Trivalent lanthanide metal ions promote formation of stacking G-quartets†

Irene C. M. Kwan, Yi-Min She and Gang Wu*

Received (in Austin, TX, USA) 6th July 2007, Accepted 25th July 2007
First published as an Advance Article on the web 10th August 2007
DOI: 10.1039/b710299b

We report the first examples of stacking G-quartet formation assisted by trivalent lanthanide metal ions (La^{3+}, Eu^{3+}, Tb^{3+}, Dy^{3+}, Tm^{3+}).

In recent years, the G-quartet has attracted considerable attention in various areas of research, ranging from molecular biology to nanotechnology.[1,2] With only a few exceptions,[3–5] G-quartet formation generally requires the presence of metal ions. To date, only monovalent (Na^+, K^+, Rb^+, NH_4^+, Tl^+) and divalent (Sr^{2+}, Ba^{2+}, Pb^{2+}) cations have been found to assist G-quartet formation.[1,2] Here we report the first examples of G-quartet formation promoted by trivalent lanthanide metal ions (La^{3+}, Eu^{3+}, Tb^{3+}, Dy^{3+}, Tm^{3+}).

Fig. 1 shows the electrospray ionization (ESI) MS spectra of 2′,3′,5′-*O*-triacetylguanosine (G) complexes with trivalent lanthanide metal ions.‡§ For the La^{3+} and Eu^{3+} complexes, the predominant species was a G dodecamer containing only one metal ion, $[G_{12} + M]^{3+}$, whereas for the Tb^{3+}, Dy^{3+} and Tm^{3+} complexes, both dodecamer and octamer formations had large relative abundances. In each case, clusters corresponding to $[G_n + M]^{3+}$ (n = 9, 10, 11, 13, 14) were also observed, but mostly with much weaker abundances (<10%). It is interesting to note that the ESI-MS spectra shown in Fig. 1 do not contain any cluster where $n < 8$, indicating that the G octamers were very stable. In the ESI-MS spectrum of the Eu^{3+} complex, an additional peak was observed between the signals for $[G_{11} + Eu]^{3+}$ (*m*/*z* 1551.418) and $[G_{12} + Eu]^{3+}$ (*m*/*z* 1686.809). This peak (*m*/*z* 1656.995) was assigned to $[G_8 + K + H]^{2+}$.

To further investigate whether the observed $[G_8 + M]^{3+}$ and $[G_{12} + M]^{3+}$ clusters were indeed related to G-quartet formation, we subsequently performed ESI-MS/MS experiments. Fig. 2 shows the ESI-MS/MS spectra of $[G_{12} + Tb]^{3+}$ (*m*/*z* 1689.476) and $[G_8 + Tb]^{3+}$ (*m*/*z* 1143.964) as examples. Collision induced dissociation of the G dodecamer at a low energy led to fragments of 11-mer, 10-mer, 9-mer and 8-mer, whereas a further breakdown of the G octamer gave rise to 7-mer, 6-mer, 5-mer and 4-mer (G-quartet). It should be noted that, because the fragments of $[G_8 + Tb]^{3+}$ are +2 ions, their MS signals do not appear on one side of the signal from the parent +3 ion. These observations are consistent with the expectation that the G-quartet is a basic unit of these clusters.

Department of Chemistry, Queen's University, 90 Bader Lane, Kingston, Ontario, Canada K7L 3N6. E-mail: gang.wu@chem.queensu.ca; Fax: +1 613 533 6669; Tel: +1 613 533 2644

† Electronic supplementary information (ESI) available: ^{1}H NMR spectra of the G complexes with Na^+, K^+, Rb^+ and La^{3+}, electrospray ionization MS/MS spectra for all complexes, excitation and emission spectra for the Tb^{3+} complex. See DOI: 10.1039/b710299b

The solution ^{1}H NMR spectrum of the diamagnetic La^{3+} complex (Fig. 1S†) exhibited complex spectral features, similar to those observed for the G complexes containing Na^+, K^+ and Rb^+.[6] For the La^{3+} complex, NOE cross peaks were observed between H_8 and the imino/amino protons, which is a signature feature of G-quartet formation. The diffusion-ordered spectroscopy (DOSY) NMR experiments[7,8] indicated that the translational diffusion coefficient of the G/La^{3+} complex in $CDCl_3$ at 298 K (2.74×10^{-10} m^2 s^{-1}) is smaller than those of the Na^+, K^+ and Rb^+ complexes (*ca.* 3.50×10^{-10} m^2 s^{-1}), suggesting that the G/La^{3+} aggregates are larger. The complex ^{1}H NMR spectrum may be due to different modes of G-quartet stacking. The sensitized luminescence excitation spectrum¶ of the Tb^{3+} complex in $CHCl_3$

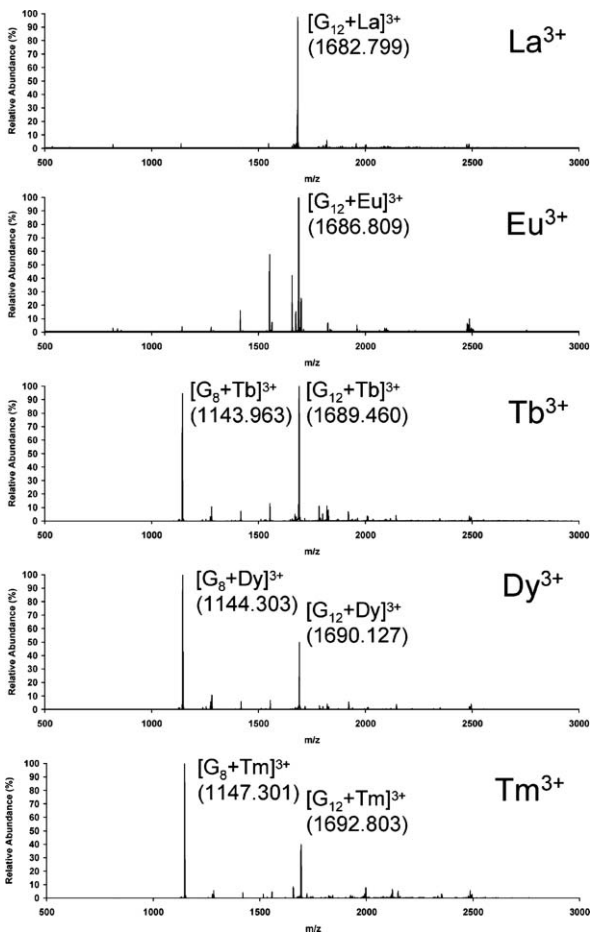

Fig. 1 High resolution ESI-MS spectra of the G complexes containing various trivalent lanthanide metal ions. The mass accuracy of these measurements is within 8 ppm.

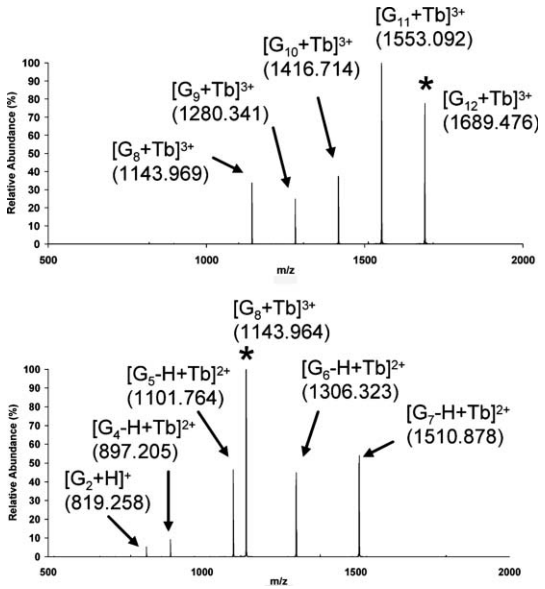

Fig. 2 ESI-MS/MS spectra of the Tb^{3+} complexes: $[G_{12} + Tb]^{3+}$ (top) and $[G_8 + Tb]^{3+}$ (bottom). The parent ion is marked by an asterisk (*).

(Fig. 2S†) exhibits characteristic peaks at 492 ($^5D_4 \rightarrow {}^7F_6$), 545 ($^5D_4 \rightarrow {}^7F_5$) and 584 ($^5D_4 \rightarrow {}^7F_4$) nm. Because guanosine monomers do not enhance Tb^{3+} fluorescence,[9] the above observation is consistent with the formation of a complex between G and Tb^{3+} that leads to G $\rightarrow$ Tb^{3+} energy transfer.

It is clear from the ESI-MS results that trivalent lanthanide metal ions can promote the formation of G octamers and G dodecamers. Fig. 3 shows the different modes of ion binding in G-quartet structures. To date, the in-plane binding mode has been observed only for Na^+ ions in ESI-MS spectra[10] and crystal structures.[11,12] The most common mode of ion binding is the sandwich mode, where a metal ion is sandwiched between two G-quartet planes.[13] For trivalent metal ions, the observation of a G dodecamer containing only one metal ion suggests a triple-decker structure, as illustrated in Fig. 3. The dodecamer structure represents a new mode of ion binding in G-quartet structures. This triple-decker cluster is quite stable, presumably due to the very strong ion dipole interactions between trivalent cations and O6 carbonyl oxygen atoms from the guanine bases. It is also likely that the G-quartets in the octamers and dodecamers containing trivalent cations are more closely stacked than those in the octamers containing mono- and divalent cations. This, in turn, would further enhance the stability of the stacking G-quartets in the former complexes.

We also noted a correlation between the relative abundance of dodecamers and octamers, and the ionic radius of the metal ion.

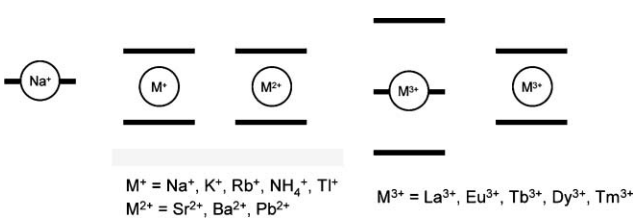

Fig. 3 Illustration of the modes of ion binding in G-quartet structures. The bar represents a G-quartet plane.

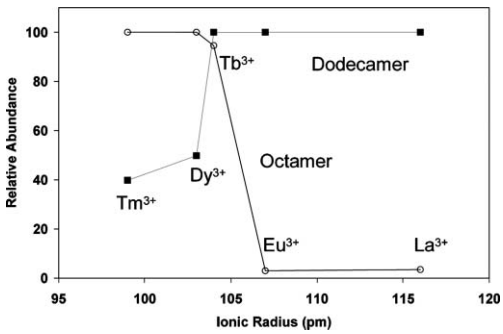

Fig. 4 Relationship between the relative abundance of G dodecamer and G octamer, observed in ESI-MS spectra, and the ionic radius of the metal ion. All ionic radii correspond to a coordination number of 8.[14]

As shown in Fig. 4, the relative abundance of dodecamers decreases as the ionic radius of the metal ion decreases from La^{3+} to Tm^{3+}. This suggests that, compared with other lanthanide ions, La^{3+} has an optimal size to fit into the G-quartet plane to form a very stable ion-filled G-quartet. This is quite similar to what has been found for Na^+ ions.[10–12] The ionic radius of Na^+ is 118 pm (for a coordination number of 8),[14] which is nearly identical to that of La^{3+}, 116 pm. Therefore, it is not surprising that the G dodecamer containing a La^{3+} ion is the most stable one. The fact that the Na^+–carbonyl interaction is much weaker than the La^{3+}–carbonyl interaction is likely to be the reason why a G dodecamer containing a single Na^+ ion has never been detected. Because all ESI-MS spectra were obtained under very similar conditions, we may use the extent of fragmentation as an indicator for G dodecamer stability. For example, in the MS spectrum of the La^{3+} complex, only the signal from the G dodecamer was observed, indicating very little fragmentation. However, in the MS spectrum of the Tb^{3+} complex, the signals from the dodecamer and octamer had almost equal intensity, suggesting that the G dodecamer containing a Tb^{3+} ion was not as stable as its La^{3+} analog. Therefore, we conclude that the stability of G dodecamer formation is in the following order: $La^{3+} > Eu^{3+} > Tb^{3+} > Dy^{3+} > Tm^{3+}$, and that the stability of octamer formation is in the reverse order.

In summary, we have discovered for the first time that trivalent lanthanide metal ions can also facilitate G-quartet formation. We have also observed a new mode of metal ion binding in G-quartet structures, *i.e.*, a triple-decker G dodecamer containing a single metal ion in the central G-quartet. Our findings open up new possibilities in regard to designing the electronic, magnetic and photonic properties of guanosine-based materials for nanotechnology. The biological relevance of our findings are unclear at present; further investigations into the possible role of trivalent metal ions in G-quadruplex DNA are under way in our laboratory.

This work was supported by the NSERC of Canada. All ESI-MS spectra were obtained at the Queen's University MassSpec Facility, which is supported by the Canada Foundation for Innovation (CFI). We thank Professor Suning Wang for access to a fluorescence spectrometer.

Notes and references

‡ 2′,3′,5′-*O*-Triacetylguanosine (98% purity) was purchased from Sigma-Aldrich and used without further purification. G–M^{3+} complexes were

prepared using a liquid–solid extraction method in $CHCl_3$. Metal chlorides were used as the source of metal ions. After 24 h of extraction, the organic phase was collected and subsequently dried under vacuum.

§ ESI-MS spectra were recorded with an Applied Biosystems QSTAR XL quadrupole time-of-flight (QqTOF) mass spectrometer in positive mode. ESI-MS data were acquired using Analyst QS 1.1 software. Samples were dissolved in anhydrous nitromethane (CH_3NO_2) and injected with a syringe pump at a flow rate of 5.0 μL min^{-1}. The mass range of single MS measurements was set at *m*/*z* 300 to 6000. To observe $[G_n + M]^{3+}$ complexes, the declustering potential (DP) was set to 20 V during the MS experiment. Subsequent tandem mass spectrometry (MS/MS) measurements were performed using nitrogen as the collision gas. A collision induced dissociation (CID) energy of 35 eV was applied to break down the G dodecamers and G octamers. The mass range of MS/MS measurements was set at *m*/*z* 100 to 4000. For spectral assignment, theoretical MS peaks were generated using Data Explorer v. 4.0.0.0 (Applied Biosystems, 1997–2000).

¶ Excitation and emission spectra were recorded on a Photon Technology International (PTI) QuantaMaster model C-60 spectrometer at room temperature using the software FeliX (v. 1.4, 1999) supplied by PTI.

1 J. T. Davis, *Angew. Chem., Int. Ed.*, 2004, **43**, 668–698.
2 *Quadruplex Nucleic Acids*, ed. S. Neidle and S. Balasubramanian, Royal Society of Chemistry, Cambridge, UK, 2006.
3 J. L. Sessler, M. Sathiosatham, K. Doerr, V. Lynch and K. A. Abboud, *Angew. Chem., Int. Ed.*, 2000, **39**, 1300–1303.
4 F. W. Kotch, V. Sidorov, K. Kayser, Y. F. Lam, M. S. Kaucher, H. Li and J. T. Davis, *J. Am. Chem. Soc.*, 2003, **125**, 15140–15150.
5 R. Otero, M. Schock, L. M. Molina, E. Laegsgaard, I. Strensgaard, B. Hammer and F. Besenbacher, *Angew. Chem., Int. Ed.*, 2005, **44**, 2270–2275.
6 X. Liu, I. C. M. Kwan, S. Wang and G. Wu, *Org. Lett.*, 2006, **8**, 3685–3688.
7 A. Wong, R. Ida, L. Spindler and G. Wu, *J. Am. Chem. Soc.*, 2005, **127**, 6990–6998.
8 M. S. Kaucher, Y.-F. Lam, S. Pieraccini, G. Gottarelli and J. T. Davis, *Chem.–Eur. J.*, 2005, **11**, 164–173.
9 C. Formoso, *Biochem. Biophys. Res. Commun.*, 1973, **53**, 1084–1087; D. P. Ringer, S. Burchett and D. E. Kizer, *Biochemistry*, 1978, **17**, 4818–4825; M. D. Topal and J. R. Fresco, *Biochemistry*, 1980, **19**, 5531–5537.
10 K. Fukushima and H. Iwahashi, *Chem. Commun.*, 2000, 895–896.
11 G. Laughlan, A. I. H. Murchie, D. G. Norman, M. H. Moore, P. C. E. Moody, D. M. J. Lilley and B. Luisi, *Science*, 1994, **265**, 520–524; K. Phillips, Z. Dauter, A. I. H. Murchie, D. M. J. Lilley and B. Luisi, *J. Mol. Biol.*, 1997, **273**, 171–182.
12 M. P. Horvath and S. C. Schultz, *J. Mol. Biol.*, 2001, **310**, 367–377.
13 G. Wu and A. Wong, in *NMR Spectroscopy of Biological Solids*, ed. A. Ramamoorthy, CRC Press, Boca Raton, FL, 2006, ch. 13, pp. 317–344.
14 R. D. Shannon, *Acta Crystallogr., Sect. A: Cryst. Phys., Diffr., Theor. Gen. Cryst.*, 1976, **32**, 751–767.