheath gas stream of a commercial mass spectrometer. In contrast to other HDX techniques in which gas phase HDX reactions are carried out on trapped ions [53,54] or on ions in low energy ion beams, the ions in these experiments are not trapped. The atmospheric pressure gas phase HDX here allows exchange to be studied in a population of metastable ions beginning immediately after their formation [26,27].

Atmospheric pressure gas phase HDX was performed for compounds A, B, and C, and the mixture of compound A with C. The mass spectra of isotopic distributions after gas phase HDX (middle) are shown in Figs. 6 (for A) and 7 (for A mixed with C). The average gas phase HDX level of compounds A, B, and C and A mixture with C after gas phase HDX are calculated using Eq. (1) and the results are shown in Table 5. The average number of gas phase H/D exchanges for the A monomer is 0.62 ± 0.03 (Fig. 6 and Table 5), while the A–A dimer has a level of H/D exchanges equal to 1.10 ± 0.04 (Fig. 6 and Table 5). In contrast to the solution phase HDX, this number is only 1.77 ± 0.11 times (Table 5) of the number of H/D exchanges observed in the compound A monomer. Since the exchange level of the dimer is less than the sum of the number of exchanges for the A monomer, it is likely that the A–A dimer formed by nanoESI exists as a gas phase duplex with intermolecular hydrogen bonds.

Compound B has an average number of gas phase H/D exchanges equal to 0.56 ± 0.02 for monomer (Table 5), while the B–B complex has a level of H/D exchanges equal to 0.76 ± 0.03 (Table 3). This number is only 1.36 ± 0.07 times the number of H/D exchanges observed in the B monomer, and is significantly less than the sum of the number of H/D exchanges for the B monomer. This is clear evidence that gas phase HDX in the B–B dimer is greatly restricted, implying that the dimer complex exists in the gas phase. In addition, the smaller dimer/monomer ratio indicates more intermolecular hydrogen bonds in the B–B dimer than in the A–A dimer. Similarly, the average number of gas phase H/D exchanges for compound C was 0.66 ± 0.03 (Table 5), while the average number of H/D exchanges for the C–C dimer was 1.14 ± 0.05 (Table 5). The exchange level of the C–C dimer was 1.73 ± 0.11 times that observed in the C monomer, indicating the C–C dimer formed during nanoESI also exists as a gas phase duplex with intermolecular hydrogen bonds.

Table 5
Average HDX level of A, B, C as well as A–C dimer in gas phase by CD_3OD

	Monomer	Dimer	Dimer/monomer
A	0.62 ± 0.03	1.10 ± 0.04	1.77 ± 0.11
B	0.56 ± 0.02	0.76 ± 0.03	1.36 ± 0.07
C	0.66 ± 0.03	1.14 ± 0.05	1.73 ± 0.11
A–C		0.86 ± 0.04	1.34[a] ± 0.10

Standard deviations were calculated from five times measurements.
[a] Value of dimer/(average of monomer A and C).

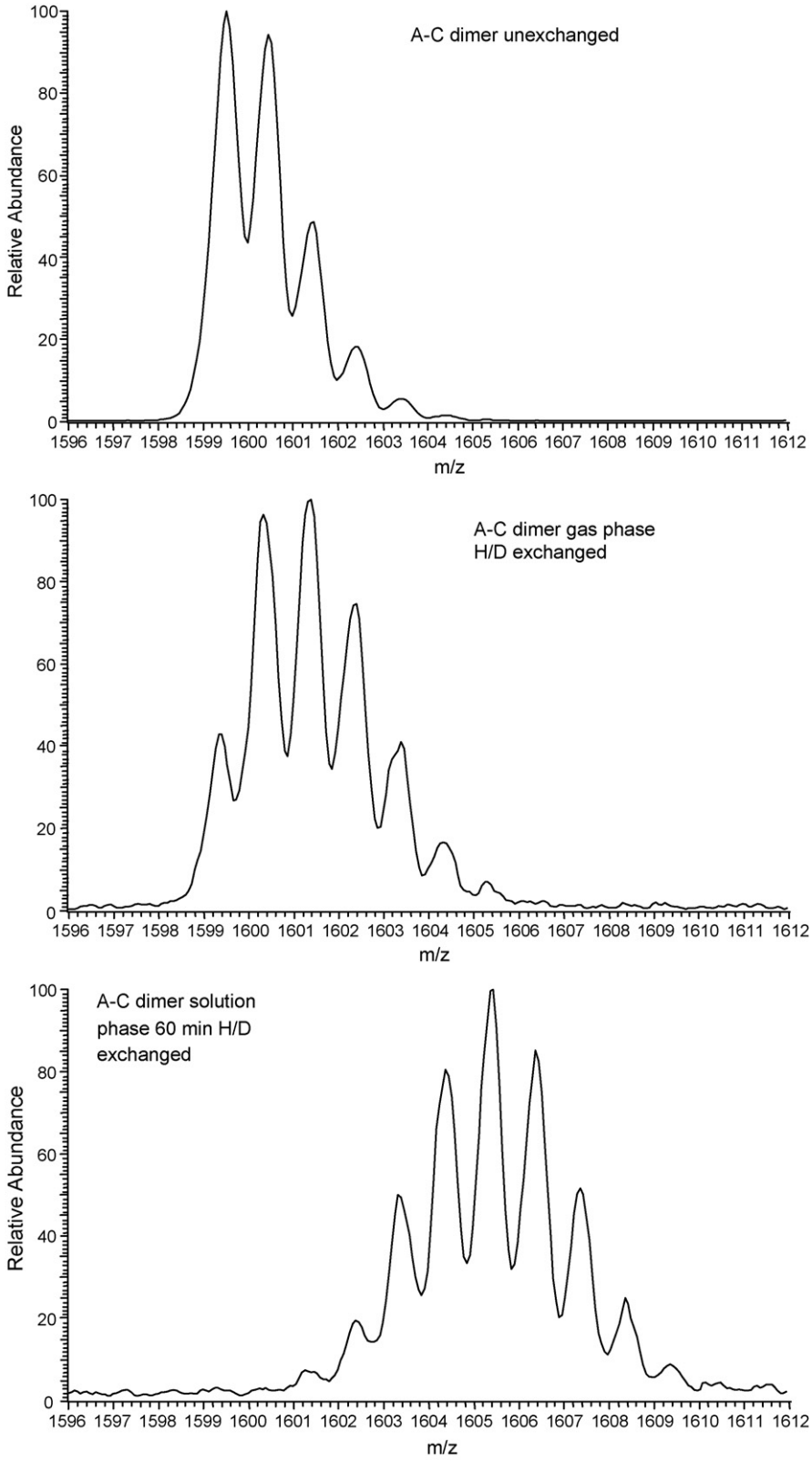

Fig. 8. Insets of isotopic distributions in the A–C dimer. Sodiated A–C dimer before HDX (top), gas phase HDX (middle), and 60 min solution HDX (bottom).

Admixing compounds A and C (both 10 μM in 90% chloroform/8% CH_3OH/2% acetic acid) for gas phase HDX resulted in the ESI mass spectrum shown in Fig. 8. The average number of exchanges for the heteromeric complex A–C is 0.86 ± 0.04 (Fig. 8 and Table 3). Since the average number of H/D exchanges for the A monomer is 0.62 ± 0.03 (Table 5) and for the C monomer is 0.66 ± 0.03 (Table 5); the average number of exchanges for the A–C complex is less than the sum of the

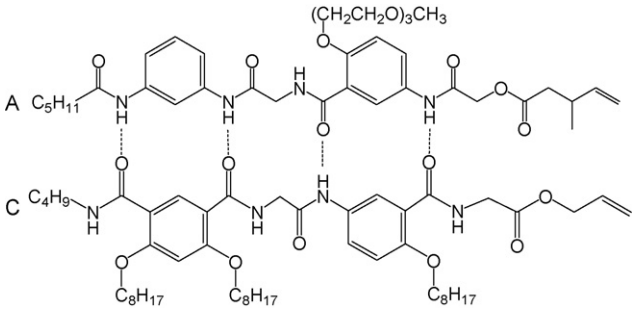

Fig. 9. Sequence specificity of the mutually complementary hydrogen-bonded molecular duplex A–C that contains the DDAD–AADA array.

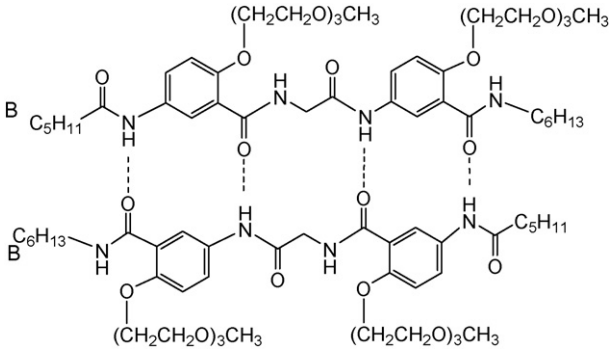

Fig. 10. Sequence specificity of the self-complementary hydrogen-bonded molecular duplex B–B that contains the DADA–ADAD array.

number of exchanges in its constituent monomers (0.86 versus 1.28). Therefore, it can be concluded that gas phase HDX in the A–C dimer is also restricted, and the A–C complex observed by nanoESI is the result of a solution phase interaction forming hydrogen bonds.

It is reasonable to envision it is easier for molecules to form supramolecular aggregation through intermolecular hydrogen bonds in the gas phase without the effect of the solution. Since compound A contains a sequence of $D_HD_HA_HD_H$ array (D_H: H-bonding donor and A_H: H-bonding acceptor) while compound C contains a sequence of $A_HA_HD_HA_H$ array, there are at most two intermolecular hydrogen bonds. For the heteromeric A–C dimer, however, the mutually complementary hydrogen-bonded molecular duplex that contains the sequence specificity of $D_HD_HA_HD_H$–$A_HA_HD_HA_H$ array shown in Fig. 9 can be formed, with up to four hydrogen bonds. Compound B contains a sequence of $D_HA_HD_HA_H$, so it can form the self-complementary hydrogen-bonded molecular duplex B–B that contains the sequence specificity of $D_HA_HD_HA_H$–$A_HD_HA_HD_H$ array of four hydrogen bonds, which is shown in Fig. 10. Both A–C and B–B duplexes show similar HDX ratios of dimer to monomer (1.68 versus 1.65 in solution (Table 3) and 1.34 versus 1.36 in gas phase HDX).

4. Conclusions

The hydrogen bond studies here are generally in agreement with earlier experiments with NMR. This study illustrates the use of solution and gas phase HDX to determine the specificity of non-covalent complexes observed by nanoESI-MS. Thus, solution and gas phase HDX provides for a convenient method in investigating the hydrogen bonding of non-covalent molecular complexes. The results here demonstrate that this nanoESI-MS system can be used to study and compare both solution and gas phase HDX reactions. Also it can be used to monitor continuous reactions for over 2 h with only 1 microliter of sample.

Acknowledgements

The authors would like to thank Dr. Sarah A. L. Caccamise and Chi-Tung Chiang for helpful discussion. Financial support of this work was provided through a subcontract of NIH SBIR Grant RR16399 (to Marie A. Moy of Nanogenesys Inc.).

References

[1] K. Müeller-Dethlefs, P. Hobza, Chem. Rev. 100 (2000) 143.
[2] H.-J. Schneider, A. Yatsimirsky, Principles and Methods in Supramolecular Chemistry, John Wiley & Sons Ltd., Chichester, England, 2000.
[3] J.W. Steed, J.L. Atwood, Supramolecular Chemistry, John Wiley & Sons Ltd., Chichester, England, 2000.
[4] J.M. Lehn, Science 227 (1985) 849.
[5] D. Tzalis, Y. Tor, Tetrahedron Lett. 37 (1996) 8393.
[6] W. Pirkle, C. Welch, Tetrahedron Assym. 5 (1994) 777.
[7] D. Barak, A. Ordentlich, Y. Segall, B. Velan, H.P. Benschop, L.P.A. De Jong, A. Shafferman, J. Am. Chem. Soc. 119 (1997) 3157.
[8] M. Karas, D. Bachmann, U. Bahr, F. Hillenkamp, Int. J. Mass Spectrom. Ion Process. 78 (1987) 53.
[9] K. Tanaka, H. Waki, Y. Ido, S. Akita, Y. Yoshida, T. Yoshida, Rapid Commun. Mass Spectrom. 2 (1988) 151.
[10] M. Yamashita, J.B. Fenn, J. Phys. Chem. 88 (1984) 4451.
[11] M. Przybylski, M.O. Glocker, Angew. Chem. Int. Ed. Engl. 35 (1996) 806.
[12] A.M. Last, C.V. Robinson, Curr. Opin. Chem. Biol. 3 (1999) 564.
[13] A.A. Rostom, C.V. Robinson, Curr. Opin. Struct. Biol. 9 (1999) 135.
[14] T.D. Veenstra, Biophys. Chem. 79 (1999) 63.
[15] J.A. Loo, Int. J. Mass Spectrom. 200 (2000) 175.
[16] L. Konermann, Can. J. Chem. 82 (2004) 1565.
[17] T.D. Wood, R.A. Chorush, F.M. Wampler, D.P. Little, P.B. O'Connor, F.W. McLafferty, Proc. Natl. Acad. Sci. U.S.A 92 (1995) 2451.
[18] N.N. Dookeran, A.G. Harrison, J. Am. Soc. Mass Spectrom. 6 (1995) 19.
[19] M.K. Green, C.B. Lebrilla, Mass Spectrom. Rev. 16 (1997) 53.
[20] S.J. Valentine, D.E. Clemmer, J. Am. Soc. Mass Spectrom. 13 (2002) 506.
[21] J.A.A. Demmers, J. Haverkamp, A.J.R. Heck, R.E. Koeppe II, J.A. Killian, Proc. Natl. Acad. Sci. U.S.A. 97 (2000) 3189.
[22] S.J. Eyles, J.P. Speir, G.H. Kruppa, L.M. Gierasch, I.A. Kaltashov, J. Am. Chem. Soc. 122 (2000) 495.
[23] B.E. Winger, K.J. Light-Wahl, A.L. Rockwood, R.D. Smith, J. Am. Chem. Soc. 114 (1992) 5897.
[24] K.A. Kellersberger, C. Dejsupa, Y. Liang, R.M. Pope, D.V. Dearden, Int. J. Mass Spectrom. Ion Process. 193 (1999) 181.
[25] J. Zhang, J.S. Brodbelt, J. Am. Chem. Soc. 126 (2004) 5906.
[26] Z. Takats, S.C. Nanita, G. Schlosser, K. Vekey, R.G. Cooks, Anal. Chem. 75 (2003) 6147.
[27] Z. Takats, G. Schlosser, K. Vekey, Int. J. Mass Spectrom. Ion Process. 228 (2003) 729.
[28] D. Suckau, Y. Shi, S.C. Beu, M.W. Senko, J.P. Quinn, F.M. Wampler III, F.W. McLafferty, Proc. Natl. Acad. Sci. U.S.A. 90 (1993) 790.
[29] S.A. Lorenz, E.P. Maziarz III, T.D. Wood, J. Am. Soc. Mass Spectrom. 12 (2001) 795.
[30] S.A. Lorenz, P.M. Bigwarfe Jr., S.V. Balasubramanian, G.J. Fetterly, R.M. Straubinger, T.D. Wood, J. Pharm. Sci. 91 (2002) 2057.
[31] S. Campbell, M.T. Rodgers, E.M. Marzluff, J.L. Beauchamp, J. Am. Chem. Soc. 117 (1995) 12840.

[32] S. Campbell, M.T. Rodgers, E.M. Marzluff, J.L. Beauchamp, J. Am. Chem. Soc. 116 (1994) 9765.
[33] S.E. Evans, N. Lueck, E.M. Marzluff, Int. J. Mass Spectrom. Ion Process. 222 (2003) 175.
[34] M.A. Freitas, A.G. Marshall, Int. J. Mass Spectrom. Ion Process. 182/183 (1999) 221.
[35] J.R. Engen, D.L. Smith, Anal. Chem. 73 (2001) 256A.
[36] M. Wilm, M. Mann, Anal. Chem. 68 (1996) 1.
[37] T.D. Wood, M.A. Moy, A.R. Dolan, P.M. Bigwarfe Jr., T.P. White, D.R. Smith, D.J. Higbee, Appl. Spectrosc. Rev. 38 (2003) 187.
[38] S. Myung, J.M. Wiseman, S.J. Valentine, Z. Takats, R.G. Cooks, D.E. Clemmer, J. Phys. Chem. B 110 (2006) 5045.
[39] R. Juraschek, T. Dulcks, M. Karas, J. Am. Soc. Mass Spectrom. 10 (1999) 300.
[40] A. Schmidt, M. Karas, T. Dulcks, J. Am. Soc. Mass Spectrom. 14 (2003) 492.
[41] T.P. White, T.D. Wood, Anal. Chem. 75 (2003) 3660.
[42] D.R. Smith, T.D. Wood, Anal. Chem. 75 (2003) 7015.
[43] H. Zeng, X. Yang, R.A. Flowers II, B. Gong, J. Am. Chem. Soc. 124 (2002) 2903.
[44] X. Yang, L. Yuan, K. Yamato, A.L. Brown, W. Feng, M. Furukawa, X.C. Zeng, B. Gong, J. Am. Chem. Soc. 126 (2004) 3148.
[45] X. Yang, S. Martinovic, R.D. Smith, B. Gong, J. Am. Chem. Soc. 125 (2003) 9932.
[46] B.M. Hossain, D.A. Simmons, L. Konermann, Can. J. Chem. 83 (2005) 1953.
[47] B.M. Hossain, L. Konermann, Anal. Chem. 78 (2006) 1613.
[48] D.R. Smith, G. Sagerman, T.D. Wood, Rev. Sci. Instrum. 74 (2003) 4474.
[49] D.R. Smith, M.A. Moy, A.R. Dolan, T.D. Wood, Analyst 131 (2006) 547.
[50] D.S. Wagner, R.J. Anderegg, Anal. Chem. 66 (1994) 706.
[51] K.R. Babu, D.J. Douglas, Biochemistry 39 (2000) 14702.
[52] M. Witt, J. Fuchser, G. Baykut, J. Am. Soc. Mass Spectrom. 13 (2002) 308.
[53] D. Mao, C. Ding, D.J. Douglas, Rapid Commun. Mass Spectrom. 16 (2002) 1941.
[54] T.G. Schaaff, J.L. Stephenson, S.A. McLuckey, J. Am. Soc. Mass Spectrom. 11 (2000) 167.