# Effect of 2′-Deoxyguanosine Oxidation at C8 Position on N-Glycosidic Bond Stability

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The influence of 2′-deoxyguanosine (dG) oxidation at the C-8 position on N-glycosidic bond stability was investigated. A kinetic analysis of dG and 8-oxo-2′-deoxyguanosine (8-oxodG) depurination reactions was carried out in water solutions at pH ranging from 2 to 7.4 and temperature of 100 °C. The results indicate that N-glycosidic bond of 8-oxodG is significantly more stable in comparison with dG at any pH applied. At pH 5.1 hydrolysis rate of dG is 4.5-fold higher than that for 8-oxodG. The chemical stability of the modified nucleoside in oxidatively damaged DNA is one of important factors contributing to its mutagenic potential. Results of our experiments indicate that 8-oxodG, potentially mutagenic and carcinogenic nucleoside, is hardly susceptible to spontaneous depurination and its removal from cellular DNA depends mostly on the activity of DNA repair enzymes.

## Introduction

8-Oxoguanine is one of the most common oxidative damage products in mammalian DNA. Recent studies demonstrate the involvement of this nucleoside in mutagenesis and carcinogenesis. The presence of 8-oxodG in DNA template leads to the point mutations consisting mainly of G => T transversions (Shibutani *et al.*, 1991; Cheng *et al.*, 1992; Shibutani *et al.*, 1994). Many *in vitro* and *in vivo* experiments show that many chemical carcinogens as well as ionizing radiation contribute to 8-oxoguanine formation. Comparative studies on different kinds of cancer indicate a higher steady-state level of 8-oxoguanine in cancerous tissues than in cancer-free surrounding tissues (Olinski *et al.*, 1992; Jaruga *et al.*, 1994).

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The stability of the N-glycosidic bond, linking modified base to a sugar-phosphate DNA backbone is one of the factors which can contribute to mutagenic potential of the base. Little is known about N-glycosidic bond stability of oxidatively damaged nucleosides in DNA. Therefore, in the present work we studied the bond stability for 8-oxo-2′-deoxyguanosine in comparison with standard 2′-deoxyguanosine investigating a kinetics of the hydrolytic decomposition of both compounds (Fig. 1) at elevated temperature.

## Materials and Methods

Acetic acid, ammonium acetate, 2′-deoxyguanosine, hydrochloric acid, Tris (hydroxymethyl)aminomethane were obtained from Sigma Chemical Company (St. Louis, MO). 8-Oxo-2′-deoxyguanosine was kindly supplied by Dr. Kazimierz Kasprzak (Laboratory of Comparative Carcinogenesis, National Cancer Institute, FCRDC, Frederic, Maryland 21702–1201). HPLC grade methanol was purchased from Aldrich Chemical Company (St. Louis, MO). In all experiments bioresearch grade water was used (Barnstead/Thermolyne NANOpure water purification system).

### *N-glycosidic bond hydrolysis experiments*

One milliliter mixtures containing appropriate buffer or hydrochloric acid, 0.75 mM dG and 0.75 mM 8-oxodG were incubated in boiling water bath in sealed reagent polypropylene tubes. In one hour increments the tubes were rapidly cooled and 20µl of the reaction mixture was chromatographed immediately.

### *HPLC analysis*

The quantitative analysis of dG and 8-oxodG was performed using HPLC system. The calibration of the method was performed using equimolar standards solutions of different concentrations. The progress of depurination reaction was assessed by determination of remained nucleosides. HPLC system consisted of LKB 2150 pump, 2140 Rapid Spectral Detector, Rheodyne 7125 injector (20µl loop), Spherisorb ODS II column (250 x 4.6

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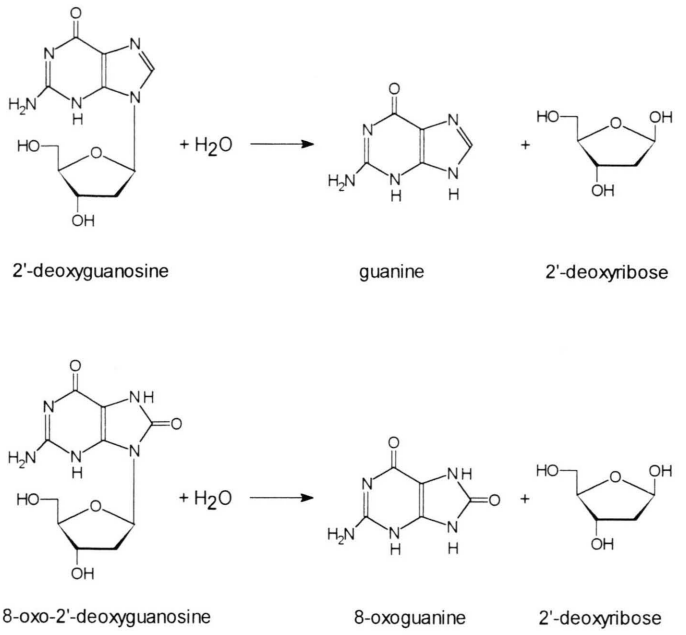


Fig. 1. Investigated reactions of hydrolytic decomposition of 2′-deoxyguanosine and 8-oxo-2′-deoxyguanosine.

mm, 5μm grain) and 10mm guard column filled by the same chromatographic medium. Elution was carried out isocratically using 20mM ammonium acetate buffer, pH 5.1 / methanol (9:1) at a flow rate of 1ml/min. Data acquisition (254nm) and evaluation were performed using LKB Wavescan EG 1.08 software and Knauer HPLC-software, version 2.21.

## Results

*Hydrolytic decomposition of dG and 8-oxodG at 100 °C and pH 7.4*

The equimolar solution of dG and 8-oxodG (0.75mM) in 20mM Tris-acetate buffer, pH 7.4 was heated in boiling water bath for five hours. Chromatographic analyses of incubation mixture were performed just before and after each hour of incubation. Just after 1st hour of incubation in the reaction mixture guanine was identified as a single decomposition product. Depurination of dG reached the 23.5% of the starting quantity after the 5th hour of heating. In these conditions no decomposition of 8-oxodG was observed. The guanine was the sole UV-absorbing product of all decomposition reactions. Kinetic curves obtained in this experiment are presented in the Fig. 2.

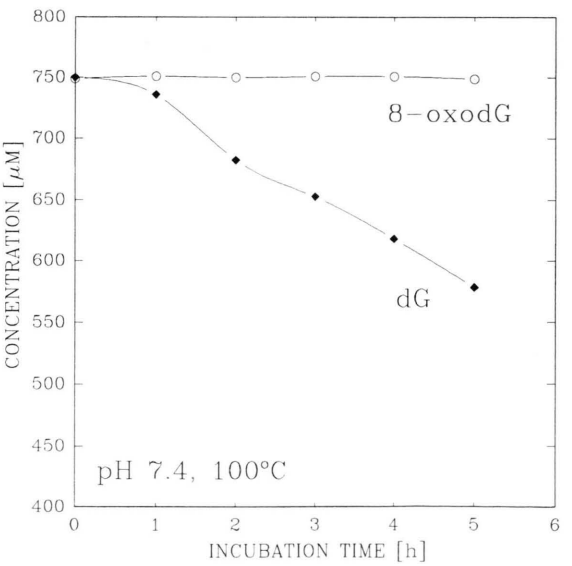


Fig. 2. Kinetic curves of 2′-deoxyguanosine (dG) and 8-oxo-2′-deoxyguanosine (8-oxodG) hydrolysis carried out at 100 °C and pH 7.4. Advance of the depurination reaction is presented as a substrate loss.

*Kinetics of hydrolytic decomposition of dG and 8-oxodG at 100 °C, pH 5.1*

The equimolar solution of dG and 8-oxodG (0.75mM) in 10mM ammonium acetate, pH 5.1 was incubated in boiling water bath for five hours. Qualitative and quantitative determinations of the incubation mixture were performed just before heating and after each incubation hour. Both nucleosides decomposed in this conditions but with the different rates (Fig. 3). After five hour incubation concentration of dG decreased from 0.75mM to 0.26mM (35% of original value). At the same time concentration of 8-oxodG decreased from 0.75 to 0.58mM (77% of original value). The increase of 8-oxoguanine peak was not proportional to 8-oxodG loss because 8-oxoguanine is hardly soluble in water.

First order kinetics equation was used as a model of the reaction in order to compare hydrolysis rate constants. Hydrolysis rate of 8-oxodG ($k_{S\text{-}1}$ = 1.25 x $10^{-5}$ [1/s]) is 4.5-fold lower than that of dG ($k_{S\text{-}1}$ = 5.64 x $10^{-5}$ [1/s]) in applied conditions.

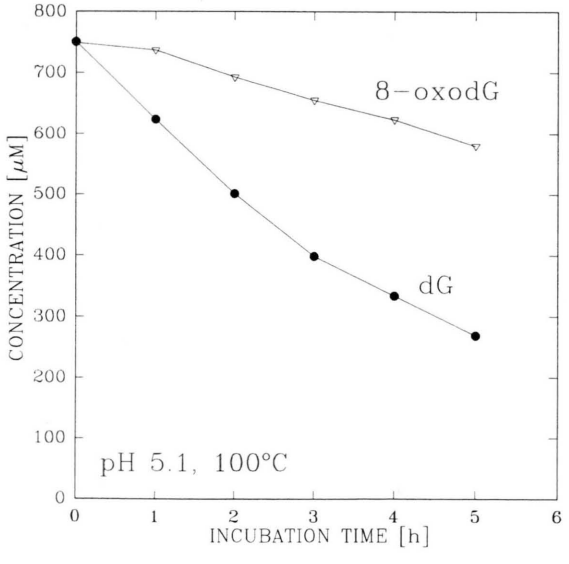


Fig. 3. Kinetic curves of 2′-deoxyguanosine (dG) and 8-oxo-2′-deoxyguanosine (8-oxodG) hydrolysis carried out at 100 °C and pH 5.1. Advance of the depurination reaction is expressed by means of substrate loss.

*Stability of dG and 8-oxodG in 10mM HCl at 100 °C*

The solution containing 0.375mM dG and 8-oxodG in 10mM HCl was heated at 100 °C for three hours. The incubation mixture was chromatographed just before and after incubation. Applied conditions resulted in complete acidic hydrolysis of dG to guanine and only slight decrease in 8-oxodG concentration from 0.375mM to 0.334mM (11% loss).

Separate experiments dealing with dG heated in different, buffered water solutions showed that there is no UV-detectable formation of 8-oxodG from dG.

## Discussion

To our knowledge the stability of N-glycosidic bonds of oxygen radical-modified deoxynucleosides have not been a research objective until now.

Laayoun *et al.* (1994) has investigated the hydrolytic stability of purine 2′-deoxynucleosides modified at C8 by electron-withdrawing substituents such as –Br, –SH, –SCH3, –S–CH2–CH2–CH3, –SO2CH3. In all cases drastic rate acceleration for N-glycosidic bond cleavage was observed in comparison with parental dG ( 29000-fold acceleration for C8-methylsulfonyl-derivative). The clear positive correspondence has been noticed between the inductive electron-withdrawing character of substituents and the rates of nucleoside hydrolysis. All investigated substituents having positive values of Hammet & Taft constant resulted in N-glycosidic bond destabilization when introduced into C8 position of dG. Results of our experiments reveal significantly higher stability of 8-oxodG N-glycosidic bond as compared with the unmodified deoxynucleoside. This observation delivers some information regarding tautomeric form of 8-oxodG. Hammet & Taft constant for -OH substituent was calculated to be +0.1. Thus, the presence of 8-enol form of 8-oxodG should be expressed by N-glycosidic bond labilization in comparison with unmodified dG. We postulate that observed N-glycosidic bond stabilization effect is connected with 8-keto tautomerisation of 8-oxodG. Such a suggestion is supported by many experimental investigations (Uesugi and Ikehara, 1977; Culp *et al.*, 1989; Kouchakdjian *et al.*, 1991; Gannett and Sura, 1993).

Several reports provide an evidence for the accumulation of 8-oxodG in DNA with the age of an organism. This event is thought to be connected to a higher incidence of the cancer and degenerative diseases in relatively old individuals. Besides the inefficient DNA repair, high stability of 8-oxodG N-glycosidic bond should be the most important requirement for the accumulation of the lesion in the long-lived cells. According to Lindahl DNA of the single cell undergoes even 10,000 depurination events per day (Lindahl, 1993). Thus, a loss of the modified purines from the damaged DNA is probably partially a spontaneous process. We have demonstrated significantly higher chemical stability of 8-oxodG in comparison with the unmodified standard DNA constituent. It suggests that 8-oxoguanine is very stabile oxidative DNA damage product, unsusceptible to spontaneous hydrolysis. The removal of this lesion from DNA is dependent mainly on activity of the repair enzymes.

This remark may be also of practical value. In many studies where 8-oxodG is analyzed DNA have been denatured (mostly for 5–15 min. at 100 °C) before nuclease P1 treatment.

Results of our experiments suggest that such a treatment carried out in the neutral pH does not result in substantial loss of 8-oxoguanine from analyzed DNA.

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