COMMUNICATION

ChemComm
www.rsc.org/chemcomm

Detection of complementary hydrogen bond complexes in water by electrospray ionization-Fourier-transform ion cyclotron resonance mass spectrometry†

Rika Iwaura,[a] Mayumi Ohnishi-Kameyama,[b] Mitsuru Yoshida[b] and Toshimi Shimizu*[acd]

[a] *Graduate School of Chemistry, University of Tsukuba, 1-1-1 Tennodai, Tsukuba, Ibaraki 305-8571, Japan*

[b] *National Food Research Institute, 2-1-2 Kannondai, Tsukuba, Ibaraki 305-8642, Japan*

[c] *Nanoarchitectonics Research Center (NARC), National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba Central 5, 1-1-1 Higashi, Tsukuba, Ibaraki 305-8565, Japan. E-mail: tshmz-shimizu@aist.go.jp; Fax: +81-298-61-4545; Tel: -81-298-61-4544*

[d] *CREST, Japan Science and Technology Corporation (JST), Tokyo, Japan*

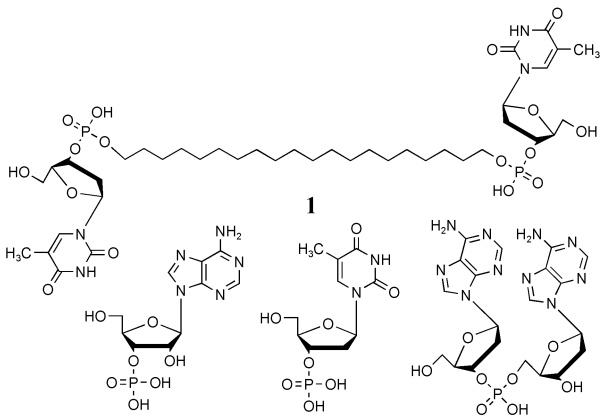
Received (in Cambridge, UK) 12th August 2002, Accepted 19th September 2002
First published as an Advance Article on the web 11th October 2002

Complementary base paired complex formation of a thymidine-appended bolaamphiphile with adenosine derivatives was able to be detected with high resolution and high sensitivity in aqueous solutions by electrospray ionization-Fourier-transform ion cyclotron resonance mass (ESI-FTICR MS) spectrometry.

Hydrogen-bond driven supramolecular assemblies are currently the focus of interest in material and analytical science.[1] The direct detection and observation of the hydrogen bond is, however, generally difficult when analytes are in aqueous environment.[2] Therefore, hydrogen bonds involved in the assemblies, especially, thymine (or uracil)–adenine complementary base pairs has only to date been studied either in nonpolar organic solvents or in the solid state using NMR, IR and XRD spectroscopy.[3] Very recently, Dingleey and Grzesiek[4] and Wuethrich and coworkers[5] reported direct observation of hydrogen bonds between nucleic acids in water using NMR scalar couplings although they had to synthesize ^{13}C and ^{15}N-enriched corresponding compounds. On the other hand, the electrospray ionization mass spectrometry (ESI-MS) technique is known to be useful to analyze low affinity complexes between biomolecules in aqueous solutions.[6] The combination of this ESI and Fourier-transform ion cyclotron resonance mass spectrometry (FTICR MS) enables us to detect hydrogen bond formation between a thymidine-appended bolaamphiphile and complementary adenosine derivatives in aqueous solutions without any modification. In this paper we first report the direct observation of complementary base paired association between small molecules with high resolution and high sensitivity in water by means of ESI-FTICR MS measurement.

We chose and synthesized a thymidine-appended bolaamphiphile **1** which self-assembles in aqueous solutions to form nanofibers according to the method reported previously.[7] We also employed two complementary adenosine phosphate derivatives **2** and **4**, and noncomplementary thymidine monophosphate **3** as hydrogen bonding counter species.‡ All compounds were treated by cation-exchange resin to completely remove sodium and potassium cations. To the aqueous solution of the thymidine bolaamphiphile **1** in Milli-Q water were added nucleotide derivatives **2**–**4** as a template and the obtained aqueous solutions were subjected to the ESI-FTICR MS spectrometry. The final concentrations of **1**, **2**, **3** and **4** were adjusted to 5.4×10^{-4}, 1.1×10^{-3}, 1.1×10^{-3} and 5.4×10^{-4} M, giving equivalent mole fractions for the thymine and adenine residues.

ESI-FTICR MS measurement§ proved to give new multiple peaks only for the complementary complexed species in addition to authentic peaks derived from **1**. Table 1 shows the MS peak data of base paired hydrogen bond complexes observed for the **1**/**2**, **1**/**3** and **1**/**4** aqueous solutions and their assignments. Figs. 1 and 2 display ESI-FTICR MS spectra obtained from the **1**/**2** and **1**/**4** aqueous solutions, respectively. The MS peaks ascribable to single component **1**, its potassium adducts dimers and trimers are observable as pointed by arrows (Figs. 1(a) and 2(a)). In Fig. 1, the relatively small signals appearing at *m*/*z* = 1268.470 and 1653.486 correspond to



1

2 **3** **4**

Table 1 Observed peaks in ESI-FTICR MS spectra

Sample	Corresponding to **1** + oligonucleotide *m*/*z* (composition) Resolution, S/N	Corresponding to **1** *m*/*z* (composition)
1/2	1268.470 [**1** + **2** − H]$^{-2-}$ 35,000, 120 1306.432 [**1** + **2** + K − 2H]$^{-}$ 1306.432 [2 (**1** + **2** + K − 2H)] 34,000, 140 1653.486 [**1** + 2(**2**) + K − 2H]$^{-}$ 26,000, 75 2266.777 [2(**1**) + **2** + 2K − 3H]$^{-}$ 18,000, 45	921.404 [**1** − H]$^{-a}$ 940.381 [2(**1**) + K − 3H]$^{2-a}$ 1420.565 [3(**1**) + 2K − 4H]$^{2-a}$
1/3	No Peaks	1900.747 [4(**1**) + 3K − 5H]$^{2-a}$ 2380.930 [5(**1**) + 4K − 6H]$^{2-b}$
1/4	1485.586 [**1** + **4** − H]$^{-}$ 27,000, 45 1523.524 [**1** + **4** + K − 2H]$^{-}$ 28,000, 80 2483.956 [2(**1**) + **4** + K − 2H]$^{-}$ 18,000, 50	2842.141 [3(**1**) + 2K − 3H]$^{-b}$

[a] Observed in all spectra. [b] Only observed in **1**/**2** spectrum.

† Electronic supplementary information (ESI) available: ESI-FTICR MS spectrum of the **1**/**3** aqueous solution. See http://www.rsc.org/suppdata/cc/b2/b207874k/

DOI: 10.1039/b207874k

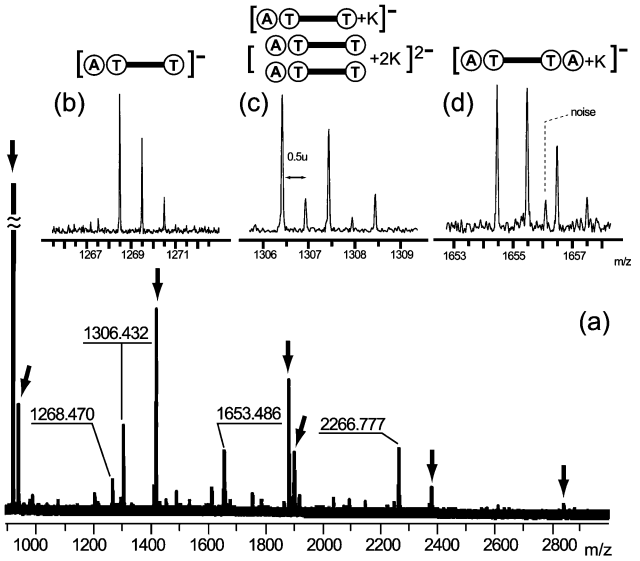


Fig. 1 (a) ESI-FTICR MS spectrum of the **1**/**2** aqueous solution. The MS peaks derived from the single component **1** are pointed by arrows; (b), (c) and (d) ESI-FTICR MS spectra showing complexed species through complementary A–T base pairs.

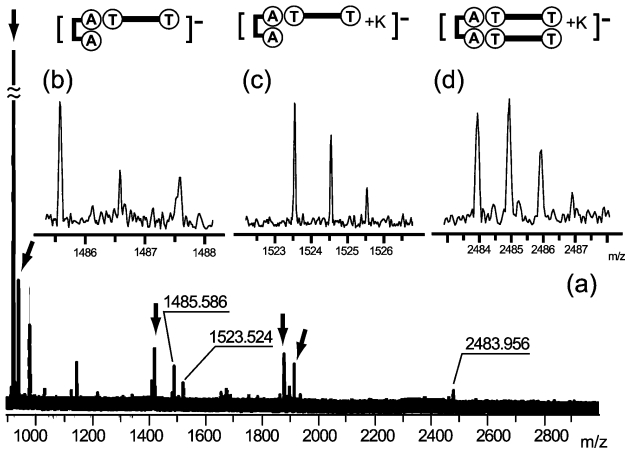


Fig. 2 (a) ESI-FTICR MS spectrum of the **1**/**4** aqueous solution. The MS peaks derived from the single component **1** are pointed by arrows; (b), (c) and (d) ESI-FTICR MS spectra showing complexed species through complementary A–T base pairs.

[**1** + **2** − H]$^-$ (Fig. 1(b)) and [**1** + 2(**2**) + K − H]$^-$ (Fig. 1(d)), respectively. We also found that the signal at *m*/*z* = 1306.432 includes two kinds of complexed species different in the charge number, *i.e.* singly charged [**1** + **2** + K − H]$^-$ and doubly charged [2(**1** + **2** + K) − H]$^{2-}$ species (Fig. 1(c)). The isotropic distribution of weakly bound species achieved by high mass resolution and high mass accuracy of FTICR MS technique enables us to distinguish these two components. We were also able to detect three kinds of complexed species for the **1**/**4** aqueous solution, [**1** + **4** − H]$^-$ (Fig. 2(b)), [**1** + **4** + K − H]$^-$(Fig. 2(c)), and [2(**1**) + **4** + K − H]$^-$ (Fig. 2(d)).

In order to verify that no complex formation is based on non-specific interaction in the gas phase, we measured the **1**/**3** aqueous solution consisting of a noncomplementary combination. Consequently, we were able to detect no significant MS peaks ascribable to the complex formation from **1** and **3**, but only found the peaks derived from the constituent thymidine monophosphate **3**, bolaamphiphile **1**, and its potassium adduct (see ESI†). These findings strongly support the view that the ESI-FTICR MS can detect only the complexed species based on complementary hydrogen bonds in aqueous solutions. Furthermore, the obtained MS data are in good agreement with the fact that the thymine–thymine interaction is much weaker than that of the thymine–adenine base pair. Kyogoku *et al.* reported association constants of various nucleic acid derivatives in chloroform solution using IR spectroscopy.[8] The estimated values are 3.2 and 130 M^{-1} for thymine–thymine and thymine–adenine complementary hydrogen bonding, respectively. Actually, the association constants between nucleic acids should drastically decrease in aqueous medium, but still show the preference of the thymine–adenine hetero-interaction over the thymine–thymine homo-interaction.[9]

The complex formation of the **1**/**2** combination through complementary hydrogen bonding of A–T base pairs is also confirmed by ATR-FTIR spectroscopy in D_2O solution. The carbonyl C=O stretching vibration at 1712 cm^{-1} attributable to the thymine–thymine or thymine–water hydrogen bonding completely disappeared upon complex formation, indicating the formation of thymine–adenine association instead.[10] It should be noted here that individual base pairing between nucleic acid components is generally difficult in aqueous solutions without a double-stranded polynucleotide chain.[11] Therefore, the present, efficient detection by the ESI-FTICR MS technique implies that the complementary complex is well stabilized by hydrophobic interaction between oligomethylene spacers of the bolaamphiphiles and π–π stacking interaction between aromatic rings.

Notes and references

‡ The 3′-adenosine monophosphate **2** and 3′-thymidine monophosphate **3** were purchased from Aldrich Chemicals (Wisconsin). Adenosine nucleotide **4** was purchased from ESPEC OLIGO (Ibaraki).

§ ESI-FTICR MS experiments have been performed with Apex II 70e (Bruker Daltonics, Billerica, MA) in the negative ion-mode. The cylinder, capillary, and endplate voltages were adjusted to 0, 4.8 and 4.8 kV, respectively, and the spray was stabilized with a gas pressure of 40 psi with nitrogen. The sample was introduced at 3 μl min^{-1}. the capillary interface was unheated. Ions were accumulated for 3 s in a hexapole ion guide prior to transfer to the trapped ion cell.

1 For example, see: J.-M. Lehn, *Makromol. Chem., Macromol. Symp.*, 1993, **69**, 1; G. M. Whitesides, E. E. Simanek, J. P. Mathias, C. T. Seto, D. N. Chin, M. Mammen and D. N. Gordon, *Acc. Chem. Res.*, 1995, **28**, 37; D. Philp and J. F. Stoddart, *Angew. Chem., Int. Ed. Engl.*, 1996, **35**, 1154; T. Shimizu, *Macromol. Rapid Commun.*, 2002, **23**, 311; T. Kato, *Science*, 2002, **295**, 2414.

2 F. Hibbert and J. Emsley, *Adv. Phys. Org. Chem.*, 1990, **26**, 255.

3 K. Nagai, K. Hayakawa, S. Ukai and K. Kanematsu, *J. Org. Chem.*, 1986, **51**, 3932; Y. Aoyama, H. Ohnishi and Y. Tanaka, *Tetrahedron Lett.*, 1990, **31**, 1177; Y. Itojima, Y. Ogawa, K. Tsuno, N. Handa and H. Yanagawa, *Biochemistry*, 1992, **31**, 4757; O. F. Schall and G. W. Gokel, *J. Am. Chem. Soc.*, 1994, **116**, 6089; J. L. Sessler and R. Wang, *J. Am. Chem. Soc.*, 1996, **118**, 9808; T. Itahara, *Bull. Chem. Soc. Jpn.*, 2002, **75**, 285; G. A. Jeffrey and W. Saenger, *Hydrogen Bonding in Biological Structures*, Springer-Verlag, Berlin, 1991.

4 A. J. Dingleey and S. Grzesiek, *J. Am. Chem. Soc.*, 1998, **120**, 8293.

5 K. Pervushin, A. Ono, C. F. T. Szyperski, M. Kainosho and K. Wuethrich, *Proc. Natl. Acad. Sci. USA*, 1998, **95**, 14147.

6 B. Ganem, *J. Am. Chem. Soc.*, 1991, **113**, 6294; V. Katta and B. T. Chait, *J. Am. Chem. Soc.*, 1991, **113**, 8534; Y.-T. Li and J. D. Henion, *J. Am. Chem. Soc.*, 1991, **113**, 7818; M. Baca and S. B. H. Kent, *J. Am. Chem. Soc.*, 1992, **114**, 3992; A. K. Ganguly, B. N. Pramanik, A. Tsarbopoulos, T. R. Covey, E. Huang and S. A. Fuhrman, *J. Am. Chem. Soc.*, 1992, **114**, 6559; R. P. O. Loo, D. R. Goodlett, R. D. Smith and J. A. Loo, *J. Am. Chem. Soc.*, 1993, **115**, 4391; K. J. Light-Wahl, B. L. Schwartz and R. D. Smith, *J. Am. Chem. Soc.*, 1994, **116**, 5271; S. A. Hofstadler and R. H. Giffey, *Chem. Rev.*, 2001, **101**, 377; J. Vincenti, *J. Mass Spectrom.*, 1995, **30**, 925.

7 R. Iwaura, K. Yoshida, M. Masuda, K. Yase and T. Shimizu, *Chem. Mater.*, 2002, **14**, 3047.

8 Y. Kyogoku, R. C. Lord and A. Rich, *Proc. Natl. Acad. Sci.*, 1967, **57**, 250.

9 M. Raszka and N. O. Kaplan, *Proc. Natl. Acad. Sci.*, 1972, **69**, 2025.

10 H. H. Mantsch and D. Chapman, *Infrared Spectroscopy of Biomolecules*, Wiley-Liss, New York, 1996.

11 J. S. Nowick and J. S. Chen, *J. Am. Chem. Soc.*, 1992, **114**, 1107; V. M. Rotello, E. A. Vianti, G. Deslongchamps, B. A. Murray and J. Rebek, *J. Am. Chem. Soc.*, 1993, **115**, 797.