1:1 Complex of guanine quartet with alkali metal cations detected by electrospray ionization mass spectrometry

Kazuaki Fukushima and Hideo Iwahashi*

*Wakayama Medical College, 811-1 Kimiidera, Wakayama 641-8509 Japan. E-mail: chem1@wakayama-med.ac.jp*

Selective 1:1 complex formation of a square quartet of 9-ethylguanine with small alkali metal cations (Li+, Na+) was detected by electrospray mass spectrometry

The guanine base is unique as a pair of hydrogen-donating groups (NH and NH_2) and a pair of hydrogen-accepting groups (C=O and N) are arranged on adjacent edges of the molecule [Fig. 1(a)]. Thus, a guanine base can form a self-complementary, hydrogen-bonded square quartet [Fig. 1(b)], which has been widely found in gels of guanosine derivatives $1^{1,2}$ and in parallel[3] and antiparallel[4] quadruple strands of guanine-rich oligonucleotides such as telomeric DNA.
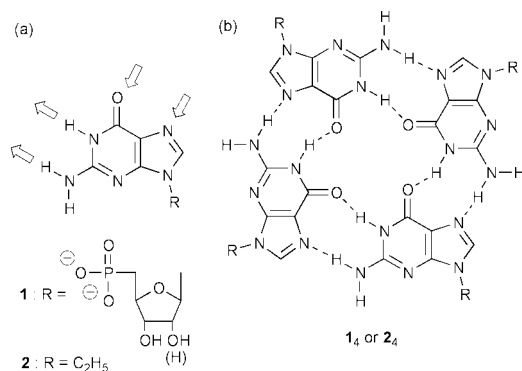

Fig. 1 (a) Guanosine-5′-monophosphate **1** and 9-ethylguanine **2**, (b) guanine quartet (1_4 and 2_4).

Pinnavaia *et al.* and Laszlo and coworkers have independently reported that the aggregate of guanosine-5′-monophosphate (5′-GMP) in the presence of K+ is a 2:1 complex (a sandwich complex) composed of two stacked 1_4 and one K+ ion [Fig. 2(a)].[5] In order to stabilize the sandwich structure, chelation of cations with phosphate groups is essential,[5b,c] although stacking interactions between guanine bases and ion–dipole interaction between K+ and carbonyl groups may also be important. In contrast to 5′-GMP, 9-alkylguanines do not have the ability to show such chelation. In this work, we have detected a 1:1 complex of guanine quartets with alkali metal cations by electrospray ionization mass spectrometry (ESI-MS)[6] in the system of 9-ethylguanine **2**.†
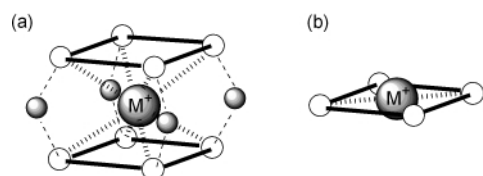
Fig. 2 Schematic representations of (a) a 2:1 complex (a sandwich complex) and (b) a 1:1 complex of a guanine quartet with alkali metal cations: O, guanine base; (······), interaction between alkali metal cation (M+) and carbonyl group; (——), hydrogen bonding between guanine bases; (-----), chelation of alkali metal cation (◉) with phosphate groups.

The ESI-MS spectrum of **2** [Fig. 3(a)] shows prominent peaks at *m/z* 202, 381 and 739, corresponding to $[2 + Na]^+$, $[2_2 + Na]^+$ and $[2_4 + Na]^+$ in the presence of 0.1 mM $NaClO_4$. Other $[2_n +$
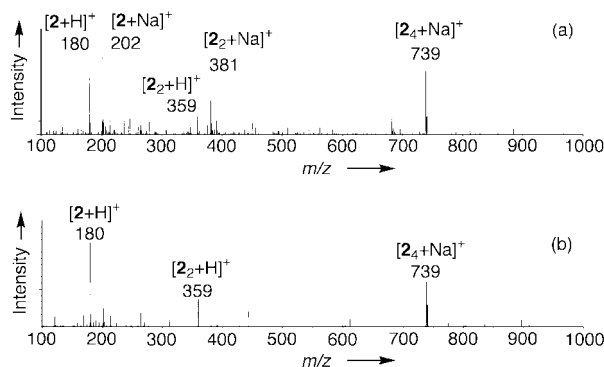
Fig. 3 ESI-MS spectra of 9-ethylguanine **2**: in the presence of (a) 0.1 mM and (b) 0.01 mM $NaClO_4$.

$Na]^+$ ($n = 3$ and $n \geqslant 5$) complexes were scarcely observed under these conditions. When the concentration of Na+ was reduced to 0.01 mM [Fig. 3(b)], only the $[2_4 + Na]^+$ ion was observed. The above results indicate that the 1:1 complex of the guanine quartet (2_4) with sodium cation, $[2_4 + Na]^+$, is remarkably stable.

Similarly, **2** also gave the corresponding $[2_4 + M]^+$ along with $[2 + M]^+$ and $[2_2 + M]^+$ in the presence of 0.1 mM of other alkali metal cations (Li+, K+, Rb+ and Cs+) (Fig. 4). The peak intensity ratios of $[2_4 + M]^+/[2 + M]^+$ for Li+ and Na+ (Li+, 0.69 and Na+, 0.80) are considerably higher than those of the other alkali
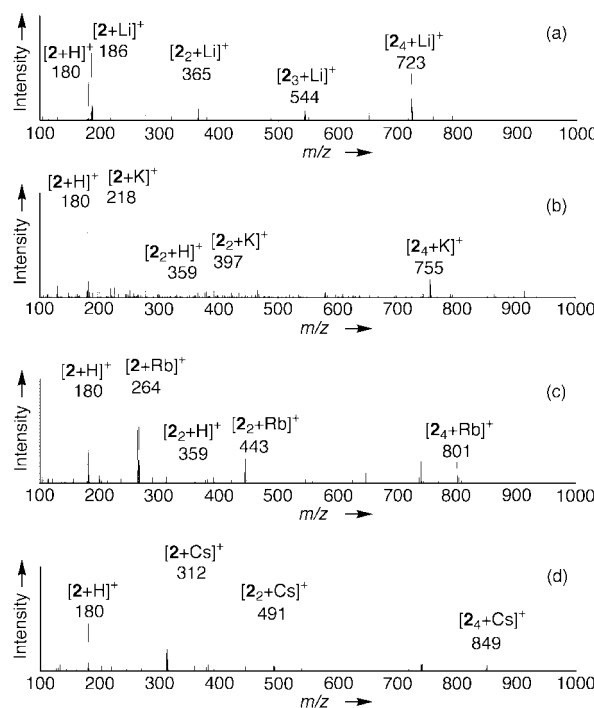
Fig. 4 ESI-MS spectra of 9-ethylguanine **2** in the presence of 0.1 mM $MClO_4$: (a) M = Li, (b) M = K, (c) M = Rb and (d) M = Cs.

metals (K$^+$, 0.20; Rb$^+$, 0.20; and Cs$^+$, 0.16), suggesting that Li$^+$ as well as Na$^+$ form a stable $[\mathbf{2}_4 + \text{M}]^+$ complex. To confirm this tendency, ESI-MS measurements were performed in the presence of a pair of cations both 0.1 mM (Na$^+$–Li$^+$, Li$^+$–K$^+$, K$^+$–Rb$^+$, or Rb$^+$–Cs$^+$). The relative peak intensities of $[\mathbf{2}_4 + \text{M}]^+$ increased as Na$^+$ > Li$^+$ > K$^+$ $\gg$ Rb$^+$, Cs$^+$.

NMR studies of 2:1 complexes $[(\mathbf{1}_4)_2 + \text{M}]^+$ showed that the stability increases in the order K$^+$ > Na$^+$, Rb$^+$ $\gg$ Li$^+$, Cs$^+$.[5a] The K$^+$-selectivity in the 2:1 complex shows that size of the cavity between two 5′-GMP quartets ($\mathbf{1}_4$) is appropriate to accommodate K$^+$ [Fig. 2(a)].[5c] On the other hand, our results indicate that Li$^+$ and Na$^+$ bind with $\mathbf{2}_4$ more strongly than does K$^+$.[7] The selectivity for Na$^+$ and Li$^+$ is possibly explained by a correspondence between the size of M$^+$ and that of the central cavity of $\mathbf{2}_4$. Average distances from the center of $\mathbf{2}_4$ to carbonyl oxygens are estimated to be 2.41 Å by molecular orbital calculation,[8,9] close to the experimentally observed Na–O distance (2.34 ± 0.02 Å) by X-ray analysis.[4c] Accordingly, Na$^+$ can fit exactly in the central cavity of $\mathbf{2}_4$. Li$^+$ (Li–O, 2.04–2.24 Å[10]) may also be located inside the cavity. On the other hand, K$^+$, Rb$^+$ and Cs$^+$ (K–O, 2.75–2.81 Å;[11] Rb–O, 2.88–2.93 Å;[11] and Cs–O, 3.06–3.18 Å[12]) are too large to fit in the central cavity of $\mathbf{2}_4$ and would be located out-of-plane.

In conclusion, we have observed 1:1 complexes of $\mathbf{2}_4$ with alkali metal cations in the systems of 9-ethylguanine $\mathbf{2}$ using ESI–MS. The sodium complex $[\mathbf{2}_4 + \text{Na}]^+$ was the most stable, in contrast to the K$^+$-selective 2:1 complex of $\mathbf{1}_4$. The differences in the composition and in the cation selectivity between $[(\mathbf{1}_4)_2 + \text{M}]^+$ and $[\mathbf{2}_4 + \text{M}]^+$ may be ascribed to the difference in stacking ability of $\mathbf{1}_4$ and $\mathbf{2}_4$. For $\mathbf{2}_4$, the absence of phosphate groups may significantly reduce the stacking interaction in comparison with $\mathbf{1}_4$. Therefore, the stability of $[\mathbf{2}_4 + \text{M}]^+$ depends on the size of M$^+$ which is located in the central cavity of $\mathbf{2}_4$.

Notes and references

† *General procedure.* 9-ethylguanine $\mathbf{2}$ was purchased from Sigma and purified by reverse phase HPLC. The column (150 mm × 4.6 mm i.d.) packed with TSKgel ODS-120T (5 μm particle size) (Tosoh) was used in the LC-MS system. The solvents used in the LC-MS system were MeOH–H$_2$O (1:9, v/v) containing MClO$_4$ (M = Li, Na, K, Rb or Cs). 100 μl of 1.0 mM of an aqueous solution of $\mathbf{2}$ was applied to the HPLC. The flow rate was 1.0 ml min^{-1}. Mass spectra of the 9-ethylguanine fraction were obtained by introducing the effluent into the ESI-MS apparatus, a Hitachi M–1200HS mass spectrometer, just before the peak was eluted. The flow-rate of the pump was kept at 50 μl min^{-1} while the effluent was introduced into the ESI-MS apparatus.

1 W. Guschlbauer, J. F. Chantot and D. Thiele, *J. Biomol. Struct. Dyn.*, 1990, **8**, 491.
2 M. Gellert, M. N. Lipsett and D. R. Davies, *Proc. Natl. Acad. Sci. USA*, 1962, **48**, 2013.
3 D. Sen and W. Gilbert, *Nature*, 1988, **334**, 364; F. Aboul-ela, A. I. H. Murchie and D. M. J. Lilley, *Nature*, 1992, **360**, 280; 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.
4 (*a*) W. I. Sundquist and A. Klug, *Nature*, 1989, **342**, 825; (*b*) F. W. Smith and J. Feigon, *Nature*, 1992, **356**, 164; (*c*) C. Kang, X. Zhang, R. Ratliff, R. Moyzis and A. Rich, *Nature*, 1992, **356**, 126.
5 (*a*) T. J. Pinnavaia, C. L. Marshall, C. M. Mettler, C. L. Fisk. H. T. Miles and E. D. Becker, *J. Am. Chem. Soc.*, 1978, **100**, 3625; (*b*) E. Bouhoutsos-Brown, C. L. Marshall and T. J. Pinnavaia, *J. Am. Chem. Soc.*, 1982, **104**, 6576; (*c*) C. Detellier and P. Laszlo, *J. Am. Chem. Soc.*, 1980, **102**, 1135.
6 ESI-MS has been successfully applied to detection of weakly associated complexes: M. Yamashita and J. B. Fenn, *J. Phys. Chem.*, 1984, **88**, 4451; M. Yamashita and J. B. Fenn, *J. Phys. Chem.*, 1984, **88**, 4671; C. M. Whitehouse, R. N. Dreyer, M. Yamashita and J. B. Fenn, *Anal. Chem.*, 1985, **57**, 675; J. B. Fenn, M. Mann, C. K. Meng, S. F. Wong and C. M. Whitehouse, *Science*, 1989, **246**, 64.
7 Successful assessment of correlation of solution and gas phase complexation of macrocyclic compounds with alkali metal cations by ESI-MS has been reported: K. Wang and G. W. Gokel, *J. Org. Chem.*, 1996, **61**, 4693; E. Leize, A. Jaffrezic and A. V. Dorsselaer, *J. Mass Spectrom.*, 1996, **31**, 537.
8 Density functional calculations (pBP/DN**) were carried out using SPARTAN 5.0, Wavefunction Inc., Irvine, CA.
9 A. D. Becke, *Phys. Rev. A*, 1988, **38**, 3098; J. P. Perdew, *Phys. Rev. B*, 1986, **33**, 8822.
10 I. L. Karle, *J. Am. Chem. Soc.*, 1974, **96**, 4000.
11 T. Sakamaki, Y. Iitaka and Y. Nawata, *Acta Crystallogr., Sect. B*, 1976, **32**, 768; Y. Iitaka, T. Sakamaki and Y. Nawata, *Chem. Lett.*, 1972, 1225.
12 T. Sakamaki, Y. Iitaka and Y. Nawata, *Acta Crystallogr., Sect. B*, 1977, **33**, 52.