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 ionization 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 \([\text{dG} + \text{Na}]^+\), \([\text{dG}_2 + \text{Na}]^+\), and \([\text{dG}_4 + \text{Na}]^+\) 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 \([8\text{-oxodG} + \text{Na}]^+\) and \([8\text{-oxodG}_2 + \text{Na}]^+\). 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 ionization 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 \(\beta\)-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].

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Fig. 1. The hydrogen-bonded guanine tetrad. R represents 2'-deoxyribose residue.
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 (tetradecanoylphorbolacetate) [37], mitochondrial 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; N2 controller pressure, 2.0 kgf/cm2; 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 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 water. Three hundred microliter of aqueous solution 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...
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]⁺, [dG₂ + Na]⁺, and [dG₄ + 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-oxodG + Na]⁺ and [8-oxodG₂ + 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]⁺, [dG₂ + K]⁺, and [dG₄ + 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-oxodG + K]⁺ and [8-oxodG₂ + 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, [dG₄ + Na]⁺ or m/z 1156, [8-oxodG₄ + 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, [dG₄+Na]⁺; (open circle), m/z 1156, [8-oxodG₄+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.
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.

References

[1] Kang, C., Zhang, X., Ratliff, R., Moyzis, R., and Rich, A.: Crystal structure of four-stranded *Oxytricha* telomeric DNA. *Nature*, **356**, 126–131, 1992.

[2] Kim, J., Cheong, C., and Moore, P.B.: Tetramerization of an RNA oligonucleotide containing a GGGG sequence. *Nature*, **351**, 331–332, 1991.

[3] Sundquist, W.I. and Klug, A.: Telomeric DNA dimerizes by formation of guanine tetrads between hairpin loops. *Nature*, **342**, 825–829, 1989.

[4] Williamson, J.R., RaghuRaman, M.K., and Cech, T.R.: Monovalent Cation-Induced Structure of Telomeric DNA: The G-quartet Model. *Cell*, **59**, 871–880, 1989.

[5] Sen, D. and Gilbert, W.: A sodium-potassium switch in the formation of four-stranded G4-DNA. *Nature*, **344**, 410–414, 1990.

[6] Hardin, C.C., Henderson, E., Watson, T., and Prosser, J.K.: Monovalent Cation Induced Structural Transitions in Telomeric DNAs: G-DNA Folding Intermediates. *Biochemistry*, **30**, 4460–4472, 1991.

[7] Fukushima, K. and Iwahashi, H.: 1:1 Complex of guanine quartet with alkali metal cations detected by electrospray ionization mass spectrometry. *Chem. Commun.*, 895–896, 2000.

[8] Blachburn, E.H.: Structure and function of telomeres. *Nature*, **350**, 569–573, 1991.

[9] Sundquist, W.I. and Heapy, S.: Evidence for interstrand quadruplex formation in the dimerization of human immunodeficiency virus1 genomic RNA. *Proc. Natl. Acad. Sci. U.S.A.*, **90**, 3393–3397, 1993.

[10] Sen, D. and Gilbert, W.: Formation of parallel four-stranded complex by guanine-rich motifs in DNA and its implications for meiosis. *Nature*, **334**, 364–366, 1988.

[11] Howell, R. and Usdin, K.: The Ability to Form Intrastrand Tetraplexes is an Evolutionarily Conserved Feature of the 3′ End of L1 Retrotransposons. *Mol. Biol. Evol.*, **14**, 144–155, 1997.

[12] Fry, M. and Loeb, L.A.: Human Werner Syndrome DNA Helicase Unwinds Tetrahelical Structures of the Fragile X Syndrome Repeat Sequence (CGG)n. *J. Biol. Chem.*, **274**, 12797–12802, 1999.

[13] Nadel, Y., Weisman-shomer, P., and Fry, M.: The Fragile X Syndrome Single Strand (CGG)n Nucleotide Repeats Readily Fold Back to Form Unimolecular Hairpin Structures. *J. Biol. Chem.*, **270**, 28970–28977, 1995.

[14] Fry, M. and Loeb, L.A.: The fragile X syndrome (CGG)n nucleotide repeats form a stable tetrahelical structure. *Proc. Natl. Acad. Sci. U.S.A.*, **91**, 4950–4954, 1994.

[15] Murchie, A.I. and Lilley, D.M.: Retinoblastoma susceptibility genes contain 5′ sequences with a high propensity to form guanine-tetrad structures. *Nucleic Acids Res.*, **20**, 49–53, 1992.

[16] Howell, R.M., Woodford, K.J., Weitzmann, M.N., and Usdin, K.: The Chicken β-Globin Gene Promotor Forms a Novel “Cliched Tetrahelical Structure. *J. Biol. Chem.*, **271**, 5208–5214, 1996.

[17] Hammond-Kosack, M.C.U. and Docherty, K.: A consensus repeat sequence from the human insulin gene linked polymorphic region adopts multiple quadruplex DNA structures in vitro. *FEBS Lett.*, **301**, 79–82, 1992.

[18] Hammond-Kosack, M.C.U., Dobrinski, B., Lurz, R., Docherty, K., and Kilpatrick, M.W.: The human insulin gene linked polymorphic region exhibits an altered DNA structure. *Nucleic Acids Res.*, **20**, 231–236, 1992.

[19] Catastì, P., Chen, X., Bradbury, E.M., and Gupta, G.: Structure-Function Correlations of the Insulin-linked Polymorphic Region. *J. Mol. Biol.*, **264**, 534–545, 1996.

[20] Lew, A., Rutter, W.J., and Kennedy, G.C.: Unusual DNA structure of the diabetes susceptibility locus IDDM2 and its effect on transcription by the insulin promoter factor P1/MAZ. *Proc. Natl. Acad. Sci. U.S.A.*, **97**, 12508–12512, 2000.

[21] Fang, G. and Cech, T.R.: Characterization of a G-Quartet Formation Reaction Promoted by the β-Subunit of the *Oxytricha* Telomere-Binding Protein. *Biochemistry*, **32**, 11646–11657, 1993.

[22] Fang, G. and Cech, T.R.: *Oxytricha* telomere-binding protein: DNA-dependent dimerization of the α and β subunits. *Proc. Natl. Acad. Sci. U.S.A.*, **90**, 6056–6060, 1993.

[23] Ames, B.N.: Dietary Carcinogens and Anticarcinogens. *Science*, **221**, 1256–1264, 1983.

[24] Teoule, R.: Radiation-induced DNA damage and its repair. *Int. J. Radiat. Biol.*, **51**, 573–589, 1987.

[25] Ames, B.N., Shigenaga, M.K., and Hagen, T.M.: Oxidants, antioxidants, and the degenerative diseases of aging. *Proc. Natl. Acad. Sci. U.S.A.*, **90**, 7915–7922, 1993.

[26] Beckman, K.B. and Ames, B.N.: The Free Radical Theory of Aging Matures. *Physiol. Rev.*, **78**, 547–581, 1998.

[27] Kasai, H. and Nishimura, S.: Hydroxylation of deoxyguanosine at the 8 position by ascorbic acid and other reducing agents. *Nucleic Acids Res.*, **12**, 2137–2145, 1984.

[28] Dizdaroglu, M.: Formation of an 8-Hydroxyguanine Moiety in Deoxyribonucleic Acid on γ-Irradiation in Aqueous Solution. *Biochemistry*, **24**, 4476–4481, 1985.

[29] Simandan, T., Sun, J., and Dix, T.A.: Oxidation of DNA...
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bases, deoxyribonucleosides and homopolymers by peroxyl radicals. Biochem. J., 335, 233–240, 1998.

[30] Ito, K. and Kawanishi, S.: Photoinduced Hydroxylation of Deoxyguanosine in DNA by Pterins: Sequence Specificity and Mechanism. Biochemistry, 36, 1774–1781, 1997.

[31] Ito, K. and Kawanishi, S.: Site-Specific DNA Damage Induced by UVA Radiation in the Presence of Endogenous Photosensitizer. Biol. Chem., 378, 1307–1312, 1997.

[32] Saito, I., Takayama, M., Sugiyama, H., Nakatani, K., Tsuchida, A., and Yamamoto, M.: Photoinduced DNA Cleavage via Electron Transfer: Demonstration That Guanine Residues Located 5' to Guanine Are the Most Electron-Donating Sites. J. Am. Chem. Soc., 117, 6406–6407, 1995.

[33] Ly, D., Kan, Y., Armitage, B., and Schuster, G.B.: Cleavage of DNA by Irradiation of Substituted Anthraquinones: Intercalation Promotes Electron Transfer and Efficient Reaction at GG Steps. J. Am. Chem. Soc., 118, 8747–8748, 1996.

[34] Hall, D.B., Holmlin, R.E., and Barton, J.K.: Oxidative DNA damage through long-range electron transfer. Nature, 382, 731–735, 1996.

[35] Liu, Z., Lu, Y., Rosenstein, B., Lebwohl, M., and Wei, H.: Benzo[a] pyrene enhances the formation of 8-hydroxy-2'-deoxyguanosine by ultraviolet A radiation in calf thymus DNA and human epidermoid carcinoma cells. Biochemistry, 37, 10307–10312, 1998.

[36] Kawanishi, S., Oikawa, S., Murata, M., Tsukitome, H., and Saito, I.: Site-Specific Oxidation at GG and GGG Sequences in Double-Stranded DNA by Benzoyl Peroxide as a Tumor Promoter. Biochemistry, 38, 16733–16739, 1999.

[37] Floyd, R.A., Watson, J.J., Harris, J., West, M., and Wong, P.K.: Formation of 8-Hydroxydeoxyguanosine, Hydroxyl Free Radical Adduct of DNA in Granulocytes Exposed to the Tumor Promoter, Tetradecylphorbolacetate. Biochem. Biophys. Res. Commun., 137, 841–846, 1986.

[38] Suter, M. and Richter, C.: Fragmented Mitochondrial DNA Is the Predominant Carrier of Oxidized DNA Bases. Biochemistry, 38, 459–464, 1999.

[39] Dandona, P., Thusu, K., Cook, S., Snyder, B., Makowski, J., Armstrong, D., and Nicotera, T.: Oxidative damage to DNA in diabetes mellitus. Lancet, 347, 444–445, 1996.

[40] Rehman, A., Nourooz-Zadeh, J., Moller, W., Tritschler, H., Pereira, P., and Halliwell, B.: Increased oxidative damage to all DNA bases in patients with type II diabetes mellitus. FEBS Lett., 448, 120–122, 1999.

[41] Shigenaga, M.K., Gimeno, C.G., and Ames, B.N.: Urinary 8-hydroxy-2'-deoxyguanosine as a biological marker of in vivo oxidative DNA damage. Proc. Natl. Acad. Sci. U.S.A., 86, 9697–9701, 1989.

[42] Brady, P.A. and Sanders, J.K.M.: Electrospray mass spectrometry and supramolecular complex: quantifying the metal ion binding properties of cholic acid derivatives. New J. Chem., 411–417, 1998.

[43] Uesugi, S. and Ikehara, M.: Carbon-13 Magnetic Resonance Spectra of 8-Substituted Purine Nucleosides. Characteristic Shifts for the Syn Conformation. J. Am. Chem. Soc., 99, 3250–3253, 1977.