Comparison of transition metal-mediated oxidation reactions of guanine in nucleoside and single-stranded oligodeoxynucleotide contexts

Pranjali Ghude, Mark A. Schallenberger, Aaron M. Fleming, James G. Muller, Cynthia J. Burrows *

Department of Chemistry, University of Utah, 315 S 1400 East, Salt Lake City, UT 84112-0850, USA

ARTICLE INFO

ABSTRACT

As the most readily oxidized of DNA's four natural bases, guanine is a prime target for attack by reactive oxygen species (ROS) and transition metal-mediated oxidants. The oxidation products of a modified guanosine nucleoside and of a single-stranded oligodeoxynucleotide, 5′-d(TTTTTTTGTTTTTTT)-3′ have been studied using oxidants that include Co^{II}, Ni^{II}, and Ir^{IV} compounds as well as photochemically generated oxidants such as sulfate radical, electron-transfer agents and singlet oxygen. The oxidized lesions formed include spiroiminodihydantoin (Sp), guanidinohydantoin (Gh), imidazolone (Iz), oxazolone (Z) and 5-carboxamido-5-formamido-2-iminohydantoin (2-Ih) nucleosides with a high degree of dependence on the exact oxidation system employed. Interestingly, a nickel(II) macrocyclic complex in conjunction with $KHSO_5$ leads to the recently reported 2-Ih heterocycle as the major product in both the nucleoside and oligonucleotide contexts.

1. Introduction

Chemical modification of guanine (G), the most readily oxidized nucleobase in DNA, has been linked to aging, cancer and degenerative diseases [1–4]. The constant attack by endogenous and exogenous oxidants on guanine generates various oxidation products (Fig. 1) [5]. Among these products, 8-oxo-7,8-dihydroguanine (8-oxo-G), guanine-derived 2,6-diamino-4-hydroxy-formamido-pyrimidine (FaPy-G) [6–8], spiroiminodihydantoin (Sp) [9] and oxazolone (Z) [10] have been found in vivo. The normal level of 8-oxo-G, the most common oxidation product of guanine is 0.3–4 lesions per 10^6 dGs [11], and it has been used as a biomarker of oxidative stress in cells [12].

The various oxidation products formed are due to hydroxylation or perhydroxylation at the C5 or C8 positions of guanine. For example, the C5 pathway involves loss of one electron from guanine leading to formation of the guanine radical cation ($G^{+\cdot}$) that has a pK_a of 3.9 [13]; $G^{+\cdot}$ undergoes rapid deprotonation in the nucleoside form, although it may be longer lived in duplex DNA (Scheme 1) [14]. The neutral guanine radical ($G–H^{\cdot}$) [15–17] is attacked by dioxygen [18–20] or superoxide anion [17,21,22] and results in a cascade of decomposition reactions leading to formation of imidazolone (Iz) and eventually oxazolone (Z) products [23–25]. On the other hand, the guanine radical cation ($G^{+\cdot}$) can be trapped by H_2O at C8 [13], followed by deprotonation and formation of an intermediate adduct ($G–OH^{\cdot}$) [26] that is prone to further oxidation and tautomerization leading to 8-oxo-G (Scheme 2). Under reducing conditions, one-electron reduction of ($G–OH^{\cdot}$) followed by ring opening leads to FaPy-G. Both 8-oxo-G and FaPy-G are formed during radiation damage to DNA [4,27]. 8-Oxo-G has a reduction potential of 0.74 V versus NHE [12], and because this is ∼0.55 V lower than that of G (E = 1.29 V versus NHE at pH 7), further oxidation can readily occur yielding the spirocyclic base Sp as well as guanidinohydantoin (Gh) [28,29]. Both of the hydantoin lesions are known to be highly mutagenic (Scheme 2) [30].

A wide range of aqueous oxidation systems, from reactive oxygen species (ROS) [31] to photochemical oxidants [32] to transition metals [24,25,33–35], are known to react with the electron-rich guanine heterocycle (Table 1). Among the sulfoxyl radicals, the sulfate radical anion ($SO_4^{\cdot-}$) is the most potent oxidant with a redox potential of 2.43 V versus NHE at pH 7, and it acts by a one-electron abstraction mechanism [36]. Another relevant oxidant is singlet oxygen formed by photoexcitation of sensitizers such as Rose Bengal (RB) or porphyrins [32]. Singlet oxygen can potentially act as a four-electron oxidant of G, bypassing 8-oxo-G to yield hydantoin products directly [37].

Furthermore, several transition metal ions act as promoters of nucleic acid oxidation. Mechanistically, the simplest of these oxidants is hexachloroiridate, $IrCl_6^{2-}$, a water-soluble, one-electron oxidant with a redox potential of 0.9 V versus NHE [38]. $IrCl_6^{2-}$ and $IrBr_6^{2-}$ are capable of facile oxidation of 8-oxo-G and of slow oxidation of G. In the presence of cobalt (II) or nickel(II), $KHSO_5$ (oxone) causes site-specific guanine oxidation [39–41]. The

* Corresponding author. Tel.: +1 801 585 7290; fax: +1 801 585 0024.
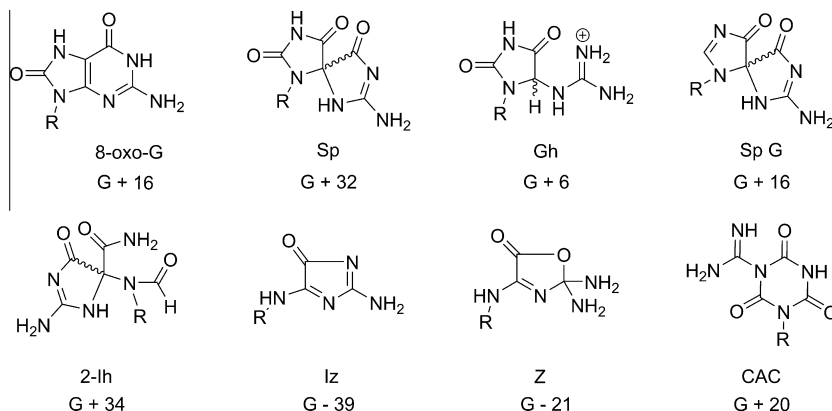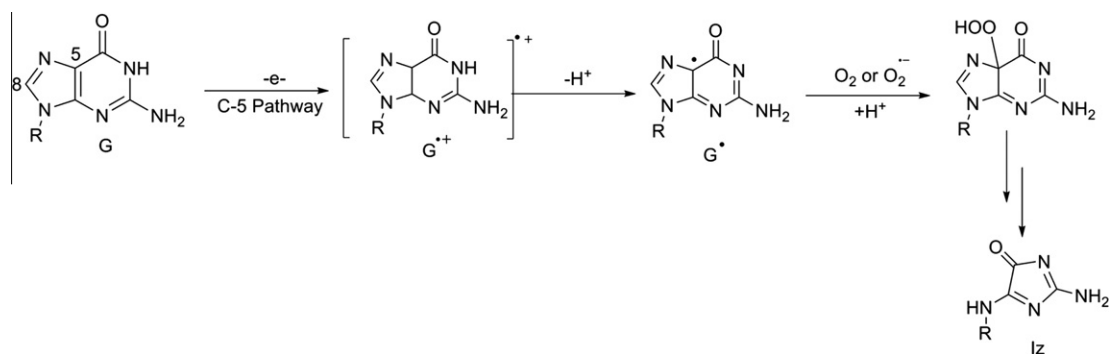E-mail address: burrows@chem.utah.edu (C.J. Burrows).

Fig. 1. Oxidation products of 2′,3′,5′-tri-O-acetylguanosine (R = 2′,3′,5′-tri-O-acetylribose) and their relative masses.

Scheme 1. C5 pathway for oxidation of 2′,3′,5′-tri-O-acetylguanosine.

Table 1
Aqueous oxidation systems.

Oxidation system	Oxidant	$E_{1/2}$ (V vs. NHE) at pH 7
Na$_2$IrCl$_6$	e$^-$	0.9
K$_2$S$_2$O$_8$/hν (256 nm)	SO$_4^{\cdot-}$	2.43
Rose Bengal/hν (360 nm)	^{1}O$_2$	NA
CoCl$_2$/KHSO$_5$	SO$_4^{\cdot-}$	>2.0
Ni(II)CR/KHSO$_5$	L$_4$–Ni–O–SO$_3^{\cdot}$	>2.0
Fe(II)EDTA/H$_2$O$_2$	OH$^{\cdot}$	1.9

decomposition of KHSO$_5$ catalyzed by these metals generates SO$_4^{\cdot-}$ that appears to be responsible for the oxidation of guanine [39]. CoCl$_2$/KHSO$_5$ generates highly diffusible sulfate radicals that induce guanine-specific modification in bulges, loops and single-stranded DNA [33].

It has been reported that simple nickel salts in the presence of H$_2$O$_2$ result in the formation of 8-oxo-G as the oxidation product [42,43]. Certain four-coordinate, square-planar nickel(II) complexes demonstrate nickel binding to chromatin and have been studied for their toxicity and carcinogenicity [44,45]. These complexes can facilitate oxidation of DNA causing strand breaks, oxidative base damage and DNA–protein cross-links [46–48]. Peptide ligands such as glycylglycylhistidine coordinate to nickel(II) and enhance the metal reactivity by stabilizing the +III oxidation state of the metal, thereby promoting guanine oxidation [49–51]. Particularly in the case of Ni(II), the ligand surrounding the metal and the redox properties of the metal play an important role in the determination of the oxidation pathway. Nickel tetraazamacrocycles that possess strong in-plane donor ligands and provide vacant coordination sites mediate oxidative damage of guanine with per-

acids like KHSO$_5$ [48]. NiCR (Fig. 2), unlike nickel peptides, does not react in the minor groove under low salt concentrations [52], but instead incurs base damage at guanines that are solvent accessible. Hence, NiCR/KHSO$_5$ acts as a probe of guanine exposure in determining the three-dimensional folded structure of DNA and RNA [53–55].

NiCR is thought to prefer oxidation at exposed guanines by binding to the most basic site, N7 in guanine, thus delivering the oxidant to the exposed base via direct metal coordination [34,56]. The intermediate is proposed to be an octahedral nickel(III)-bound sulfate radical in which the two additional ligands, guanine and sulfate, are cis coordinated (Scheme 3) [46]. Subsequent reductive elimination of these groups results in an oxidized G in DNA that leads to strand scission upon treatment with hot piperidine [34,48,57]. The Co(II)/KHSO$_5$ oxidation system has somewhat less specific requirements for guanine exposure suggesting the generation of a free SO$_4^{\cdot-}$ species induced by CoCl$_2$ as compared to nickel-coordinated SO$_4^{\cdot-}$ in the case of NiCR [33].

Square-planar nickel peptide complexes, Ni^{II}Gly-Gly-His and Ni^{II}Arg-Gly-His, appear to generate a similar reactive complex with sulfate radical via autoxidation of sulfite. In this mechanism, nickel(II) catalyzes the formation of HSO$_5^-$ via autoxidation of HSO$_3^-$ [51,52,57,58]. Curiously, the product of G oxidation by the Ni^{II}Gly-Gly-His/HSO$_3^-$/O$_2$ system appears to be 8-oxo-G [56], which has not been observed from G oxidation by the NiCR/KHSO$_5$ system.

Despite its popularity as a structural tool, the NiCR/KHSO$_5$ system remained elusive in terms of its eventual chemistry with guanine. The final product formed by G oxidation in oligodeoxynucleotides with NiCR/KHSO$_5$ had a mass that was higher than the starting material by 34 amu, although a transient product of mass