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Review

Recent developments in analytical methodology for 8-hydroxy-2′-deoxyguanosine and related compounds

Michael C. Peoples, H. Thomas Karnes*

Department of Pharmaceutics, Virginia Commonwealth University Medical Center, P.O. Box 980533, Richmond, VA 23298-0533, USA

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Abstract

When biomolecules such as proteins, lipids, and DNA are subjected to oxidative attack by free radicals or other reactive species, a number of measurable biomarkers may be produced. The study of oxidative DNA damage is valuable in research concerning cancer and aging. The current review includes methodology involving various separation science techniques for the analysis of DNA oxidation biomarkers, mainly 8-hydroxy-2′-deoxyguanosine. This review will present recent analytical developments with respect to sample preparation and instrumental considerations, noting key outcomes and biological relevance where appropriate.

Keywords: Oxidative stress; 8-Hydroxy-2′-deoxyguanosine; DNA damage; Analysis

Contents

1. Introduction

The study of oxidative stress in biological systems generally involves the measurement of biomarkers that reflect damage induced from an attack by free radicals or other reactive species. Reactive species can occur from normal cellular metabolism or exogenous sources and are countered by antioxidants. When antioxidant defenses are overwhelmed, oxidative stress persists and causes damage to biomolecules, such as proteins, lipids, and DNA [1,2]. Direct measurement of reactive species and free radicals is impractical because they are short-lived, due to their highly reactive nature. Thus, biomarkers have been used to reflect the degree of oxidative damage in light of a particular clinical or research interest, i.e. disease or disorder state. Oxidative stress has been linked to neurological disorders, atherosclerosis, diabetes, cancer, and other age-related diseases [1,3,4].

Oxidative stress can lead to a variety of measurable protein modifications as well as protein carbonyl derivatives, which

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* Corresponding author. Tel.: +1 804 828 3819; fax: +1 804 828 8359.
E-mail address: tom.karnes@vcu.edu (H. Thomas Karnes).

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have been most commonly studied [5]. The measurement of protein markers as indicators of oxidative stress may involve several products due to the fact that there are 20 amino acids available for an oxidative attack. Therefore, there is a need for technology capable of analyzing larger numbers of protein modifications to identify which oxidative products are relevant to the desired study. In light of this need for protein modification targets, proteomic-based methods are emerging as useful techniques for exploring specific proteins as markers of oxidative damage [4,6]. The process of lipid peroxidation includes oxidative chain reactions of fatty acids, where several measurable products may be produced [3]. Among the most frequently investigated are malondialdehyde (MDA), 4-hydroxynonenal (HNE), and the isoprostanes. The prostaglandin-like isoprostanes are thought to be specific markers of lipid peroxidation since their production is non-enzymatic. F_2-isoprostanes, formed from the peroxidation of arachidonic acid, have represented the bulk of isoprostanes research [7–9]. Recently, there has been a greater focus on F_4-neuroprostanes, which originate from peroxidation of docosahexaenoic acid, the major fatty acid in the brain [10]. F_4-neuroprostanes may therefore reflect oxidative injury to nervous system tissue [11].

The role of oxidative damage to DNA is considered important in studies involving aging and the development of cancer [12,13]. An overall schematic representation of oxidative stress and DNA damage is shown in Fig. 1. Reactive oxygen species (i.e. hydroxyl radical) can alter the deoxyribose-phosphate backbone, cause DNA-protein cross-links, and modify both purine and pyrimidine bases. Repair of oxidized DNA in vivo is accomplished by glycosylases (bases) and endonucleases (deoxynucleotides). Deoxynucleotides are excreted in the urine as deoxynucleosides [1].

Guanine most readily undergoes an oxidative attack, possessing the lowest oxidation potential of the four bases. Consequently, the nucleoside 8-hydroxy-2′-deoxyguanosine (8-OHdG) is the most often studied biomarker of oxidative DNA damage [2,14]. The presence of the modified base, 8-hydroxyguanine (8-OHGua), during DNA replication can cause G:C-T:A transversion mutations. Therefore, oxidative lesions not repaired before replication can become mutagenic [15,16].

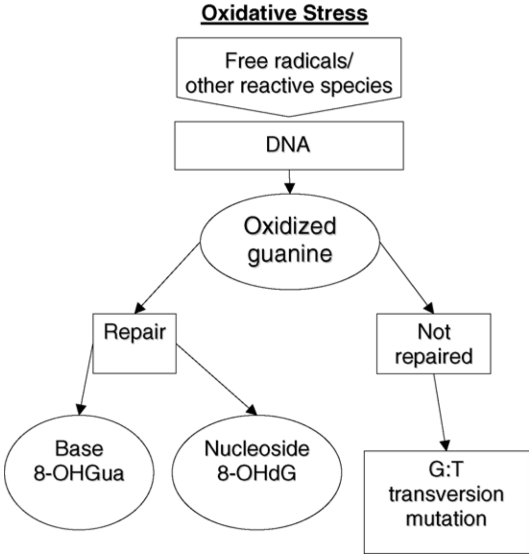

Fig. 1. Pathway of commonly measured biomarkers of oxidative stress.

In cellular DNA, detecting oxidative lesions without artificially oxidizing the normal base during sample preparation is a concern [4]. Results of 8-OHdG analysis from nuclear DNA samples such as tissue or cells are often expressed normalized to the unmodified base (8-OHdG/dG) and enzymatic DNA digestion is required to liberate and measure free 8-OHdG. Measurements of this type represent oxidative damage at the specific sampling site at the time of sampling [2].

Alternatively, analysis of 8-OHdG as a repair product in urine reflects the amount of total body oxidative DNA damage from a non-invasive sample [4]. Urinary levels of 8-OHdG in healthy human subjects have been reported at levels of approximately 10–30 nM [17-19]. Complex sample cleanup methods are often required due to interferences inherent to the urine matrix. However, once formed, 8-OHdG is a stable product and not subjected to further metabolism [2]. Furthermore, urine samples present no danger of artifactual production of 8-OHdG and do not require enzymatic digestion [19]. Table 1 summarizes urinary sample preparation procedures for methods covered in this review.

Table 1
Urine sample preparation procedures

Sample Size	Pretreatment	Analysis time or throughput	Separation or assay	Ref.
30 μL	Dilute and inject[a]	34 min run time	HPLC-EC	[35]
2 mL	Double SPE	25 min chromatogram shown	HPLC-EC	[19]
50 μL	Dilute and inject[a]	∼45 min chromatogram shown	HPLC-EC	[36]
10 mL	Single SPE	12 min chromatogram shown	HPLC-EC	[37]
Unclear	Single SPE	Unclear	CE-EC	[18]
1 mL	Single SPE	Unclear	CE-EC	[39]
2 mL	Single SPE	Unclear	CE-EC	[40]
100 μL	Dilute and inject	<15 min total analysis	CE-UV	[42]
0.8–3.2 mL	Single SPE	<10 min run time	GC/MS	[46]
3 mL	Single SPE	∼50 samples/day throughput	LC/MS/MS	[47]
15 mL, 100 μL injection	Untreated	15 min chromatogram shown	LC/MS/MS	[50]
50 μL	Untreated	3.5–4 h per 18 samples total analysis	ELISA	[b]

[a] Automated valve-initiated column switching.

[b] “New 8-OHdG Check” from Japan Institute for the Control of Aging, Fukuroi, Shizuoka (www.jaica.com/biotech/e).