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8-Oxo-7,8-dihydro-2'-deoxyguanosine Forms a Relatively Unstable Tetrameric Structure Compared with 2'-Deoxyguanosine

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Summary **The hydrogen-bonded guanine tetrad, or G-quartet has been implicated in a variety of biological roles, including the function of chromosome telomeres. Here effect of the hydroxylation of guanosine at the 8 position on the G-quartet formation was examined. Electrospray inonization mass (ESI-MS) spectra of 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG) and 2'-deoxyguanosine (dG) were measured in order to know whether or not 8-oxodG forms a tetrameric structure as 2'-deoxyguanosine forms in teromeres. The ESI-MS spectra of dG shows prominent peaks at m/z 290, m/z 557, and m/z 1092, corresponding to $[dG + Na]^+$, $[dG2 + Na]^+$, and $[dG_4 + Na]^+$ in the presence of 0.1 mM NaCl. On the other hand, the ESI-MS spectra of 8-oxodG in the presence of 0.1 mM NaCl shows prominent peaks at m/z 306 and m/z 589, corresponding to $[8\text{-oxodG} + Na]^+$ and $[8\text{-oxodG}_2 + Na]^+$. The results showed that 8-oxodG forms a relatively unstable tetrameric structure compared with dG.**

Key Words**: 8-oxodG, guanine tetrad, G-quartet, teromeres, electrospray inonization mass spectrum**

Introduction

DNA and RNA containing runs of consecutive guanine bases may adopt four-stranded conformations based on the hydrogen-bonded guanine tetrad, or G-quartet (Fig. 1) [*1–3*]. The hydrogen-bonded guanine tetrad, or G-quartet are stabilized by monovalent ions such as sodium and potassium [*4–7*]. Such tetraplexes have been implicated in a variety of biological roles, including the function of chromosome telomeres [*8*], the dimerization of the human immunodeficiency virus RNA genome [*9*], the site-specific recombination of immunoglobulin genes [*10*], L1 retropositions [*11*], promoter regions of DNA such as the triplet repeat sequence that causes fragile-X syndrome [*12–14*], the retinoblastoma susceptibility gene [*15*], the chicken β-globulin gene [*16*], and the insulin gene-linked polymorphic region (ILPR) [*17–20*]. Their functional importance is supported by the isolation of proteins that bind and promote the formation of tetraplex structure [*21*, *22*].

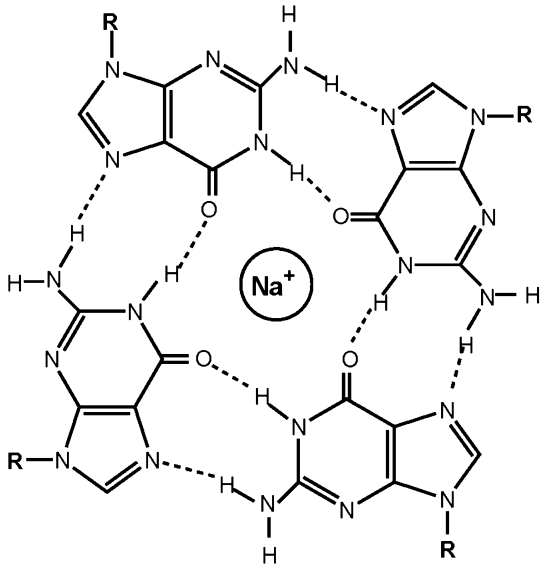

Fig. 1. The hydrogen-bonded guanine tetrad. R represents 2'-deoxyribose residue.

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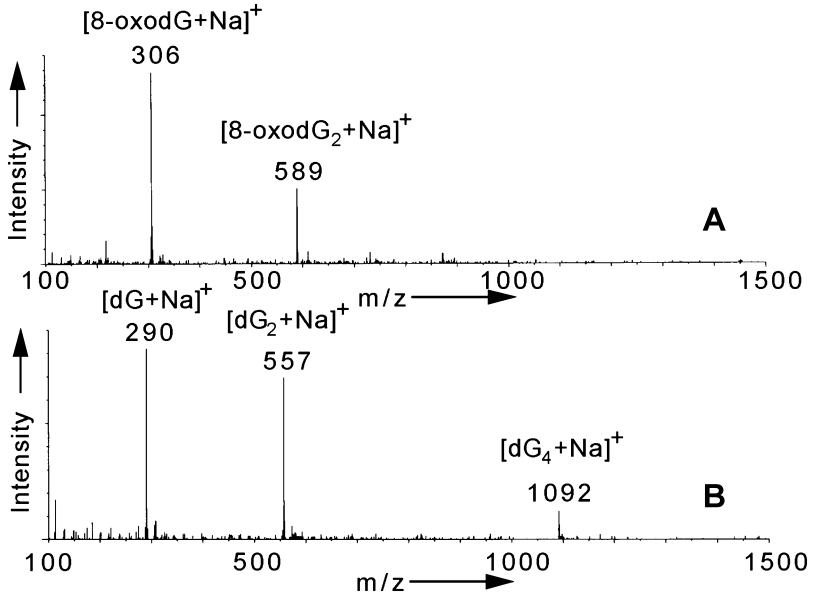

Fig. 2. Electrospray inonization mass (ESI-MS) spectra of mixtures of 8-oxodG [or 2'-deoxyguanosine (dG)] with NaCl. HPLC-ESI-MS conditions are as described in Materials and Methods. A, an ESI-MS spectrum of a mixture of 8-oxodG with NaCl; B, an ESI-MS spectrum of a mixture of dG with NaCl.

On the other hand, reactive oxygen species, which are generated in cellular metabolism [*23*] and ionization radiation [*24*], produce irreversible modification to DNA. The damage caused by these reactive free radical species has been proposed to contribute to aging, cancer, and other age-related degenerative processes [*25*, *26*]. 8-Oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG) was identified in the DNA exposed to oxygen radicals [*27*], or γ-irradiation [*28*], or peroxyl radicals [*29*]. The irradiation of UVA has resulted in the hydroxylation specifically at C-8 of the 5' site of GG and GGG sequence in DNA in the presence of endogeneous photosentizers [*30–35*]. Site-specific oxidation at GG and GGG sequences in DNA has also been induced by benzoyl peroxide [*36*]. Increases in the levels of its oxidation product, 8-oxodG have been reported in granulocute exposed to the tumor promoter (tetradeconylphorbolacetate) [*37*], mitochondorial DNA [*38*], mononuclear cells from patients with both insulin- and non-insulin-dependent diabetes [*39*, *40*], and the urine [*41*].

In this paper, effect of the hydroxylation of guanosine at 8 position on the G-quartet formation was examined by using electrospray ionization mass (ESI-MS) spectrometer. ESI-MS was employed in this study because ESI-MS is a very powerful technique for the analysis of binding interactions [*7*, *42*].

Materials and Methods

Materials

2'-Deoxyguanosine (dG) was from NAKARAI CHEMICALS (Kyoto, Japan). 8-oxodG was purchased from Sigma (St. Louis, MO). All other chemicals used were of analytical grade.

HPLC-ESI-MS

The high performance liquid chromatograph-electrospray ionization-mass spectrometer (HPLC-ESI-MS) consisted of a model 7125 injector (Reodyne Cotati, CA) with a 5 ml sample loop, a model L-7100 pump (Hitachi Ltd., Ibaragi, Japan), and a model M-1200AP LC-MS system with an electrospray ionization (ESI) (Hitachi Ltd., Ibaragi, Japan).

The operating conditions of the mass spectrometer were: nebulizer, 180°C; aperture 1, 120°C; N_2 controller pressure, 2.0 kgf/cm^2; drift voltage, 70 V; multiplier voltage, 1800 V; needle voltage, 3000 V; polarity, positive; resolution, 48.

For the analyses of a mixture of NaCl with dG (or 8-oxodG), the HPLC was performed at flow rate of 50 µl/min without a column. The mobile phase used was water. Three hundred microliter of aqueous solution of 0.1 mM NaCl with 1.0 mM dG (or 1.0 mM 8-oxodG) was injected to the HPLC-ESI-MS.

For the analyses of a mixture of KCl with dG (or 8-oxodG), the HPLC was performed with a column (150 mm long × 4.6 mm i.d.) packed with TSKgel ODS-120T (TOSOH Co., Tokyo, Japan) at flow rate of 50 µl/min. The mobile phase used was 0.1 mM KCl aqueous solution. One milliliter of a mixture of 0.1 mM KCl with 1 mM dG (or 1 mM 8-oxodG) was injected to the HPLC-ESI-MS. The HPLC fraction of dG (or 8-oxodG) was introduced to the HPLC-ESI-MS. Thus, Na^+ ions contaminated in a mixture of

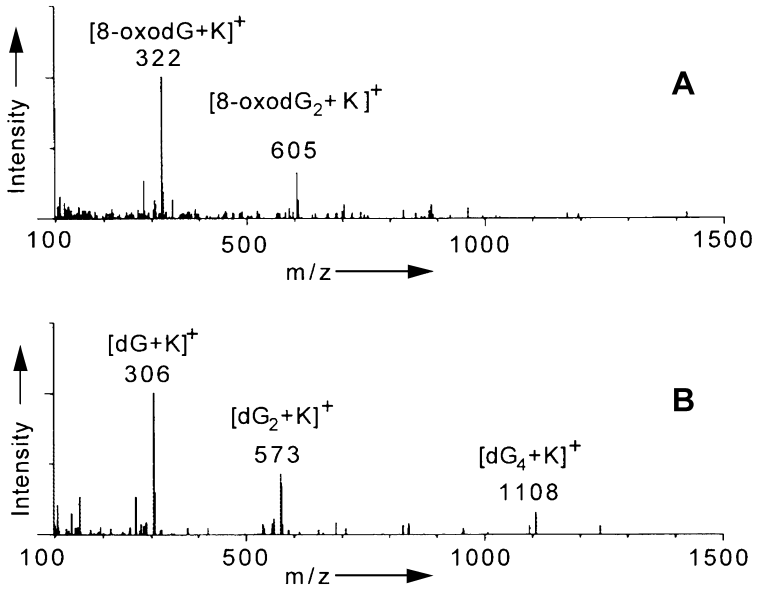

Fig. 3. Electrospray inonization mass (ESI-MS) spectra of a mixture of 8-oxodG [or 2'-deoxyguanosine (dG)] with KCl]. HPLC-ESI-MS conditions are as described in Materials and Methods. A, an ESI-MS spectrum of a mixture of 8-oxodG with KCl; B, an ESI-MS spectrum of a mixture of dG with KCl.

KCl with dG (or 8-oxodG) were replaced by K^+ ions.

For the analyses of 1 mM 2'-deoxyguanosine (or 8-oxodG) with various concentrations of NaCl, the HPLC was performed at flow rate of 50 µl/min without a column. One milliliter of acetonitrile solutions of 1 mM dG (or 1 mM 8-oxodG) with various concentration of NaCl were injected to the HPLC-ESI-MS. The one milliliter acetonitrile solutions contained 50 µl of water.

Results and Discussion

ESI-MS spectra of the solutions of 8-oxodG with NaCl or dG with NaCl (or KCl) were measured in order to know whether or not 8-oxodG forms a tetrameric structure as dG forms in teromeres.

The ESI-MS spectra of the mixture of dG with NaCl showed prominent peaks at m/z 290, m/z 557, and m/z 1092 (Fig. 2B), corresponding to $[dG + Na]^+$, $[dG_2 + Na]^+$, and $[dG_4 + Na]^+$. On the other hand, ESI-MS spectra of the mixture of 8-oxodG with NaCl showed prominent peaks at m/z 306 and m/z 589 (Fig. 2A), corresponding to $[8\text{-}oxodG + Na]^+$ and $[8\text{-}oxodG_2 + Na]^+$.

The ESI-MS spectra of the mixture of dG with KCl showed prominent peaks at m/z 306, m/z 573, and m/z 1108 (Fig. 3B), corresponding to $[dG + K]^+$, $[dG_2 + K]^+$, and $[dG_4 + K]^+$. On the other hand, ESI-MS spectra of the mixture of 8-oxodG with KCl showed prominent peaks at m/z 322 and m/z 605 (Fig. 3A), corresponding to $[8\text{-}oxodG + K]^+$ and $[8\text{-}oxodG_2 + K]^+$.

The analyses of 1 mM dG (or 8-oxodG) with various concentrations of NaCl were performed (Fig. 4). Relative peak heights of m/z 1092, $[dG_4 + Na]^+$ were much larger than those of m/z 1156, $[8\text{-}oxodG_4+Na]^+$ throughout this experiments.

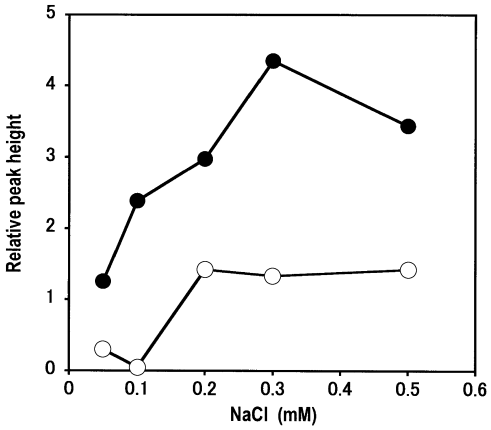

Fig. 4. Relative intensities of m/z 1092, $[dG_4 + Na]^+$ or m/z 1156, $[8\text{-}oxodG_4 + Na]^+$ observed in the mixtures of 1 mM dG or 1 mM 8-oxodG with various concentration of NaCl. HPLC-ESI-MS conditions are as described in Materials and Methods. (closed circle), m/z 1092, $[dG_4+Na]^+$; (open circle), m/z 1156, $[8\text{-}oxodG_4 + Na]^+$.

8-OxodG seems to be difficult to form a tetrameric structure as dG does. The equilibrium of 8-oxodG lies so far to the 8-keto form (Fig. 5) [*43*]. The N (7) nitrogen atom of 8-oxodG may be difficult to participate in the hydrogen bonds.

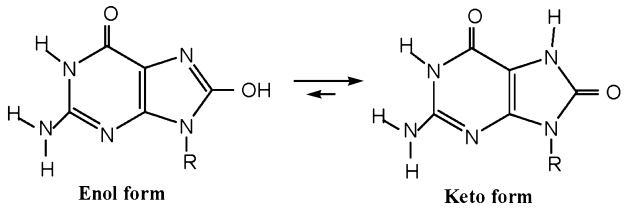

Fig. 5. Equilibrium between enol form and keto form of 8-oxodG.

Since the site-specific hydroxylation at GG and GGG sequences in DNA has been induced by various oxygen stresses [*30–36*], the hydrogen-bonded guanine tetrad, or G quartet, which is related to a variety of biological roles, is possibly disintegrated by the oxygen stresses in the biological systems.

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