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PAPER

The formation of thymidine-based T-tetramers with remarkable structural and metal ion size effects†

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We present direct ESI Q-TOF MS and X-ray evidence for remarkable structural and metal ion size effects on the formation of thymidine-based T-tetramers. The conventional H-bond acceptors on the ribose and deoxyribose may disfavor the formation of T-tetramers, and in the series of alkali metal ions, lithium did not induce T-tetramer due to its small ion size. Sodium, potassium, rubidium and caesium could produce thymidine-based T-tetramers. Furthermore, rubidium and caesium could induce T-pentamers and dimeric T-pentamers probably due to their larger ion sizes.

Introduction

G-Quartets have attracted considerable attention in areas ranging from molecular biology to nanotechnology, in particular in nucleic acid telomers of potential interest to cancer therapy and HIV-1 inhibition.[1,2] G-Quartets are formed by the hydrogen-bonding self-assembly of four guanosine (G) residues and stabilized by alkali-metal cations.[1,3] G-Quadruplexes are four-stranded helical DNA or RNA structures, comprising stacks of G-tetrads (or called G-Quartet) stabilized by Hoogsteen hydrogen bonding and monovalent cations,[4,5] which have become a focus of attention in recent years because of their role in important biological processes, such as replication, recombination transcription, and translation,[4] and potential as a therapeutic target for cellular ageing diseases, cancer and HIV.[6,7] The most important element in G-Quadruplex formation is the monovalent cations needed to stabilize the negative electrostatic potential created by the guanine oxygen(O6) atoms within the quadruplex core, and without cations, G-Quadruplexes are unstable.[8] Inspired by the structures and functions of G-Quartets and G-Quadruplexes, it is of interest whether other types of quartet and quadruplex aggregates can be formed under certain circumstances. In addition to G-Quartets and G-Quadruplexes, other types of quartet and quadruplex species formed from homotetrameric or heterotetrameric nucleobases may be also of great importance in the field of biology, medicine and nanotechnology. Although the structural differences between nucleic acid bases are small, the differences in their chemistry and electronic properties are significant.[6] A variety of supramolecular structures formed by nucleic acid bases can be formed by changing the experimental conditions.[6] It was reported that A-T-A-T Quartets could be observed at the liquid/solid interface by scanning tunneling microscopy.[6] A G^C Quartet was reported by X-ray, ESI-MS and NMR observations.[9] Very recently Xu and co-workers provided NMR evidence for a U_4 tetrad which stabilizes human telomeric RNA G-Quadruplex structure.[10] As for T-tetramers, T-tetrads and T-Quartets, although thymine and its derivatives-based T-quartets[11] and T-quintets[12] templated by alkali metal ions have been reported based on MS observations[11,12] and X-ray analysis,[13] and thymidine-based T-tetrads have been found in G-quadruplex environments by NMR techniques,[14,15] the independent formation of T-Quartets from thymidine and its derivatives is not clear. Indeed, thymidine-based T-quartet structures may be of real significance. One hand, thymidine is more associated with DNA structures than thymine, in the other hand, thymidine and its derivatives have been used for the treatment of cancer[16,17] and HIV/AIDS.[17,18]

Many detection techniques such as NMR,[2,14,15,19] X-ray,[3,19] MS,[11,12,20] fluorescence,[19] CD spectra[8,21] and IR[22] have been developed to determine these supramolecular structures. In this regard, as a popular, minimally fragmenting tool for investigating supramolecular noncovalent interactions, matrix-assisted laser desorption/ionization (MALDI) time of flight (TOF) mass spectrometry and electrospray ionization (ESI) mass spectrometry, can provide various information for these possible supramolecular complexes present in solution.[23,24] Nevertheless, only ESI is commonly used for qualitative and quantitative analysis of low molecular weight compounds. This is because, in contrast to MALDI, ESI does not require the addition of matrix molecules; such matrix molecules may undergo association reactions leading

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to interference peaks in the low-mass range.[25] ESI-MS has been successfully used in the characterization of G-Quartet and G-Quadruplex assemblies.[20,26,27] In this work ESI-MS and X-ray results showed that the formation of thymidine-based T-tetramers (tetrads and/or quartets) is structure and metal ion size dependent.

Results and discussion

Three ribose- and deoxyribose-modified thymidine derivatives ($\mathbf{T_1}$, $\mathbf{T_2}$ and $\mathbf{T_3}$) were prepared[28] for this investigation.‡ $\mathbf{T_1}$ has no acetyl group on the ribose; one acetyl group on the deoxyribose of $\mathbf{T_2}$ and two with $\mathbf{T_3}$. (^{1}H, ^{13}C) NMR spectroscopic and elemental analysis results suggested an excellent purity for the three compounds obtained (Figures S1–S11 in Supporting Information†). Fig. 1 presents the ESI-MS of three samples ($\mathbf{T_1}$, $\mathbf{T_2}$, $\mathbf{T_3}$). In the ESI-MS of $\mathbf{T_1}$, monomer ($\mathbf{T_1}$+Na$^+$), dimer (2$\mathbf{T_1}$+Na$^+$), trimer (3$\mathbf{T_1}$+Na$^+$) and tetramer (4$\mathbf{T_1}$+Na$^+$) were observed in chloroform solution. Although quartet structures such as G-Quartets are an important species in the self-assembly of purine nucleosides and nucleotides, and the quartet species from tetrameric pyrimidine-pyrimidine nucleosides and nucleotides usually are considered unstable under natural conditions, in this case, the occurrence of the tetramer (4$\mathbf{T_1}$+Na$^+$) suggested that T-tetramers can be stable in solution. Furthermore, since the substituents attached to the ribose are nearly nonpolar, it is believed that the formation of pyrimidine-pyrimidine tetramer (4$\mathbf{T_1}$+Na$^+$) is based on base-base hydrogen bonding and ion-carbonyl interactions, behaving like T-Quartet and T-tetrad assemblies.

Several common features occurred in the ESI-MS of $\mathbf{T_2}$. Monomer ($\mathbf{T_2}$+Na$^+$) and dimer (2$\mathbf{T_2}$+Na$^+$) were observed in this sample. The signal for trimer (3$\mathbf{T_2}$+Na$^+$) was very weak. No tetramer (4$\mathbf{T_2}$+Na$^+$) was observed probably due to the presence of polar acetyl group on the deoxyribose skeleton, disfavoring the formation of tetramer (tetrad or quartet) structures between thymine-thymine bases.

In contrast to the above $\mathbf{T_1}$ and $\mathbf{T_2}$ samples, $\mathbf{T_3}$ produced significantly different ESI-MS results. As seen in Fig. 1(bottom), many supramolecular complexes but no tetramer (4$\mathbf{T_3}$+Na$^+$) species were observed probably due to the presence of two polar acetyl groups on the ribose. Two distinct features occurred in this sample. One is that both monovalent and divalent cation

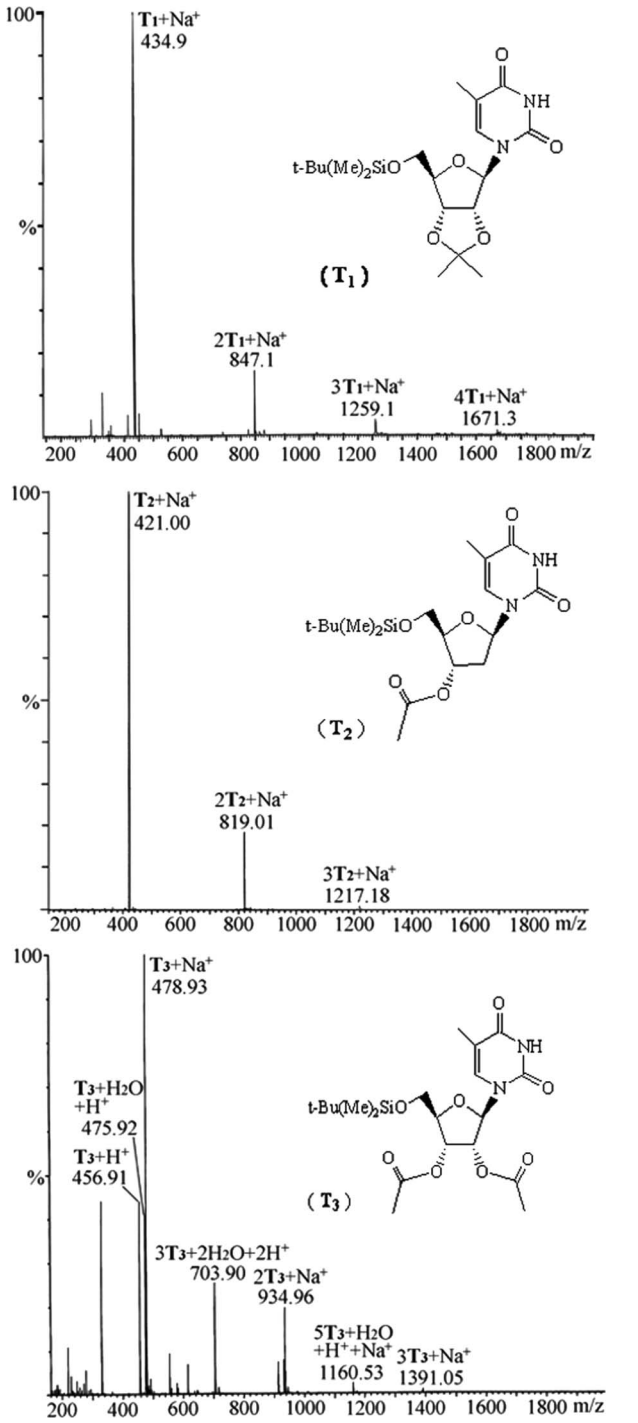

Fig. 1 ESI-MS measurements showing the influences of ribose- and deoxyribose-modified skeletons on the formation of T-tetramer species templated by sodium cation (NaCl) in chloroform.

signals were observed, and the other is that water molecules readily participated in the formation of these supramolecular complexes.

The slow evaporation of the 2-propanol solution of $\mathbf{T_2}$ at 5 °C yielded a single crystal suitable for X-ray analysis after a period of 7–8 weeks (Fig. 2).[29] CCDC reference number for $\mathbf{T_2}$ is 622349. In contrast to conventional hydrogen-bonding patterns occurring between nucleobases, as seen in Fig. 2, the H-bond donor arises from imido NH on the thymine base, and the H-bond acceptor

‡ Three samples ($\mathbf{T_1}$, $\mathbf{T_2}$ and $\mathbf{T_3}$) were prepared at the same concentration of 5×10^{-3} M in chloroform containing several grains of NaCl. ESI mass spectra were obtained by using Applied Biosystems/MDS Sciex QSTAR XL (ESI, TurboIonspray, APCI, nanospray) with an Agilent HP1100 Cap-LC system. All experiments were carried out in the positive-ion mode and the scanned mass range was set at 0—2000 u. Ring lens voltage, as an important parameter, was set at 30 V. Further electrospray ionization MS analysis for the influences of alkali metal ions on the formation of $\mathbf{T_1}$-tetramers was performed on a Micromass Q-TOF mass spectromer (Waters). Five samples for $\mathbf{T_1}$ were prepared at the same concentration of 5×10^{-3} M in acetonitrile containing several grains of alkali metal chloride salt (LiCl, NaCl, KCl, RbCl and CsCl, respectively). After ultrasonic agitation, each solution was used for ESI-MS measurements. The sample was eluted with acetonitrile and the eluent was directly infused the mass spectrometer through the ESI probe. The spray voltage of the mass spectrometer was 3.30 kV and the cone voltage 35 V. The source and desolvation temperatures were set at 353 K and 373 K, respectively. Nitrogen was used as both cone gas and desolvation gas with a flow rate of 50 L h^{-1} and 500 L h^{-1}, respectively. The collision energy was set up to 10 V. Mass Lynx (ver. 4.0) software was used for analysis and post processing.

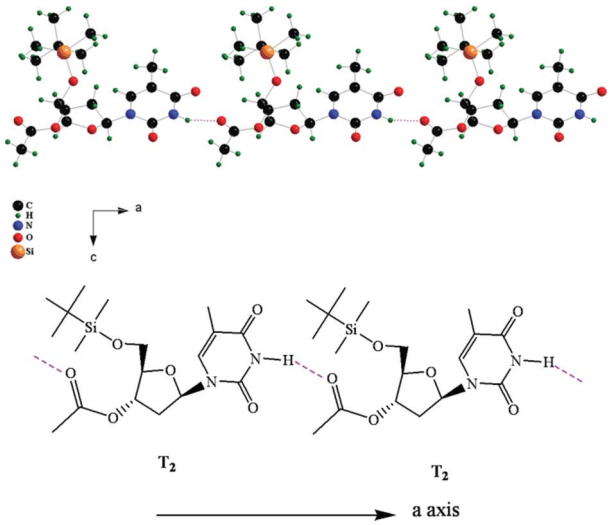

Fig. 2 A view of intermolecular NH…O hydrogen-bond for $\mathbf{T_2}$ (purple dot line, H2A…O6 = 1.99 Å. Symmetry code: −1+x, y, z. N2—H2A…O6: 159.8 (deg); N2…H2A: 0.88 Å; N2…O6: 2.8313(17) Å) (see Supporting Information†).

comes from the acetyl oxygen atom on the deoxyribose skeleton rather than the thymine base, forming a 1 : 1 intermolecular acceptor-to-donor hydrogen bonding chain along a axis.

The solid structue of $\mathbf{T_2}$ could provide further explanation for the ESI-MS behaviors of three thymidine derivatives ($\mathbf{T_1}$, $\mathbf{T_2}$ and $\mathbf{T_3}$). Since $\mathbf{T_1}$ does not have the conventional H-bond acceptor on the ribose, the conventional H-bond interactions occur between thymine bases. The situations would be different in samples $\mathbf{T_2}$ and $\mathbf{T_3}$ due to the presence of the conventional H-bond acceptor on the deoxyribose and ribose skeletons, respectively. This unexpected H-bond mode will disfavor the formation of regular T-tetramer (tetrad or quartet) structures. As the ESI-MS of $\mathbf{T_3}$ showed, many aggregates could be observed due to the presence of two conventional H-bond acceptors on the ribose. Interestingly, the ESI-MS of $\mathbf{T_2}$-NaCl in CH_3CN did not exhibit more signals except $\mathbf{T_2}$+Na^+ (Figure S12†).

Further ESI Q-TOF MS observations concerning the influences of alkali metal ions on the formation of $\mathbf{T_1}$-tetramer species in acetonitrile were presented in Fig. 3 and Fig. 4. The detailed assigments for each signal were arranged in Table S1 and Figures S13–S24.† In this series of alkali metal ions, lithium did not induce $\mathbf{T_1}$-tetramer probably due to its small ion size; sodium, potassium, rubidium and caesium could produce combined MS peaks for $\mathbf{T_1}$-tetramers ($4\mathbf{T_1}$+Na^+, $4\mathbf{T_1}$+K^+, $4\mathbf{T_1}$+Rb^+ and $4\mathbf{T_1}$+Cs^+ monomers) and corresponding dimeric $\mathbf{T_1}$-tetramers ($[4\mathbf{T_1}+Na^+]_2$, $[4\mathbf{T_1}+K^+]_2$, $[4\mathbf{T_1}+Rb^+]_2$ and $[4\mathbf{T_1}+Cs^+]_2$), where the most distinct MS peak was observed for $[4\mathbf{T_1}+Rb^+]_2$ (Fig. 4). Although the $\mathbf{T_1}$-tetramers did not exhibit base peak intensities, it is evident that these supramolecular aggregates do occur in solution. In addition, rubidium and caesium produced very weak MS peaks for $\mathbf{T_1}$-pentamers ($5\mathbf{T_1}$+Rb^+ and $5\mathbf{T_1}$+Cs^+ monomers) and corresponding dimeric $\mathbf{T_1}$-pentamers ($[5\mathbf{T_1}+Rb^+]_2$ and $[5\mathbf{T_1}+Cs^+]_2$) as well as heterodimeric multimers such as ($[3\mathbf{T_1}+Rb^+]$+$[4\mathbf{T_1}+Rb^+]$, $[4\mathbf{T_1}+Rb^+]$+$[5\mathbf{T_1}+Rb^+]$, $[3\mathbf{T_1}+Cs^+]$+$[4\mathbf{T_1}+Cs^+]$, $[4\mathbf{T_1}+Cs^+]$+$[5\mathbf{T_1}+Cs^+]$). The formation of tetramers and pentamers templated by rubidium and caesium

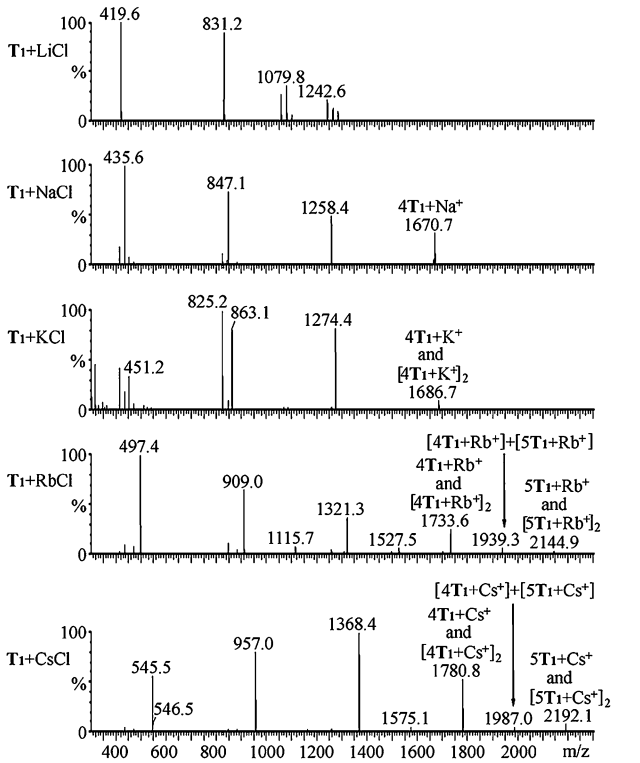

Fig. 3 TOF-ESI-MS measurements showing the influences of alkali metal ions on the formation of $\mathbf{T_1}$-tetramers and dimeric $\mathbf{T_1}$-tetramers in acetonitrile.

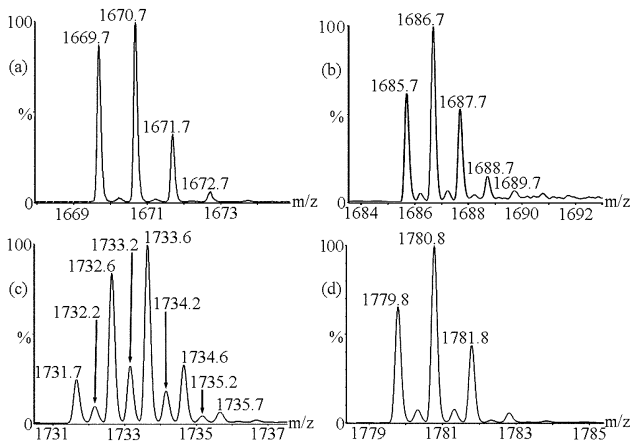

Fig. 4 Positive ion-mode TOF-ESI-MS sections for $\mathbf{T_1}$-tetramers and corresponding dimeric $\mathbf{T_1}$-tetramers in acetonitrile. (a): combined signal of $4\mathbf{T_1}$+Na^+ monomer and dimer; (b) combined signal of $4\mathbf{T_1}$+K^+ monomer and dimer; (c) combined signal of $4\mathbf{T_1}$+Rb^+ monomer and dimer; (d) combined signal of $4\mathbf{T_1}$+Cs^+ monomer and dimer.

might be due to their relative larger cation sizes. These results were significantly in contrast to the very recent report that thymine derivatives trend to form quintets by potassium, rubidium and caesium cation templates,[12] suggesting remarkable structural and metal ion size effects on the formation of $\mathbf{T_1}$-tetramer and $\mathbf{T_1}$-pentamer aggregates.

As seen in Table S1 and Figures S13–S24,† the experimental values were well consistent with calculated ones for each monomer and dimer (Figures S13 and S14†), gradual larger

differences between experimental values and calculated ones occurred through trimers to pentamers. These differences might be attributed to the fact that there are less H-bond interactions in $\mathbf{T_1}$-tetramer species so that they may behave as relatively looser assemblies relative to G-Quartets and G-Quadruplexes. Indeed, these significant differences between the experimental ESI-MS values and the corresponding calculated ones could also be observed in G-Quartets,[26] G-Quadruplexes[30,31] and other supramolecular systems.[32,33]

Conclusions

Ribose- and deoxyribose-modified thymidine derivatives have been investigated for the formation of T-Quartets (T-tetrads or T-tetramers) by ESI Q-TOF MS and X-ray observations. It was demonstrated that the formation of T-tetramers is structure and metal ion size dependent, that is, the conventional H-bond acceptors on the ribose and deoxyribose skeletons may disfavor the formation of T-tetramers, and in the series of alkali metal ions, lithium did not induce T-tetramer due to its small ion size so that one lithium ion could not correlate with four thymidine molecules simultaneously. Sodium, potassium, rubidium and caesium could produce T-tetramers, where the most distinct MS signal was observed for rubidium-induced $4\mathbf{T_1}+Rb^+$ dimer. Nevertheless, as tetramer, tetrad and quartet structures represent gradual ordered aggregates, in this case, alkali metal ion-templated T-tetramers may behave like T-Quartet or T-tetrad assemblies. In addition, rubidium and caesium could also induce T-pentamers and dimeric pentamers probably due to their larger ion sizes. Thus, This finding may help to understand the structural and functional discrepancies of DNA and RNA components.

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