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Gas-phase formation and reactions of radical cations of guanosine, deoxyguanosine and their homodimers and heterodimers☆

Linda Feketeová[a,b,c], Elizabeth Yuriev[d], John D. Orbell[e,*], George N. Khairallah[a,b], Richard A.J. O'Hair[a,b,c,**]

[a] School of Chemistry, The University of Melbourne, Victoria 3010, Australia
[b] Bio21 Institute of Molecular Science and Biotechnology, The University of Melbourne, Victoria 3010, Australia
[c] ARC Centre of Excellence for Free Radical Chemistry and Biotechnology, Australia
[d] Medicinal Chemistry and Drug Action, Monash Institute of Pharmaceutical Sciences, Monash University, Parkville, Victoria 3052, Australia
[e] School of Engineering & Science, Victoria University, Werribee, Victoria 3030, Australia

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ABSTRACT

Electrospray ionisation of methanolic solutions containing a mixture of the nucleoside deoxyguanosine, dG, incubated with $Cu(NO_3)_2$ resulted in the formation of a range of ions, including doubly charged copper nucleoside complexes $[Cu^{II}dG_n]^{2+}$, with n ranging from 2 to 10. Collision-induced dissociation of these complexes proceeds via a number of different pathways that depend on the size of the cluster, *n*. When $n = 3$, monomeric radical cations are formed via redox processes. When $n = 4$, dimeric radical cations are formed. Related complexes are formed for the nucleoside guanosine, Gs, and these $[Cu^{II}Gs_n]^{2+}$ complexes fragment in similar fashions to their $[Cu^{II}dG_n]^{2+}$ counterparts. A key finding is that the radical cations of dG and Gs have fragmentation patterns that depend on the way they are formed. Thus radical cations, $dG^{\bullet+}$ and $Gs^{\bullet+}$, formed directly in the electrospray ionisation source or via collision-induced dissociation of $[Cu^{II}dG_3]^{2+}$ and $[Cu^{II}Gs_3]^{2+}$ complexes fragment in the same way, giving the radical cation of the guanine base at *m/z* 151 via cleavage of the *N*-glycosidic bond. In contrast, the collision-induced dissociation spectra of radical cations formed via the sequences $[Cu^{II}dG_4]^{2+} \rightarrow dG_2^{\bullet+} \rightarrow dG^{\bullet+}$ and $[Cu^{II}Gs_4]^{2+} \rightarrow Gs_2^{\bullet+} \rightarrow Gs^{\bullet+}$ are dominated by the loss of CH_2O and further loss of $C_2H_3O_2$ from the sugar moiety. These different fragmentation reactions are attributed to different tautomeric structures of the radical cations. Quantum chemical calculations were carried out on possible structures of the radical cation dimer of the model 9-methylguanine. Three low energy structures were found. Two of these represent base pairs of the kind found in supramolecular motifs of guanine derivatives, and one of these possesses a novel tautomeric structure that may have important biological implications.

1. Introduction

Metal ions and metal complexes bind to nucleic acids at a range of different sites [1–8], and can also promote supramolecular complex formation *via* hydrogen bonding and/or base stacking interactions [9–17]. Electrospray ionisation (ESI) mass spectrometry of mixtures of metal ions/metal complexes and nucleic acids has proven to be a useful way of uncovering such supramolecular complexes (for a recent review on ESI/MS of metal ion-nucleic acid complexes, see [18]). For example, ESI of solutions of silver(I) and the nucleobase adenine resulted in the formation of polymeric silver adenine clusters of the type $[Ad_x + Ag_y - zH]^{(y-z)+}$ [19,20]. Guanine (G) and its derivatives exhibit a diverse range of supramolecular architectures in the condensed phase, including alkali earth metal templated tetramers, **A**, and "ribbon" structures, **B** [9–11]. ESI mass spectrometry studies of guanine and its derivatives have also revealed a range of complexes, some of which exhibit "magic numbers" [21–26]. An example of such a magic number is the well-known guanosine quartet, which has been observed to assemble around various cations including NH_4^+ and alkali metal cations [12–17]. ESI of mixtures of either deoxyguanosine (dG, **C1**) or guanosine (Gs, **C2**) and transition metals often yields a range of complexes of different stoichiometry, and the gas-phase chemistry of mass-selected complexes can be dependant on the size and nature of the complex. For example, the collision-induced dissociation (CID) spectra of $[Pt^{II}LdG_n]^{2+}$ complexes (where L = 2,2′:6′,2″ terpyridine, terpy, or diethyltriamine, dien) give primary fragmentation channels arising from loss of dG and protonated dG to

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* Corresponding author.
** Corresponding author at: School of Chemistry, The University of Melbourne, Victoria 3010, Australia. Tel.: +61 3 8344 2452; fax: +61 3 9347 5180.
E-mail addresses: John.Orbell@vu.edu.au (J.D. Orbell), rohair@unimelb.edu.au (R.A.J. O'Hair).

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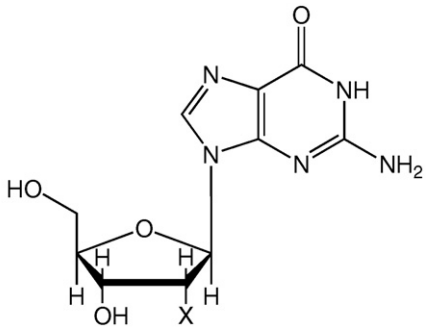
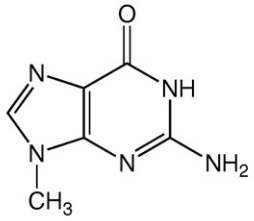
yield the fragment ions $[Pt^{II}LdG_{n-x}]^{2+}$ and $[Pt^{II}LdG_{n-x}\text{-}H]^{+}$, respectively [26]. The relative abundances of the $[Pt^{II}LdG_{n-x}]^{2+}$ fragments depend on the nature of the ligand, L, with the most abundant peaks being observed for $n-x=5$ when L = terpy and $n-x=4$ when L = dien. When a redox active metal such as Cu(II) is used, Cheng and Bohme have shown that monomeric and dimeric radical cations of guanosine, Gs, can be formed via CID of $[Cu^{II}Gs_n]^{2+}$ complexes [25]. This contrasts with previous studies on the fragmentation of copper ternary complexes, $[Cu^{II}LdG]^{2+}$ (L = 2,2′:6′,2″ terpyridine, terpy, or diethyltriamine, dien), where cleavage of the *N*-glycosidic bond occurred in preference to loss of the radical cation [24]. Cheng and Bohme found that the fragmentation of the $[Cu^{II}Gs_n]^{2+}$ complexes is size dependant [25]. When $n=2$, loss of the protonated nucleoside (Eq. (4)) and cleavage of *N*-glycosidic bond (Eqs. (6) and (7)) occurs. With $n=3$, the complexes undergo only charge separation with formation of the monomeric radical cation (Eq. (1)). And with $n=4$, the fragmentation entails the loss of the neutral nucleoside (Eq. (3)) and formation of the dimeric radical cation (Eq. (2)).

Nucleobases recognise one another by specific patterns of hydrogen bonding. Besides the Watson–Crick pairing observed in DNA, a number of non-Watson–Crick/non-canonical hydrogen-bonding motifs have been found to mediate RNA–RNA interactions and create binding sites for proteins and small molecule ligands [27]. It is not clear, however, what roles hydrogen bonding and tautomerism have on the assembly of metal complexes of guanine and its derivatives via ESI/MS and how these factors might influence their subsequent gas-phase chemistry. Thus, here we examine: (i) the CID reactions of a range of homo ($[Cu^{II}dG_n]^{2+}$ and $[Cu^{II}Gs_n]^{2+}$) and hetero $[Cu^{II}dG_nGs_m]^{2+}$ complexes; (ii) the fragmentation reactions of the monomeric nucleoside radical cations; (iii) the fragmentation reactions of the nucleoside homodimeric and heterodimeric radical cations; (iv) possible structures of the dimer radical cation for the model 9-methylguanine (9-MeG, **C3**). Our results suggest an unusual mode of base pairing that plays a role in the formation and fragmentation chemistry of nucleoside dimeric radical cations.



C1 = deoxyguanosine (dG): X = H
C2 = guanosine (Gs): X = HO



C3 = 9-methylguanine (9-MeG)

$$[Cu^{II}Gs_n]^{2+} \rightarrow [Cu^{II}Gs_{n-1}]^{+} + Gs^{\bullet+} \quad (1)$$

$$[Cu^{II}Gs_n]^{2+} \rightarrow [Cu^{II}Gs_{n-2}]^{+} + Gs_2^{\bullet+} \quad (2)$$

$$[Cu^{II}Gs_n]^{2+} \rightarrow [Cu^{II}Gs_{n-1}]^{2+} + Gs \quad (3)$$

$$[Cu^{II}Gs_n]^{2+} \rightarrow [Cu^{II}Gs_{n-2}(Gs\text{-}H)]^{+} + GsH^{+} \quad (4)$$

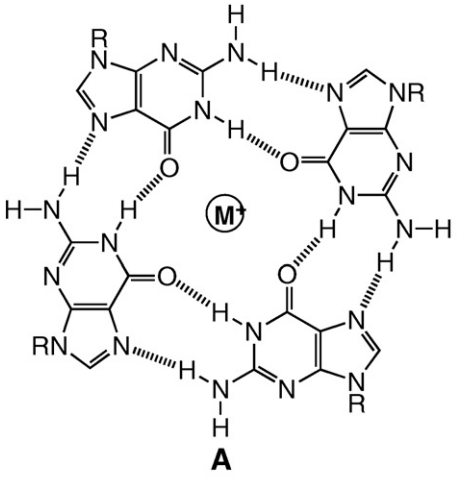
$$[Cu^{II}Gs_n]^{2+} \rightarrow [Cu^{II}Gs_{n-3}(Gs\text{-}H)]^{+} + Gs_2H^{+} \quad (5)$$

$$[Cu^{II}Gs_n]^{2+} \rightarrow [Cu^{II}Gs_{n-1}G]^{2+} + R\text{-}H \quad (6)$$

$$[Cu^{II}Gs_n]^{2+} \rightarrow [Cu^{II}(Gs_{n-1}G)\text{-}H]^{+} + R^{+} \quad (7)$$

2. Experimental

All reagents were used as supplied: $Cu(NO_3)_2$ (Ajax chemicals, 99%), guanosine hydrate (Aldrich, 98%) and deoxyguanosine (Sigma, 99%). Complexes were prepared by mixing 2:1 mM solutions of the nucleosides:$Cu(NO_3)_2$, dissolved in 3:1 methanol:water, right before infusing the reaction mixture into the mass spectrometer.



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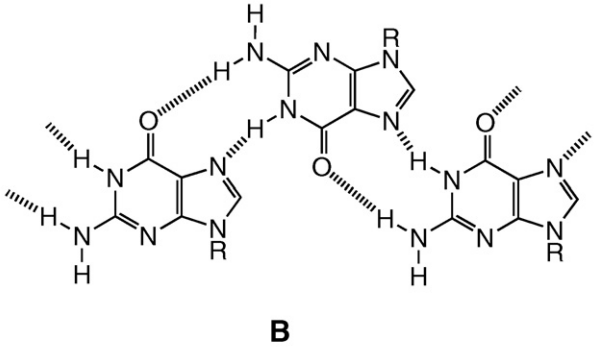

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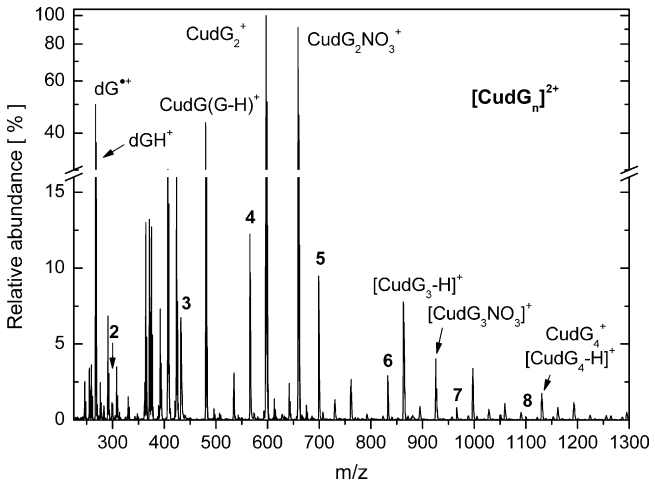


Fig. 1. ESI mass spectrum of a mixture of $Cu(NO_3)_2$ and deoxyguanosine. The doubly charged copper complexes of the form $[Cu^{II}dG_n]^{2+}$ are labelled by the integer numbers $n = 1–8$.

2.1. Mass spectrometry

All experiments were carried out using a commercially available Finnigan-LTQ-FT (Thermo, Bremen, Germany) mass spectrometer equipped with ESI source [28,29] described in detail elsewhere [30]. The samples were introduced into the mass spectrometer at 5.0 μL/min via ESI. Typical ESI conditions used were: spray voltage, 3.3–5.0 kV; capillary temperature, 250 °C; nitrogen sheath pressure, 8–40 (arbitrary units). The capillary voltage and the tube lens offset were tuned to maximise the desired peak. The injection time was set using the automatic gain control function. The LTQ-FT mass spectrometer consists of (i) a linear ion trap (LTQ); (ii) ion transfer optics; and (iii) a FT-ICR mass analyser. For the tandem mass spectrometry (MS/MS) experiments, the desired ions produced via ESI were trapped in the LTQ and subjected to CID at a He bath gas pressure of ca. 5×10^{-3} Torr. CID was carried out by mass selecting the desired ions with a 1.5–6 m/z units window and subjecting them to the following typical conditions: normalised collision energy between 16% and 40%, which determines the translational kinetic energy of the ions; activation (Q), 0.25–0.35, which assigns the radio-frequency (RF) used to fragment ions, and activation time of 30 ms that is the time set to excite the ions via CID. The high resolution of the FT-ICR mass spectrometer was used to confirm the charge states of the mass-selected precursor ions. For high-resolution mass analysis, the ions were transferred via the ion optics transfer region (2×10^{-7} Torr) into a FT-ICR cell at a pressure below 1.5×10^{-9} Torr.

2.2. Molecular modelling of the dimer radical cation

Taking into account various tautomeric forms, a range of possible structures for the dimer radical cation of 9-MeG were initially explored at the PM3 level of theory [31]. From these calculations, three low energy structures were selected for input into DFT optimization at the M052x/6-311+G(3df,2p) level of theory (Gaussian 03; Revision E01 [32]). Frequency calculations were carried out after all PM3 and DFT calculations to confirm that the refined structures corresponded to stable minima.

3. Results and discussion

ESI of mixtures of the nucleosides incubated with $Cu(NO_3)_2$ resulted in the formation of a range of ions and an illustrative spectrum is shown for dG in Fig. 1. The types of ions formed included the

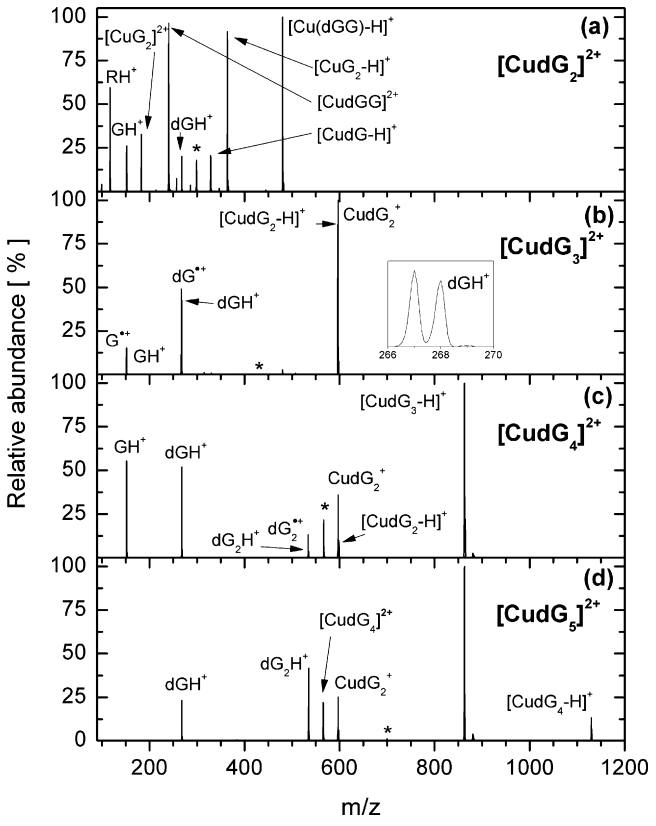


Fig. 2. CID reactions of the homo complexes $[Cu^{II}dG_n]^{2+}$ of deoxyguanosine: (a) $n = 2$; (b) $n = 3$; (c) $n = 4$; (d) $n = 5$. The mass-selected precursor ion is designated with a *.

radical cation and protonated nucleoside and the doubly charged copper nucleoside complexes $[Cu^{II}dG_n]^{2+}$, where n ranges from 2 to 10. Other complexes of high relative abundance present in the ESI spectrum include $[Cu(dGG)-H]^+$ (m/z 480), $CudG^+$ (m/z 597) and $[CudG_3-H]^+$ (m/z 863).

3.1. CID of the homo complexes $[Cu^{II}dG_n]^{2+}$ and $[Cu^{II}Gs_n]^{2+}$

CID of all doubly charged deoxyguanosine complexes $[Cu^{II}dG_n]^{2+}$, where $n = 2–5$, were investigated and are shown in Fig. 2a–d, respectively. The fragmentation of the copper complexes with deoxyguanosine is similar to that of the copper complexes with guanosine previously studied by Cheng and Bohme [25]. However, the relative abundances of fragments are different, mainly for $n = 2$. In the case of dG, the most abundant fragments correspond to *N*-glycosidic bond dissociation, which gives $[Cu^{II}dGG]^{2+}$ (Eq. (6)), $[Cu^{II}G_2]^{2+}$ and $[Cu^{II}(dGG)-H]^+$ (Eq. (7)). The doubly charged copper complex $[Cu^{II}dG_2]^{2+}$ (Fig. 2a) also dissociates by charge separation after interligand proton transfer to give dGH^+ and $[Cu^{II}(dG-H)]^+$ (Eq. (4)) but these are not the most abundant fragments as in the case of Gs reported by Cheng and Bohme [25]. In the case of $n = 3$ (Fig. 2b), the doubly charged complex $[Cu^{II}dG_3]^{2+}$ dissociates by charge separation to give the radical cation of the deoxyguanosine $dG^{\bullet+}$ (cf. Eq. (1)) and charge separation after interligand proton transfer to form dGH^+ and $[Cu^{II}dG_2\text{-}H]^+$ (cf. Eq. (4)). An examination of Fig. 2c reveals that the complex $[Cu^{II}dG_4]^{2+}$ fragments via the following competing processes: intramolecular electron transfer to form radical cation $dG_2^{\bullet+}$ (cf. Eq. (2)); charge separation of the complex (cf. Eq. (4)) to form dGH^+ and its complimentary fragment $[Cu^{II}dG_3\text{-}H]^+$; and another charge separation to give dG_2H^+ and $[Cu^{II}dG_2\text{-}H]^+$ (cf. Eq. (5)). The CID spectrum of the doubly charged copper

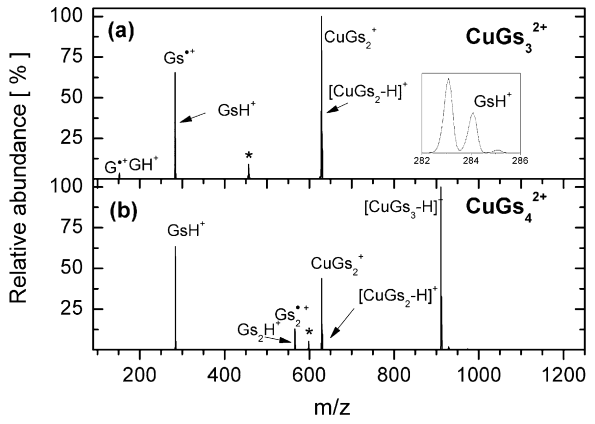


Fig. 3. CID reactions of the homo complexes $[Cu^{II}Gs_n]^{2+}$ of guanosine: (a) $n = 3$; (b) $n = 4$. The mass-selected precursor ion is designated with a *.

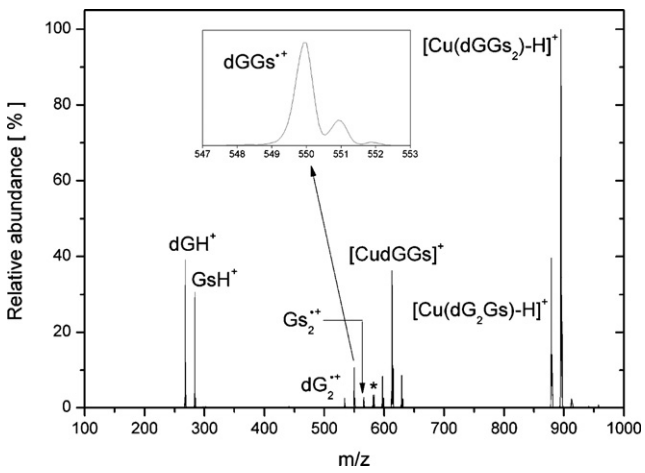


Fig. 4. CID reactions of the hetero complexes $[Cu^{II}dG_2Gs_2]^{2+}$. The mass-selected precursor ion is designated with a *.

complex $[Cu^{II}dG_5]^{2+}$ is shown in Fig. 2d. The fragmentation of this complex proceeds through loss of dG ligand (cf. Eq. (3)) to form $[Cu^{II}dG_4]^{2+}$ and by charge separation after interligand H transfer to give $[Cu^{II}dG_4\text{-}H]^+$ and $[Cu^{II}dG_3\text{-}H]^+$ (cf. Eqs. (4) and (5)). The fragment ion $[Cu^{II}dG_2\text{-}H]^+$ is also observed and the fragmentation of $[Cu^{II}dG_4]^{2+}$ shown in Fig. 2c suggests $[Cu^{II}dG_2\text{-}H]^+$ is a result of charge separation of $[Cu^{II}dG_4]^{2+}$ complex rather than the $[Cu^{II}dG_5]^{2+}$, as the complimentary fragment dG_3H^+ is not observed in the spectra of the $[Cu^{II}dG_5]^{2+}$.

We also re-examined the CID of each of the doubly charged guanosine copper complexes $[Cu^{II}Gs_n]^{2+}$, where $n = 2$–5, and the spectra for $n = 3$, 4 are shown in Fig. 3a and b, respectively. In contrast to dG, in the case of Gs and $n = 2$ (data not shown) the most abundant fragments include GsH^+ (Eq. (4)) and GH^+ as previously observed by Cheng and Bohme [25]. This result suggests that the dG binds more strongly to copper than Gs and is also consistent with the *N*-glycosidic bond in ribose being stronger than that in deoxyribose [33]. Cheng and Bohme reported that $[Cu^{II}Gs_3]^{2+}$ dissociates solely by charge separation to give the radical cation of the guanosine (Eq. (1)) [25]. However, in the present study (Fig. 3a) we find that $[Cu^{II}Gs_3]^{2+}$ also undergoes interligand proton transfer to form GsH^+ and $[Cu^{II}Gs_2\text{-}H]^+$ (Eq. (4)), but that this pathway is less prominent than in the case of dG (Fig. 2b). The CID of the complex $[Cu^{II}Gs_4]^{2+}$ shown in Fig. 3b reveals intramolecular electron transfer to form the dimer radical cation $Gs_2^{\bullet+}$. One of the most abundant fragments of the complex is formation of GsH^+. This fragment does not appear to largely arise from dissociation of the radical dimer $Gs_2^{\bullet+}$ as suggested by Cheng and Bohme [25], but rather seems to result from charge separation of the complex (Eq. (4)), since the complimentary fragment $[Cu^{II}Gs_3\text{-}H]^+$ is also present in the CID spectrum as the most abundant fragment ion. Finally, another charge separation reaction occurs to give the complementary product ions Gs_2H^+ and $[Cu^{II}Gs_2\text{-}H]^+$ (Eq. (5)). The fragmentation of the complex for $n = 5$ ($[Cu^{II}Gs_5]^{2+}$, data not shown) is very similar to the case of dG complex and proceeds through loss of the Gs ligand (Eq. (3)) and by charge separation after interligand H transfer (Eqs. (4) and (5)).

3.2. CID of the hetero $[Cu^{II}dG_nGs_m]^{2+}$ complexes

ESI of mixtures of the dG and Gs nucleosides incubated with $Cu(NO_3)_2$ resulted in the formation of doubly charged complexes including all different combinations of n and m, for $n + m = 4$. An example of CID of a hetero complex $[Cu^{II}dG_nGs_m]^{2+}$ is shown for $n = m = 2$ in Fig. 4. Intramolecular electron transfer occurs to form radical cations of two nucleosides (Eq. (2)), with all possible combinations being present (i.e., $dG_2^{\bullet+}$, $Gs_2^{\bullet+}$ and $dGGs^{\bullet+}$) in close to the statistically expected abundances. Charge separation after intramolecular proton transfer (cf. Eqs. (4) and (5)) leads to the formation of dGH^+ and GsH^+ and their complimentary fragments $[Cu^{II}(GsdG_2)\text{-}H]^+$ and $[Cu^{II}(dGGs_2)\text{-}H]^+$ as the most abundant fragments. The charge separation (cf. Eq. (5)) is confirmed through observation of minor fragments corresponding to $[Cu^{II}dG_2\text{-}H]^+$, $[Cu^{II}(dGGs)\text{-}H]^+$ and $[Cu^{II}Gs_2\text{-}H]^+$.

3.3. Fragmentation reactions of the nucleoside homodimeric and heterodimeric radical cations

Each of the homodimeric and heterodimeric nucleoside radical cations, formed via CID on the appropriate copper complexes (Figs. 2c, 3b and 4), was isolated via mass selection and subjected to CID. Fig. 5a–c shows the CID spectra for $Gs_2^{\bullet+}$, $dG_2^{\bullet+}$ and $dGGs^{\bullet+}$, respectively. For these dimeric radical cations, the major fragmentation channel is loss of a monomer to give radical cations $Gs^{\bullet+}$, $dG^{\bullet+}$ or both in the case of the heterodimeric nucleoside radical cation (Eqs. (8) and (9), Fig. 5c). Additionally, minor amounts of the protonated monomers, GsH^+ and dGH^+, are observed and these most likely arise from proton transfer (Eqs. (10) and (11)). The low abundance of this channel is consistent with our previous observation and supports the suggestion that the fragment GsH^+, observed in the dissociation of the $[Cu^{II}Gs_4]^{2+}$ described in Section 3.1, is mainly formed by charge separation of the doubly charged complex rather than by the dissociation of the radical dimer as previously suggested by Cheng and Bohme [25]. The absence of the radical cation $Gs^{\bullet+}$ in the CID spectra of the $[Cu^{II}Gs_4]^{2+}$ complex suggests that the fragment GsH^+ comes solely from a charge separation reaction. Other fragment ions observed in the dissociation of the dimeric radical cations (Fig. 5a–c) are at m/z values below those of $Gs^{\bullet+}$ and $dG^{\bullet+}$, and we will show in the next section that these arise from fragmentation of $Gs^{\bullet+}$and $dG^{\bullet+}$. For comparison, Fig. 5d and f display the fragmentation reactions of protonated nucleoside homodimers and heterodimers Gs_2H^+, dG_2H^+ and $dGGsH^+$, respectively. These spectra are dominated by the formation of the protonated monomers, GsH^+ and dGH^+. An examination of Fig. 5f suggests that proton affinity (PA) of the dG is higher than the PA of the Gs, consistent with previous studies [34].

$$dGGs^{\bullet+} \rightarrow dG + Gs^{\bullet+} \quad (8)$$

$$dGGs^{\bullet+} \rightarrow dG^{\bullet+} + Gs \quad (9)$$

$$dGGs^{\bullet+} \rightarrow (dG\text{-}H)^{\bullet} + GsH^+ \quad (10)$$

$$dGGs^{\bullet+} \rightarrow dGH^+ + (Gs\text{-}H)^{\bullet} \quad (11)$$

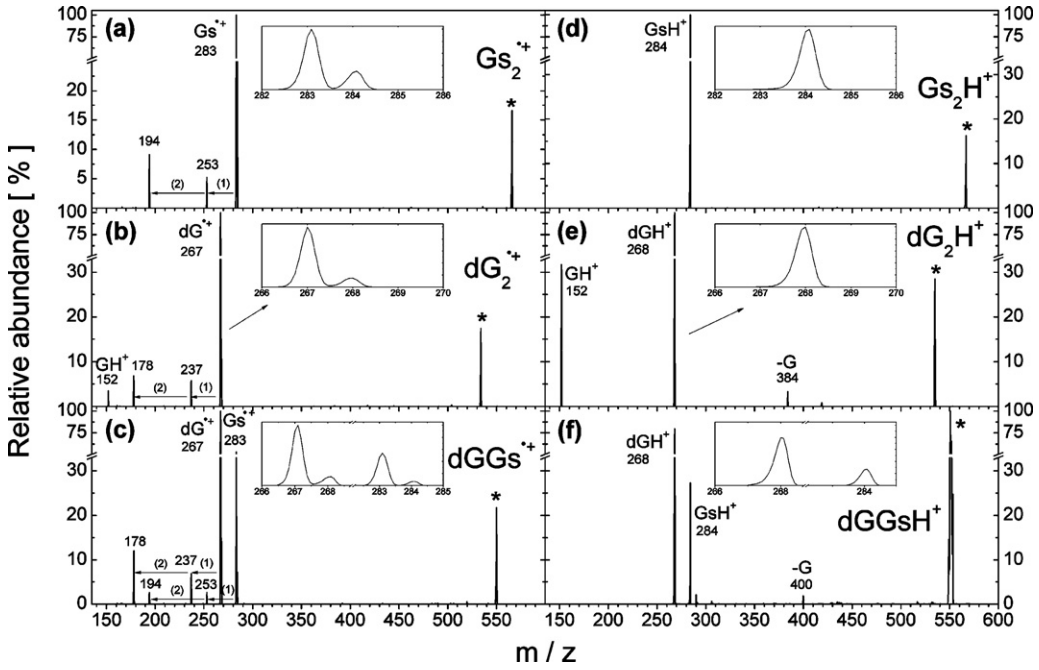


Fig. 5. CID reactions of the nucleoside homodimers and heterodimers: (a) radical cation homodimer of guanosine; (b) radical cation homodimer of deoxyguanosine; (c) radical cation heterodimer of guanosine and deoxyguanosine; (d) protonated homodimer of guanosine; (e) protonated cation homodimer of deoxyguanosine; (f) protonated cation heterodimer of guanosine and deoxyguanosine. The mass-selected precursor ion is designated with a *. Arrow (1) corresponds to loss of CH_2O from the parent ion and arrow (2) corresponds to the further loss of $C_2H_3O_2$.

Fig. 5 appears to represent a rare example in which CID is used to study the unimolecular chemistry of a mass-selected radical cation of a biomolecular dimer. An examination of the literature reveals only one other previous study, in which Ke et al. probed the fragmentation reactions of mass-selected dimer radical cations of *N*-acetylated derivatives of the aromatic amino acids tryptophan and tyrosine, which were also formed via redox reactions of copper complexes [35]. They also observed the competition between formation of the monomer radical cation (cf. Eqs. (8) and (9)) and the protonated monomers (cf. Eqs. (10) and (11)).

The relative abundances of $Gs^{\bullet+}$ and $dG^{\bullet+}$ radical cations arising from CID of the heterodimer radical cation, $dGGs^{\bullet+}$, should reflect the relative ionisation energies (IEs) of Gs and dG [36–39], provided that they both exist in the same tautomeric form within the complex. An examination of Fig. 5c reveals that $dG^{\bullet+}$ is more abundant than $Gs^{\bullet+}$ suggesting that dG has a lower IE than Gs. This contrasts with the observation of Liguori et al., who noted higher yields of $Gs^{\bullet+}$ over $dG^{\bullet+}$ in fragmentation reactions of radical cations of dimers of nucleosides with substituted napthalenes [39]. In order to address whether this discrepancy is the result of different tautomers being present in the dimer, in the next section we compare the CID reactions of $Gs^{\bullet+}$ and $dG^{\bullet+}$ formed from the dimer radical cations to those formed in the source and from the $[Cu^{II}Gs_3]^{2+}$ and $[Cu^{II}dG_3]^{2+}$complexes.

3.4. Do the dimeric radical cations have a unique structure containing two different tautomers? Evidence from CID studies and PM3/DFT calculations

The CID spectra of the monomeric radical cations $dG^{\bullet+}$ and $Gs^{\bullet+}$ formed from the dissociation of the dimers (see section above and Fig. 5) are shown in Fig. 6a and c, respectively. As noted already by Cheng and Bohme [25] and in the present study, the radical cations $dG^{\bullet+}$ and $Gs^{\bullet+}$ can be readily formed in the ESI as well as in the dissociation of the copper complex $[Cu^{II}dG_3]^{2+}$ and $[Cu^{II}Gs_3]^{2+}$. Fig. 6b and d show the CID spectra of the monomeric radical cations $dG^{\bullet+}$ and $Gs^{\bullet+}$ formed from the dissociation of the $[Cu^{II}dG_3]^{2+}$ and $[Cu^{II}Gs_3]^{2+}$ complexes, respectively. Thus, the main dissociation channel of the monomeric radical cations formed from the $[Cu^{II}dG_3]^{2+}$ and $[Cu^{II}Gs_3]^{2+}$ precursor complexes (Fig. 6b and d) is the *N*-glycosidic bond breakage to give the radical cation of the guanine base at *m*/*z* 151. The same channel and intensity is observed in the dissociation of the monomeric radical cations formed from the ESI (data not shown). The CID of the monomeric radical cations $dG^{\bullet+}$ and $Gs^{\bullet+}$ formed from the dissociation of the dimers shown in Fig. 6a and c, also shows this channel to be present. However, this channel is only a minor one, with new fragmentation reactions involving the loss of CH_2O and further loss of $C_2H_3O_2$ from the sugar moiety dominating the spectra.

The experiments described above suggest that the radical cations of dG and Gs have structures that depend on the way they are formed. Thus $dG^{\bullet+}$ and $Gs^{\bullet+}$, formed either in the source or from the $[Cu^{II}dG_3]^{2+}$ and $[Cu^{II}Gs_3]^{2+}$ precursor complexes, have *different* structures to those formed from the dimer radical cations. Although this appears to be a novel finding in terms of tautomerisation within gas-phase supramolecular complexes, a search of the literature reveals examples of different gas-phase tautomeric forms of neutral dG and Gs that arise from the way that they are generated in the gas-phase [40–42]. Thus IR spectroscopy with resonant two-photon ionisation has been used to show that neutral dG and Gs formed via laser desorption exist in the enol forms **D1** and **D2** [40], but when hydrated by one or two water molecules, exist in the keto forms **C1** and **C2** [41,42]. It is also worth mentioning that CID of metal complexes of amino acids and peptides can give rises to isomeric radical cations that depend on the metal complex [43–45]. For example, Siu has generated histidine, lysine, and arginine radical cations via CID of $[Cu^{II}(\text{auxiliary ligand})_n(\text{amino acid})]^{\bullet 2+}$ complexes using tri-, bi-, as well as monodentate auxiliary ligands [43]. The existence of two isomeric amino-acid populations was postulated, based on the observed CID products. Type 1 radical cations were proposed to result from coordination of canonical amino-acid, while Type 2 radical cations were suggested to arise from zwitterionic amino-acid coordination to the copper centre. The ratio of Type 1/Type 2 ions was found to be dependent on the

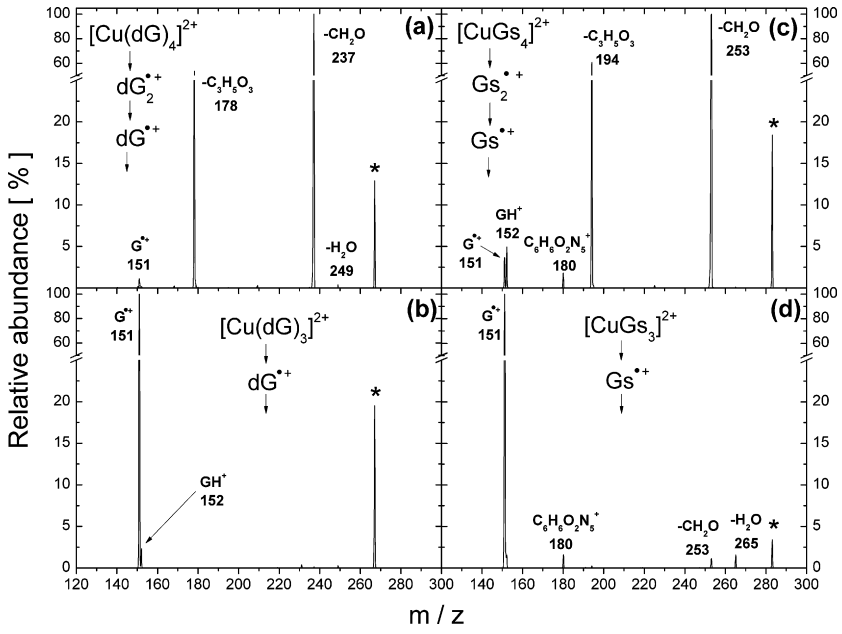
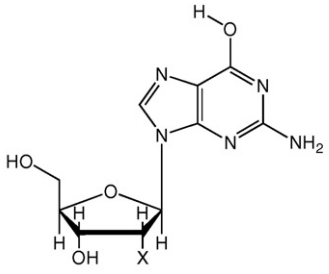


Fig. 6. CID reactions of the monomeric nucleoside radical cations: (a) deoxyguanosine formed from the dissociation of the $dG_2^{\bullet+}$ dimer; (b) deoxyguanosine formed from the dissociation of the $[Cu^{II}dG_3]^{2+}$ complex; (c) guanosine formed from the dissociation of the $Gs_2^{\bullet+}$ dimer; (d) guanosine formed from the dissociation of the $[Cu^{II}Gs_3]^{2+}$ complexes. The mass-selected precursor ion is designated with a *.

auxiliary ligand, thereby providing a way to control which radical cation is formed. The nature of the metal centre can also have an influence on the isomeric structure of peptide radical cations formed via CID of [Metal(salen)M]$^+$ complexes (where Metal = Cr, Mn, Fe and Co) [45].



D1 = deoxyguanosine (dG): X = H
D2 = guanosine (Gs): X = HO

Since the radical cations $dG^{\bullet+}$ and $Gs^{\bullet+}$ formed from the dissociation of the dimers exhibit unique chemistry, we were interested in using quantum chemical calculations to shed some light on how such dimers might be formed from their monomeric tautomers. Therefore we have carried out preliminary PM3 calculations, followed by high level DFT calculations on the model system 9-MeG, **C3**, as described previously. PM3 allows a wide range of possible structures to be rapidly screened, incorporating both stacking and hydrogen bonding interactions. The exploration of the latter structures was guided by known experimental dimer structures of guanine derivatives [46,47] and recent theoretical calculations [48–50]. Structures of interest were then refined at the higher DFT level of theory in order to extract more reliable relative energies and dissociation energies. Three stable dimeric structures were identified and re-optimised using the M052x/6-311+G(3df,2p) basis set. These were confirmed to correspond to stable minima by carrying out frequency calculations. The structures of these three dimers are shown in Fig. 7.

The lowest energy structure is a non-canonical *Trans* Watson–Crick/Hoogsteen base pair, Fig. 7a. This structure is found in RNA and is listed in the NCIR database [47]. This bonding arrangement, involving two hydrogen bonds, has been observed in earlier experimental work on isolated neutral guanine dimers [46] and was suggested as a likely structure of the Gs radical dimer cation in the work of Cheng and Bohme [25]. It is a type of bonding that appears in ribbon-like assemblies of guanosine derivatives, **B** [10,11]. The dissociation of this dimer into its respective neutral and cationic monomers was calculated to require 1.80 eV.

The second low energy structure, Fig. 7b, is a *Cis* Watson–Crick/Hoogsteen base pair and includes the same two hydrogen bonds as in structure (a), but with one of the guanines being flipped. This type of bonding appears in quartet assemblies of guanosine derivatives, **A** [9,10], and was recently investigated in the theoretical work of Sun et al. [48]. This structure is higher in energy than the *Trans* by 0.17 eV and the dissociation of this dimer into its respective neutral and cationic monomers requires 1.63 eV.

The third lowest energy structure, represented in Fig. 7c, is higher in energy than (a) by 0.32 eV. This unique structure can only occur by one of the nucleobases adopting a different tautomeric structure (the left hand nucleobase in Fig. 7c) in which the N1 hydrogen atom is transferred to the N7 position, which is described as the 7H tautomer of 9-MeG. 7H tautomers have been already hypothesised in the theoretical work of Hud and Morton on aminopurine homodimers [51]. The key outcome of this tautomerisation reaction is that it converts the N1 position from a H bond donor to a H bond acceptor, and thus allows the 7H tautomer of 9-MeG to adopt three Watson–Crick-like hydrogen bonds with the 1H tautomer of 9-MeG (the right hand nucleobase in Fig. 7c). An examination of the literature reveals that this type of structure has not been previously observed in natural nucleic acids, and represents a novel base pair that could have important biological implications.

Two immediate questions arise from this novel dimer structure: (1) How might it be formed during our experiments? (2) Does it account for the differences in the CID spectra of monomers formed from the dimer (Fig. 6a and c) versus those formed from the ESI source or the $[Cu^{II}dG_3]^{2+}$ and $[Cu^{II}Gs_3]^{2+}$ precursor complexes (Fig. 6b and d)? While we have no definitive answer to question (1), it is worth noting that hydrogen atom abstraction at N1 of

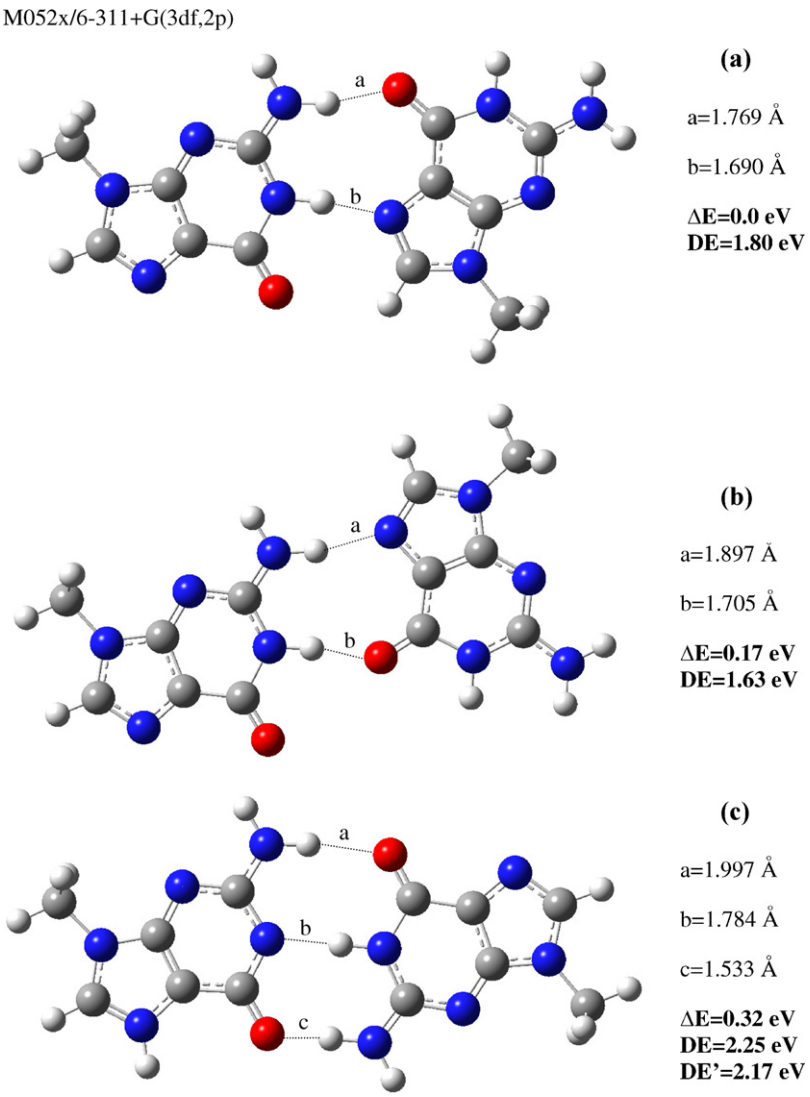


Fig. 7. Three most stable DFT-modelled structures of the dimer radical cation of guanosine optimised using the M052x/6-311+G(3df,2p) basis set: (a) *Trans* Watson–Crick/Hoogsteen base pairing (ribbon-like), (b) *Cis* Watson–Crick/Hoogsteen base pairing (quartetlike), and (c) novel structure involving two different tautomers of guanosine which hydrogen bond via Watson–Crick-like base pairing. ΔE is the relative energy difference between the various structures, DE is the dissociation energy of the dimer into the radical cation of 9-MeG (DE) or the radical cation of the 7H tautomer (DE′).

guanine may be facilitated by metal ion coordination at N7 and preliminary calculations (unpublished) suggest that the N1–H bond is significantly labilized with respect to homolytic dissociation by coordination of Cu^{2+} to N7. This is consistent with experimental observations that the N1–H bond is labilized by two orders of magnitude (pK_a) with respect to heterolytic cleavage (deprotonation) as a result of either alkylation or Pt(II) binding to N7 [52]. It is tempting to suggest that Cu^{2+} coordination to N7 promotes hydrogen atom abstraction at N1. Ensuing redox chemistry could result in the formation of the Cu^{+} species that are observed in these experiments.

With regards to the second question, a key issue is when dissociation of the dimer occurs, which of the tautomers is preferentially lost as the radical? Additional DFT calculations on the structures and energies of both tautomers as their monomeric neutrals and radical cations reveal that the dissociation of the dimer represented in Fig. 7c requires 2.25 eV if the 9-MeG radical cation is formed, but only 2.17 eV if the radical cation of the 7H tautomer of 9-MeG is formed. These calculations suggest a neat rationale for the different CID spectra: adoption of a Watson–Crick base pair structure for the dimer provides access to a new tautomer, which is retained in the monomeric radical cation formed from the dimer structure. A key, and not unreasonable assumption, is that the 7H tautomers of dG and Gs fragment differently to their conventional 1H tautomeric structures, **C**.

4. Conclusions

Upon ESI, dG and Gs form gas-phase doubly charged copper nucleoside complexes; $[Cu^{II}dG_n]^{2+}$ and $[Cu^{II}Gs_n]^{2+}$ ($2 \leq n \leq 10$). Depending on the size of the cluster, n, different fragmentation pathways can be observed upon CID. For example, in the case of dG, monomeric radical cations are formed via redox processes when $n = 3$, while when $n = 4$, dimeric radical cations are formed. The latter also generate monomer radical cations upon an additional stage of CID. The key experimental finding in this work is that the structures of the monomer radical cations of guanosine and deoxyguanosine are dependent on the way in which they are formed as shown via their CID spectra. This finding suggests the possibility of tautomer specific molecular recognition within the copper nucleoside supramolecular complexes $[Cu^{II}dG_n]^{2+}$. Quantum chemical calculations using high level DFT calculations suggested a novel Watson–Crick-type base-pairing structure for the dG, Gs and their

hetero dimer radical cations that may have important biological implications. Further work is underway to examine the issue of tautomerisms in non-covalent complexes of nucleobases.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ijms.2010.04.012.

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