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Real-time analysis of self-assembled nucleobases by Venturi easy ambient sonic-spray ionization mass spectrometry

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ABSTRACT

In this study, the real-time analysis of self-assembled nucleobases was employed by Venturi easy ambient sonic-spray ionization mass spectrometry (V-EASI-MS). With the analysis of three nucleobases including 6-methyluracil (6MU), uracil (U) and thymine (T) as examples, different orders of clusters centered with different metal ions were recorded in both positive and negative modes. Compared with the results obtained by traditional electrospray ionization mass spectrometry (ESI-MS) under the same condition, more clusters with high orders, such as $[6MU_7+Na]^+$, $[6MU_{15}+2NH_4]^{2+}$, $[6MU_{10}+Na]^+$, $[T_7+Na]^+$, and $[T_{15}+2NH_4]^{2+}$ were detected by V-EASI-MS, which demonstrated the soft ionization ability of V-EASI for studying the non-covalent interaction in a self-assembly process. Furthermore, with the injection of K^+ to the system by a syringe pumping, the real-time monitoring of the formation of nucleobases clusters was achieved by the direct extraction of samples from the system under the Venturi effect. Therefore, the effect of cations on the formation of clusters during self-assembly of nucleobases was demonstrated, which was in accordance with the reports. Free of high voltage, heating or radiation during the ionization, this technique is much soft and suitable for obtaining the real-time information of the self-assembly system, which also makes it quite convenient for extraction samples from the reaction system. This "easy and soft" ionization technique has provided a potential pathway for monitoring and controlling the self-assembly processes.

1. Introduction

The self-assembly of nucleobases, nucleosides, nucleotides and oligonucleotides has already been reported to be of considerable interest in biochemistry and supramolecular chemistry [1]. By the self-assembly of nucleobases, the clustering of nucleic acid components such as quintets has been discovered, which obtained increased interest in biology or nanotechnology [2,3]. In addition, some nucleobases such as uracil, thymine, guanine or adenine were reported to form the clusters in the cation-binding environment thanks to hydrogen bonds. The cations included Ca^{2+}, Ba^{2+}, Na^+, K^+, Cs^+, Rb^+, Li^+, Co^{2+} or NH_4^+, which were significant to the stabilization of self-assembled nucleobase and responsible for multi-strand stabilization within the telomere [4–12]. In fact, the formation of nucleobases cluster species such as quartet and quadruplex is formed in the telomers, the end of the chromosomes, which are reported to be involved in some tumors [4,13]. Thus, the study on the self-assembly of nucleobases may be of great importance in the field of biology, medicine and nanotechnology.

Several techniques have been employed in the characterization of nucleobases clusters in aqueous solution, including atomic force microscopy (AFM) [14], X-ray diffraction (XRD) [15], nuclear magnetic resonance (NMR) [6,16,17], gel electrophoresis [18], circular dichroism (CD) [19] and other spectroscopic methods [20]. However, these methods were usually destructive and normally employed by the end-point analysis of the final product, which could not provide the on-line or near on-line changes of cation-nucleobase clusters during the self-assembly process. Therefore, for examining non-covalent bonding of biomolecules and controlling the self-assembled process, the methods of monitoring the self-assembly process in real-time are significant.

Mass spectrometry (MS) has the ability of providing chemical structure information of molecules by recording mass-to-charge ration of charged particles from the analyst. The introduction of ambient MS ionization techniques, first introduced by electrospray ionization mass spectrometry (ESI), has overcome the limitation of getting samples into the vacuum environment of the spectrometer

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in the form of ions suitable for mass analysis [21]. Nowadays, the ambient MS techniques have coming to be great importance for drug discovery, doping control, forensic identification, food safety and quality control [22]. Recently, several ambient MS techniques including electrospray ionization mass spectrometry (ESI-MS) [2,5,13,23,24], desorption electrospray ionization mass spectrometry (DESI-MS) [25], or reactive DESI-MS [26] have been applied for investigating non-covalent interaction in self-assembly to form cation–nucleobase clusters. As reported, by ESI, DESI or reactive DESI, different clusters of cation-nucleobases were observed, which would vary with nucleobases, solution chemistry and the ionization conditions [23,25]. However, although these ambient ion sources have the facility for introducing samples into a closed or vacuum device under ambient conditions, these methods are still not convenient for coupling or integration with reaction systems for on-line analysis. Recently, some ambient techniques have been applied into the real-time monitoring of chemical reactions, such as atmospheric pressure chemical ionization mass spectrometry (APCI-MS) [27], low-temperature plasma mass spectrometry (LTP-MS) [28], probe electrospray ionization mass spectrometry (PESI-MS) [29], extractive electrospray ionization mass spectrometry (EESI-MS) [30,31], ion mobility-mass spectrometry (IM-MS) [32], ultrasound-assisted spray ionization (UASI) [33], etc. However, none of these studies are related to the real-time monitoring of the nucleobases self-assembly.

Venturi easy ambient sonic-spray ionization (V-EASI), using the self-pumping phenomenon of Venturi effect, is simple and easy to assemble, which operates solely via the assistance of a sonic stream of nitrogen or air [34,35]. Operating in voltage-, heat-, and radiation-free mode, V-EASI is therefore free of thermal, electrical, or discharge interferences, which is a gentle ionization to produce very minute charged droplets. Although the absolute signal intensity of the produced ions for V-EASI was about 2–3 times lower than that for ESI, V-EASI displayed superior signal-to-noise ratios, with the reducing chemical noise and fragmentation [34]. In addition, according to the comparation of EASI and DESI, a comparable sensitivities of these techniques were resulted [33]. Thus, V-EASI could be an ideal method for real-time monitoring of nucleobase self-assembly, which obtained the continuously extraction of liquid samples from self-assembly system to MS inlet. Although the gas-phase ions were introduced to MS inlet after extraction from the liquid system, the clusters examined might arise in solution or, at least, they demonstrate trends that are also applicable in solution similar or better than an ESI method [5].

This study proposed to gain the insight into the formation of self-assembled nucleobases clusters by V-EASI-MS. Nucleobases of uracil (U), thymine (T) and 6-methyluracil (6MU) were selected as models for examining the formation of different orders of clusters. The self-assembled behaviors were also studied in the cations binding environment, which demonstrated the effect of cations on the self-assembly of nucleobases. By V-EASI-MS, the real-time monitoring of cluster structures were carried out according to the extracted ion chronograms, which exhibited different stages in self-assembly. Due to the free of thermal, electrical, or discharge interferences during the ionization process, V-EASI-MS is soft and suitable for monitoring the process mainly contained weakly bond clusters. This could provide some guidance in the study of self-assembled nucleobases.

2. Experimental

2.1. Reagents and materials

HPLC-grade methanol was obtained from Spectrum Chemical Mfg. Corp. (Brunswick, Germany). The uracil (U, 99+%), thymine (T, 97%) and 6-methyluracil (6MU, 97%) were purchased from Alfa Aesar (Tianjin, China). The molecular structures of three nucleobases are shown in Fig. 1. NaCl, KCl, and NH_4Cl were supplied by Beijing Chemical Works (Beijing, China). All the chemicals in the experiments were used without further purification. The ultrapure water used for all the experiments was from Milli-Q water purification system (Millipore, Milford, MA).

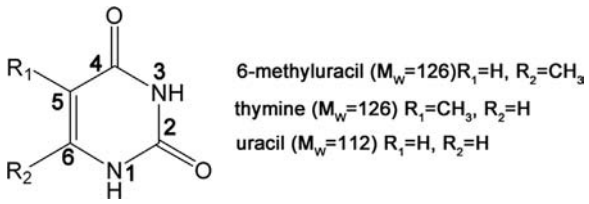

Fig. 1. Molecular structure of 6MU, T and U.

The stock solutions of uracil, thymine and 6-methyluracil were prepared by dissolving in methanol (5×10^{-3} M). Before detection, the nucleobases were diluted to the desired concentration by methanol–water (1:1, vol/vol).

2.2. V-EASI-MS

All the MS experiments were performed on an LTQ linear ion trap mass spectrometer (Thermo Fisher Scientific, San Jose, CA, USA) with a V-EASI source. The source was fabricated according to the lecture [34]. Different from DESI, V-EASI needs neither electrical power source nor syringe pumping, but an inner fused silica capillary casing with an outer stainless steel tube to generate the Venturi effect with N_2 flowing through. As shown in Fig. 2, with N_2 passing through the stainless steel tube, the samples in a glass bottle can be directly extracted by the Venturi self-pumping through the fused silica capillary. Then the dense cloud of minute droplets containing the produced ions was formed and subjected to the MS inlet for the detection. Simultaneously, the salt solution was injected into the self-assembly system through a syringe pumping. The main experimental parameters were as follows: the sample solution was suctioned at about 20 μL/min in the inner capillary (i.d. 75 μm, o.d. 365 μm), which was achieved by a N_2 flow of 1.0 MPa through the outer stainless steel tube (i.d. 550 μm, o.d. 1588 μm).

The LTQ operating parameters were as follows: mass spectra displayed from *m*/*z* 50 to 2000, capillary temperature was 250 °C, maximum injection and microscans were 50 ms and 2, respectively. All the mass results were obtained and processed by Xcalibur 2.0 software.

3. Results and discussion

3.1. Detection of nucleobases by V-EASI-MS

Three nucleobases, 6MU, T and U, were selected as samples for the detection. As shown in Fig. 1, 6MU is isomeric with T, and they are alkyl-substituted homologues of U. Fig. 3 shows the mass spectra of three analytes (3×10^{-4} M) using V-EASI-MS in both positive and negative modes. During the test, we obtained the few ions in the negative mode, most of which were deprotonated monomers and dimers. In addition, the sodium, potassium and even ammonium clusters, especially quintet clusters of $[6MU_5+NH_4]^+$, $[6MU_5+K]^+$, $[T_5+K]^+$ and $[U_5+NH_4]^+$, were formed without any salt added. As reported, the clusters were generated from the stable structure of a planar cycle by self-complementary hydrogen bonds, which have cations in the middle of the carbonyl oxgen atoms from nuleobases molecules [2].

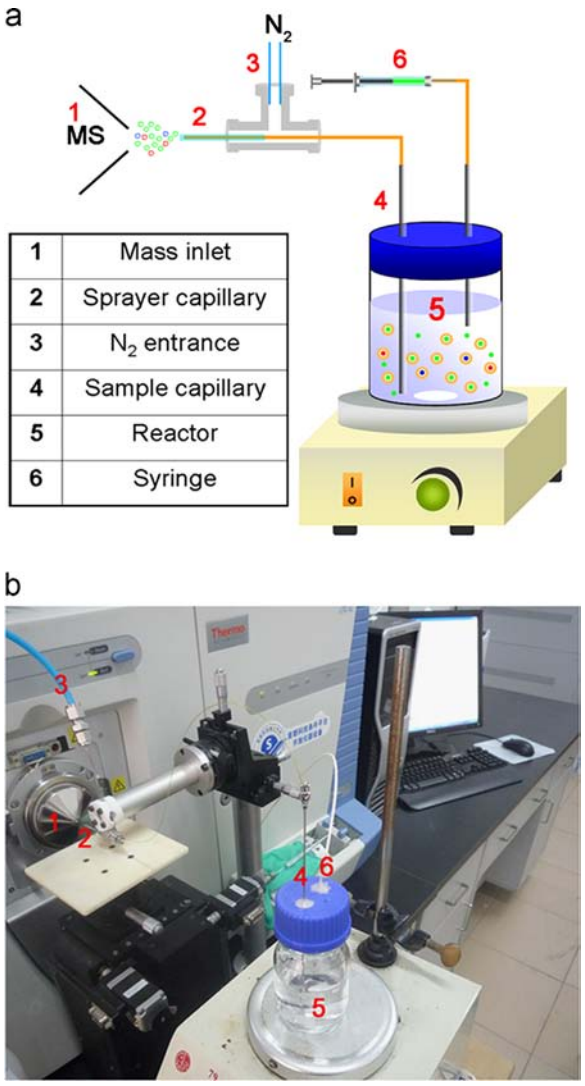

Fig. 2. (a) Schematic diagram of V-EASI-MS real-time analysis device. (b) Photograph of V-EASI-MS marked by the same labels with (a).

Table 1 lists the corresponding clusters detected in both positive and negative modes. We got cluster ions of 6MU and T with different orders, including $[6MU_4+Na]^+$ (m/z 527), $[6MU_5+NH_4]^+$ (m/z 648), $[6MU_{15}+2NH_4]^{2+}$ (m/z 963), $[T_4+Na]^+$ (m/z 527), $[T_5+NH_4]^+$ (m/z 648), $[U_4+Na]^+$ (m/z 471) and $[U_5+NH_4]^+$ (m/z 578). Interestingly, all these peaks were corresponding to quartet clusters or quintet clusters, which were in accordance with the reports (as shown in Fig. 4) [2]. Due to the smaller size of Na^+ than the sizes of K^+ and NH_4^+, the quartet clusters centered with Na^+ formed more easily than K^+ and NH_4^+ owing to the smaller cavity in quartet than quintet. In addition, the sizes of K^+ and NH_4^+ are similar ($r_{K^+} \approx 1.33$ Å, $r_{NH_4^+} \approx 1.43$ Å), which might result the similar formation of quintets with these cations as the central ions.

In addition, different orders of clusters centered with different cations were obtained by the self-assembly of three different nucleobases. Compared with T, two more clusters of $[6MU_{10}+Na]^+$ and $[6MU_{15}+2NH_4]^{2+}$ were recorded by the self-assembly of 6MU. While for the self-assembly of U, clusters with orders lower than five were detected, which resulted the less stability of the U clusters. These might be attributed to their distinct structures. As reported, the formation of nucleobases clusters centered with cations was generated from the orientation of the hydrogen-bond donor and acceptor groups [36], and the extra oxygen of nucleobases played a role as hydrogen acceptor in the hydorgen bonding between the bases [5,37]. The exo-cyclic alkyl groups also have an effect on the formation and stabilization of the cluster structures [2]. As shown in structures of three molecules (Fig. 1), 6MU and T have the similar molecular structures with an electron-donating group of methyl at C-6 or C-5, which could increase the stability of clusters. Furthermore, the group of methyl could increase the dipole moment and polarizability of the molecule and thus lead to stronger binding between cations and nucleobases. Considering the higher stable ability of methyl at C-6 than C-5, 6MU could form much higher orders of nucleobases clusters than T. Thus, although it is difficult to make the case that gas phase studies reflect in anyway condensed chemistry, we still could gain the insight into the formation of self-assembled nucleobases clusters by V-EASI-MS.

3.2. Comparison between V-EASI-MS and ESI-MS

Compared with ESI-MS, V-EASI-MS was described to be extreme soft for the ionization without heating, voltage and laser radiation, which would be expected to provide the structure changes during self-assembly of nucleobases [33]. Considering both methods could be used for studying the non-covalent bonding of biomolecules due to the possibility of transferring the non-covalent bonded clusters from solution into the gas phase, the comparison of V-ESAI-MS and ESI-MS was carried out under the same condition.

As shown in Fig. 5, lower orders of nucleobases clusters were recorded by ESI-MS. In the low mass range, the similar corresponding ions were obtained comparing with the data by V-EASI-MS. Taking 6MU as an example, the clusters included the singly charged proton monomer (m/z 127), dimer (m/z 253), sodium and potassium clusters of monomer (m/z 149 and m/z 165), dimer (m/z 275 and m/z 291), trimer (m/z 401 and m/z 417), and quartet (m/z 527 and m/z 543). However, the higher order of clusters detected by V-EASI-MS, such as $[6MU_7+Na]^+$ (m/z 905), $[6MU_{15}+2NH_4]^{2+}$ (m/z 963) and $[6MU_{10}+Na]^+$ (m/z 1283), could not be observed by ESI-MS. This might be attributed to the energy of ESI. It should be noted that the stability of nucleobases clusters will be affected by various conditions, such as temperature, pH, as well as pK, solute concentration and solute solubility, which have been demonstrated by ESI-MS [23] and reactive DESI-MS experiments [26]. In addition, the electrospray source operating conditions, such as sample flow rate, spray voltage, drying gas temperature and flow rate of ESI have also been found to have substantial impact on clusters as well [23]. However, compared with ESI related-techniques, V-EASI is much simple, without thermal, electrical, or discharge interferences during the ionization process. This might be the reason that we obtained the less ion information by ESI-MS than by V-EASI-MS.

As reported, under a certain condition, the high order clusters such as bisphosphine ligated gold clusters can be observed by ESI [38], and even the similar amino acid clusters can also be recorded by ESI [2]. Thus, the V-EASI-MS technique could be a well complementary technique for ESI in the monitoring of the self-assembly of nucleobases. Although the dynamics in an evaporating drop and process that occur through the transition to gas phase ions is different from the process that occur in a solvated condensed phase system, both V-EASI and ESI still could partly demonstrate trends that are also applicable in solution.

3.3. Real-time analysis

Without electrical syringe pumping, the V-EASI-MS has the advantage of self-extraction of samples from solution in real-time, which would be applied for the real-time monitoring of the

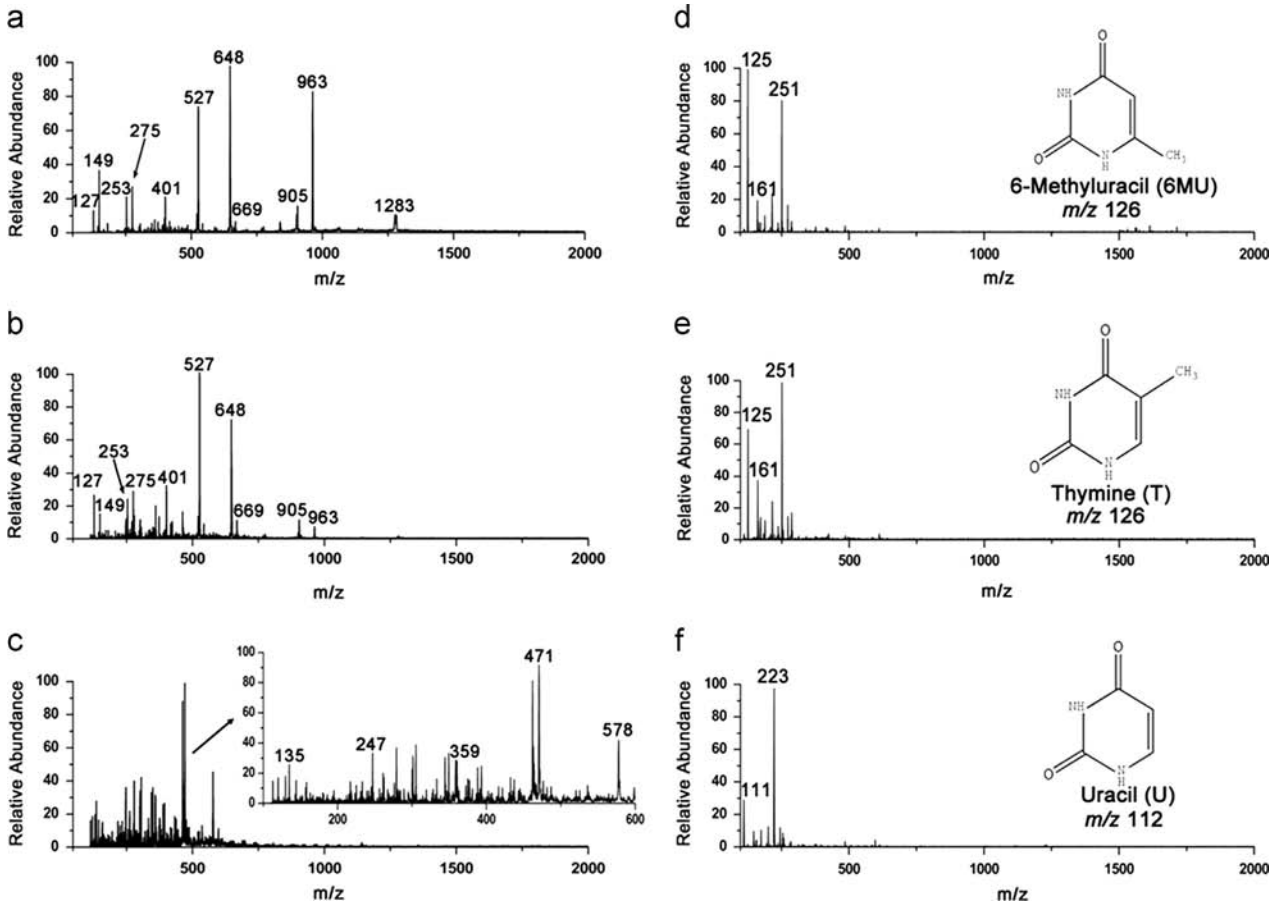

Fig. 3. V-EASI mass spectra in positive ion mode: (a) 6MU, (b) T, (c) U, the inset of (c) shows corresponding enlarged view of *m*/*z* 100–600. Mass spectra in negative ion mode: (d) 6MU, (e) T, and (f) U. The concentration of nuclebases was 3×10^{-4} M.

Table 1
Cluster of nucleobases in positive and negative ion modes detected by V-EASI-MS.

	6-Methyluracil (6MU)	*m*/*z*	Thymine (T)	*m*/*z*	Uracil (U)	*m*/*z*
Positive mode	$[6MU+H]^+$	127	$[T+H]^+$	127	$[U+Na]^+$	135
	$[6MU_2+H]^+$	253	$[T_2+H]^+$	253	$[U_2+Na]^+$	247
	$[6MU+Na]^+$	149	$[T+Na]^+$	149	$[U_3+Na]^+$	359
	$[6MU_2+Na]^+$	275	$[T_2+Na]^+$	275	$[U_4+Na]^+$	471
	$[6MU_3+Na]^+$	401	$[T_3+Na]^+$	401	$[U_5+NH_4]^+$	578
	$[6MU_4+Na]^+$	527	$[T_4+Na]^+$	527		
	$[6MU_7+Na]^+$	905	$[T_7+Na]^+$	905		
	$[6MU_{10}+Na]^+$	1283	$[T_5+NH_4]^+$	648		
	$[6MU_5+NH_4]^+$	648	$[T_{15}+2NH_4]^{2+}$	963		
	$[6MU_{15}+2NH_4]^{2+}$	963	$[T_5+K]^+$	669		
	$[6MU_5+K]^+$	669				
Negtive mode	$[6MU-H]^-$	125	$[T-H]^-$	125	$[U-H]^-$	111
	$[6MU_2-H]^-$	251	$[T_2-H]^-$	251	$[U_2-H]^-$	223

formation of nucleobases clusters. Therefore, the real-time monitoring of structure changes with introducing cations into the self-assembly system was employed by the flexible, operable V-EASI-MS. In the experiment, the potassium salt of 0.5 M was selected as the adding reagent, which was injected to the reaction system through a syringe pumping at a flow rate of 10 μL/min (as shown in Fig. 2). Therefore, the concentration of K^+ could be estimated according to the concentration, flow rate, and the monitoring time. While Na^+ and NH_4^+ ions originally existed in the solution without any reagent addition.

Fig. 6 displays the extracted ion chronograms of fourteen kinds of clusters by injecting 0.5 M KCl into 3×10^{-4} M 6MU. The clusters are divided into three groups: sodium clusters, potassium clusters, and ammonium clusters. As demonstrated, with the addition of K^+, the signal intensity of sodium and ammonium clusters is declined, while the abundant of potassium clusters increase. Especially, $[6MU_5+K]^+$ showed the highest ion signal at about 2.5 min after the injection of K^+. These changes of ion signals could be attributed to the competition of K^+ to Na^+/NH_4^+ during the formation of nucleobases clusters.

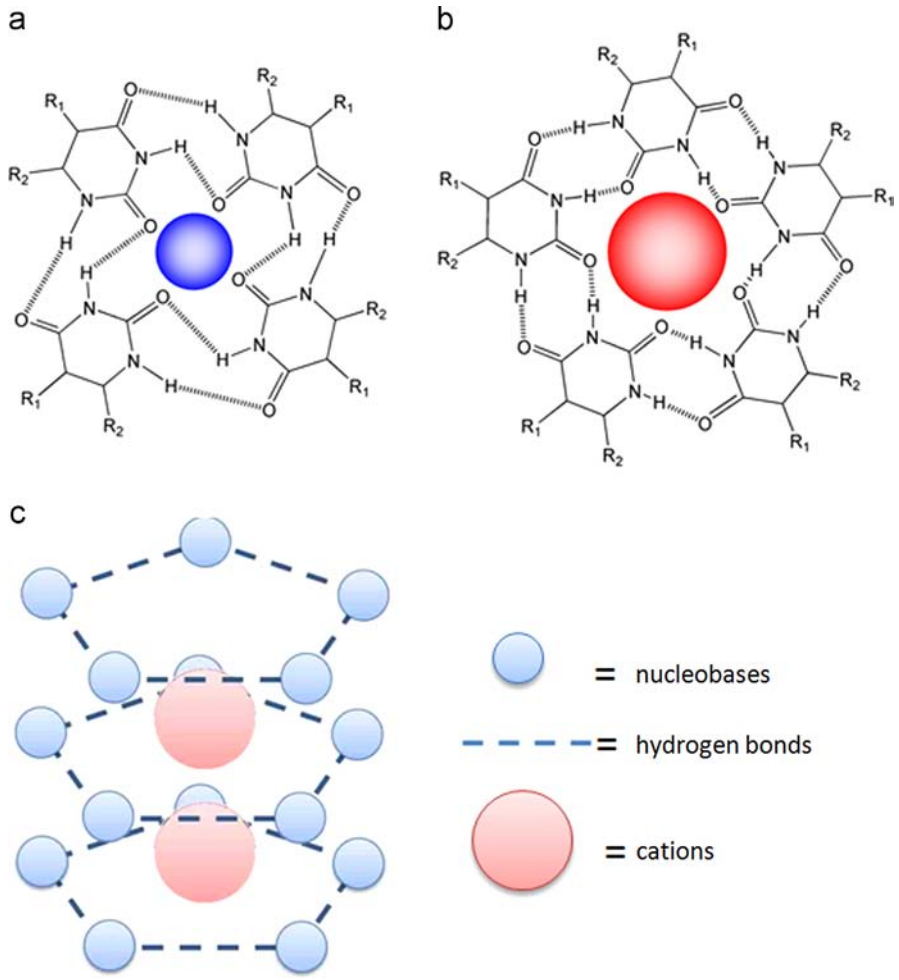

Fig. 4. The structures of nucleobases clusters: (A) quartet, (B) quintet and (C) doubly charged clusters. [2,37].

In detail, the whole analysis could be divided into four stages (the corresponding spectra are shown in Fig. 7):

(a) 0–0.7 min. At the beginning, there was only a small quantity of K^+ entered the system ($0–3.5\times10^{-5}$ M), which maintained the stable ion signals for a while. The typical mass spectrum is shown in Fig. 6a.

(b) 0.7–2.5 min. More K^+ entered the self-assembly system (3.50×10^{-5}–1.25×10^{-4} M), which led to the dramatically changes of each cluster abundance. As demonstrated, the potassium clusters increased while the sodium and ammonia clusters decreased. For example, $[6MU_5+K]^+$ (m/z 669) increased from 10.5% to 100%; while $[6MU_4+Na]^+$ (m/z 527) decreased from 76.6% to 13.9%, $[6MU_5+NH_4]^+$ (m/z 648) from 100% to 29.8%, and $[6MU_{15}+2NH_4]^{2+}$ (m/z 963) from 83.1% to 8.97%. The typical mass spectrum is shown in Fig. 6b.

(c) 2.5–4.3 min. The amount of K^+ was further increased (1.25×10^{-4}–2.15×10^{-4} M), and $[6MU_5+K]^+$ (m/z 669) was still the base peak. Although potassium clusters increased and the sodium and ammonia clusters decreased as well, the total ion chronograms were basically stable during 0–4.3 min. This indicated that most of the added K^+ participated in the competition of K^+ to Na^+/NH_4^+ for the clusters formation. The typical mass spectrum is shown in Fig. 6c.

(d) 4.3–7.8 min. With the increase of added K^+ amount, no remarkable change was observed on relative abundance of each cluster. However, the signal intensity of both total ion chronograms and extracted ion chronograms decayed, which might be attributed to the decreased ionization in the high concentration of salt solution. The typical mass spectrum is shown in Fig. 6d.

3.4. Effect of cations on nucleobases clusters

The size and species of cations were reported to be responsible for the formation and stability of nucleobase clusters, which might be related to multi-strand stabilization of telomere [39]. Different stabilities of K^+, NH_4^+ and Na^+ for different orders of clusters were also demonstrated from Fig. 6. For further studies, different clusters were monitored with the addition of K^+, Na^+ and NH_4^+ into the system, respectively. As shown in Table 2, most of the observed clusters had the same type of central cations as added cations, and different orders of nucleobases clusters were obtained with the addition of different cations. With the addition of Na^+, monomeric, and dimeric, trimeric and quartet clusters centered with Na^+ were recorded. However, the higher orders of clusters such as quintet clusters were not observed. Nevertheless, with the larger size of K^+ added, much higher orders of nucleobases clusters were obtained, which included $[T_5+K]^+$, $[U_5+K]^+$ and $[6MU_{15}+2K]^{2+}$. The cluster of $[6MU_{15}+2K]^{2+}$ was attributed to three quintets of 6MU centered with two K^+ (shown in Fig. 4C).

This might be generated from the different sizes of the cations [5], the nucleobase type [23], as well as the magic number of the clusters [37]. As reported, the sizes of K^+ and NH_4^+ are much

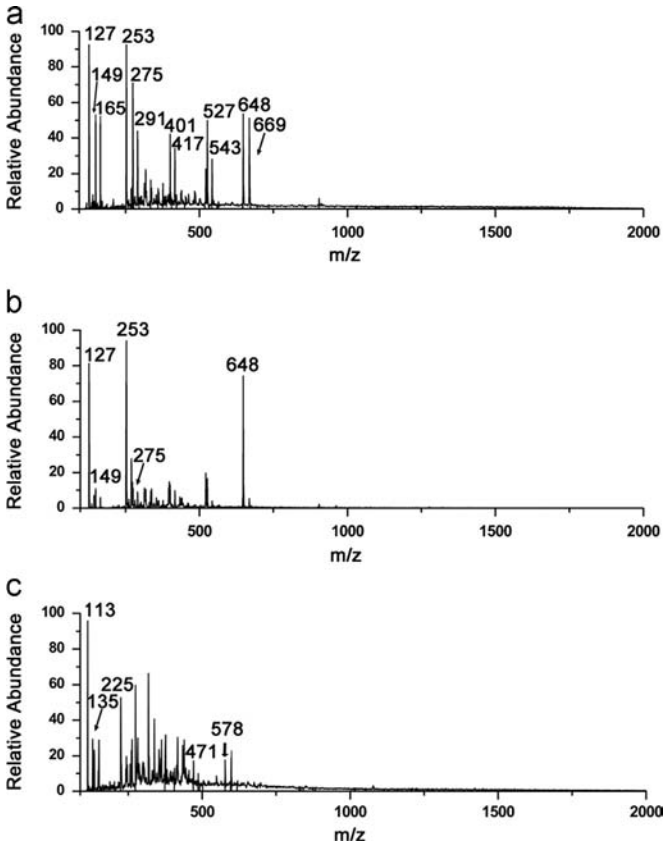

Fig. 5. ESI mass spectra of (a) 6MU, (b) T, and (c) U in the positive ion mode. The concentration of nucleobases was 3×10^{-4} M.

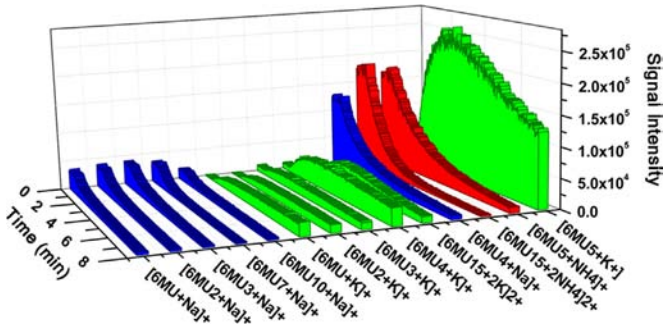

Fig. 6. Extracted ion chronograms of 6MU clusters by adding K^+. The concentration of added KCl and 6MU were 0.5 M and 3×10^{-4} M, respectively. The flow rate of KCl solution was 10 μL/min.

larger than that of Na^+, which ranked as NH_4^+ (1.43 Å) ≈ K^+ (1.38 Å) > Na^+ (0.95 Å) [40], or K^+ (1.49 Å) ≈ NH_4^+ (1.48 Å) > Na^+ (1.17 Å) [41]. Although different references showed the different data for the cation sizes, the much larger sizes of K^+ and NH_4^+ than Na^+ can always be concluded. This might be the direct causes for the formation of tetramers by Na^+, as well as pentamers by K^+ and NH_4^+ [5]. In addition, the tendency of forming tetramers with high abundance with NH_4^+ has also been found [37]. Therefore, the much stable pentamer clusters of K^+ were obtained than NH_4^+ and Na^+, which is also in accordance with the data in Fig. 6.

Therefore, the different self-assembly behaviors in the presence of different cations were preliminary confirmed. This is also in accordance with the reports, which similarly demonstrated the effects of cation sizes and species on the formation and stabilization of the clusters [2]. The different self-assembly behaviors in the presence of different cations might be related to multi-stand

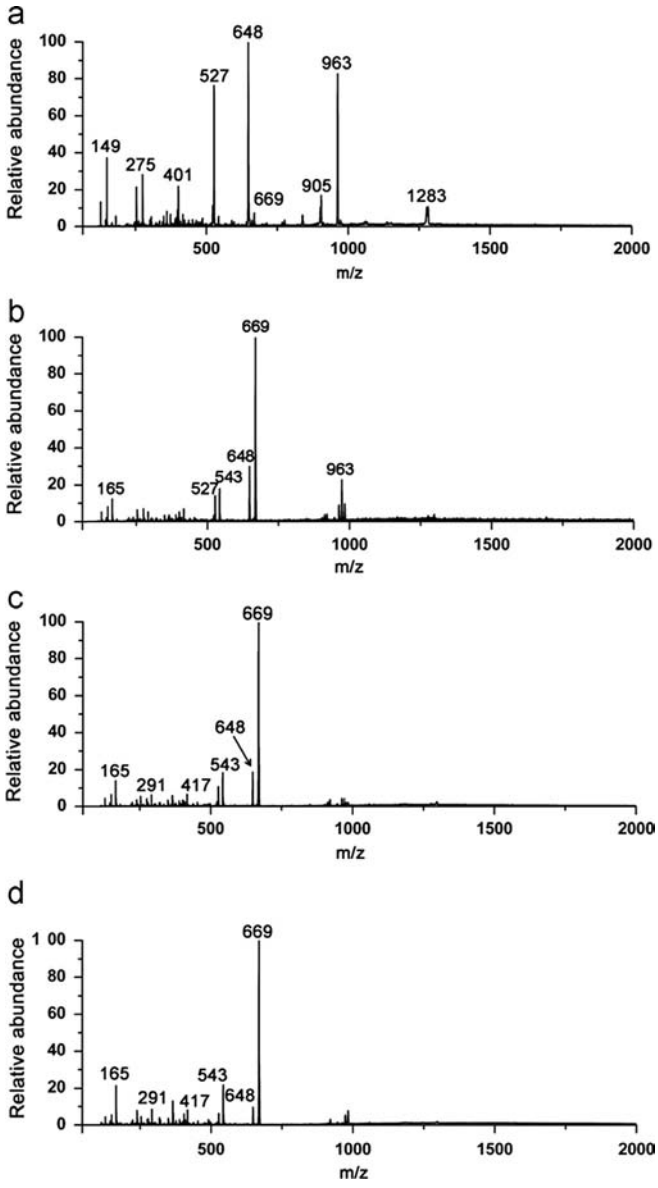

Fig. 7. The mass spectra of 6MU with adding K^+. (a) 0–0.7 min, (b) 0.7–2.5 min, (c) 2.5–4.3 min, and (d) 4.3–7.8 min.

stabilization of telomere, which could allow comparison with previously reported solution-phase results [42–45]. Although the data were not carried out under physiological conditions, the findings contributed to the fundamental understanding of the interactions between cations and nucleobases [5]. However, the further studies are still needed to further clarify the multi-strand stabilization in the telomere.

4. Conclusions

In conclusion, the real-time analysis of the self-assembly of nucleobases has been employed by V-EASI-MS. Due to the free of high voltage, heating or radiation during the ionization, this technique was much soft for obtaining the real-time information of the self-assembly system, which also makes it quite convenient for extraction samples from the reaction system. The present technique not only obtained the structure information rapidly by the simple Venturi injection, but also achieved the real-time monitoring of clusters with the addition of metal ions to the

Table 2
Cluster of nucleobases formed in presence of Na^+, K^+, and NH_4^+ detected by V-EASI-MS.

	6-Methyluracil (6MU)	*m*/*z*	Thymine (T)	*m*/*z*	Uracil (U)	*m*/*z*
Na^+	$[6MU+H]^+$	127	$[T+H]^+$	127	$[U+H]^+$	113
	$[6MU+Na]^+$	149	$[T+Na]^+$	149	$[U+Na]^+$	135
	$[6MU_2+Na]^+$	275	$[T_2+Na]^+$	275	$[U_4+Na]^+$	471
	$[6MU_3+Na]^+$	401	$[T_3+Na]^+$	401	$[U_4+H_3O]^+$	467
	$[6MU_4+Na]^+$	527	$[T_4+Na]^+$	527		
K^+	$[6MU+K]^+$	165	$[T+K]^+$	149	$[U+H]^+$	113
	$[6MU_2+K]^+$	291	$[T_2+K]^+$	291	$[U_2+H]^+$	225
	$[6MU_3+K]^+$	417	$[T_3+K]^+$	417	$[U_3+H]^+$	337
	$[6MU_4+K]^+$	543	$[T_4+K]^+$	543	$[U+K]^+$	151
	$[6MU_5+K]^+$	669	$[T_5+K]^+$	669	$[U_2+K]^+$	263
	$[6MU_{15}+2K]^{2+}$	984			$[U_3+K]^+$	375
					$[U_4+K]^+$	487
					$[U_5+K]^+$	599
NH_4^+	$[6MU+H]^+$	127	$[T+H]^+$	127	$[U+H]^+$	113
	$[6MU_2+H]^+$	253	$[T_2+H]^+$	253	$[U_2+H]^+$	247
	$[6MU+NH_4]^+$	144	$[T_4+NH_4]^+$	521	$[U_5+NH_4]^+$	578
	$[6MU_4+NH_4]^+$	522	$[T_5+NH_4]^+$	648		
	$[6MU_5+NH_4]^+$	648	$[T_{15}+2NH_4]^{2+}$	963		
	$[6MU_{15}+2NH_4]^{2+}$	963				

self-assembly system. The present work has demonstrated the potentials of V-EASI-MS in the field of self-assembly.

Author contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

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