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# Effects of the melanin precursor 5,6-dihydroxy-indole-2-carboxylic acid (DHICA) on DNA damage and repair in the presence of reactive oxygen species

Maria Carolina Pellosi [a], Andréia Akemi Suzukawa [a], Alexsandra Cristina Scalfo [b], Paolo Di Mascio [b], Carolina Parga Martins Pereira [b], Nadja Cristhina de Souza Pinto [b], Daniela de Luna Martins [c], Glaucia Regina Martinez [a,*]

[a] Programa de Pós-graduação em Ciências (Bioquímica), Departamento de Bioquímica e Biologia Molecular, Universidade Federal do Paraná, Curitiba, PR, Brazil
[b] Departamento de Bioquímica, Instituto de Química, Universidade de São Paulo, São Paulo, SP, Brazil
[c] Instituto de Química, Universidade Federal Fluminense, Rio de Janeiro, RJ, Brazil

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ABSTRACT

Eumelanin is a heterogeneous polymer composed of 5,6-dihydroxyindole-2-carboxylic acid (DHICA) and 5,6-dihydroxyindole (DHI). Studies have shown that DHICA promotes single strand breaks in plasmid DNA exposed to ultraviolet B radiation (UVB, 313 nm) and in DNA from human keratinocytes exposed to ultraviolet A radiation (UVA, 340–400 nm). Singlet molecular oxygen ($^1O_2$) is the main reactive species formed by UVA radiation on the skin. In this context, we now report that DHICA can cause single strand breaks in plasmid DNA even in the absence of light radiation. Interestingly, when DHICA was pre-oxidized by $^1O_2$, it lost this harmful capacity. It was also found that DHICA could interact with DNA, disturbing Fpg activity and decreasing its recognition of lesions by ~50%. Additionally, the free nucleoside deoxyguanosine (dGuo) was used to evaluate whether DHICA would interfere with the formation of 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodGuo) and spiroiminodihydantoin (dSp) by $^1O_2$ or with the formation of 8-oxodGuo by hydroxyl radical (·OH). We observed that when dGuo was oxidized by $^1O_2$ in the presence of DHICA, 8-oxodGuo formation was increased. However, when dGuo was oxidized by ·OH in the presence of DHICA, 8-oxodGuo levels were lower than in the absence of the precursor. Overall, our data reveal an important role for this eumelanin precursor in both the promotion and the protection of DNA damage and imply that it can impair DNA repair.



## Introduction

Ultraviolet radiation (UV)[1] can lead to the formation of reactive oxygen species (ROS) in the cellular environment. Increased levels of singlet oxygen ($^1O_2$) upon exposure to UVA radiation were found in human keratinocytes [1]. Additionally, in the same cellular lineage, increased levels of superoxide anion ($O_2^{\cdot-}$) and nitric oxide ($NO^{\cdot}$) were revealed upon exposure to UVA and UVB radiation [2]. These species can react with and damage many biomolecules, including proteins, lipids, carbohydrates and DNA [3]. DNA is of particular importance because it stores all of the information that directs the synthesis of other cellular constituents. Investigations into the mechanisms that prompt the formation of DNA lesions and the ways they can contribute to the onset of many diseases, such as cancer and neurodegenerative diseases, are ongoing and still present challenges to the field.

Singlet oxygen reacts with duplex DNA almost exclusively at the guanine base to form 8-oxo-7,8-dihydroguanine (8-oxoGua) [4]. $^1O_2$ can also react with free 2′-deoxyguanosine to produce 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodGuo) and the pair of diastereomers of spiroiminodihydantoin (dSp), which are generated in an approximately 7-fold excess over 8-oxodGuo [5–7]. dSp can also be generated when 8-oxodGuo is oxidized by $^1O_2$, as

* Corresponding author. Address: Departamento de Bioquímica e Biologia Molecular, Setor de Ciências Biológicas, UFPR, CP 19046, CEP 81.531-980 Curitiba, PR, Brazil. Fax: +55 041 3266 2042.
*E-mail address:* grmartinez@pq.cnpq.br (G.R. Martinez).

[1] *Abbreviations used:* DHICA, 5,6-dihydroxy-indole-2-carboxylic acid; DHI, 5,6-dihydroxyindole; dGuo, deoxyguanosine; UV, ultraviolet radiation; UVA, ultraviolet A radiation; UVB, ultraviolet B radiation; ROS, reactive oxygen species; Fapy-dG, 2,6-diamine-4-hydroxy-5-formamidopyrimidine-2′-deoxyguanosine; SOD, superoxide dismutase; EtBr, ethidium bromide; HPLC, high performance liquid chromatography; 8-oxodGuo, 8-oxo-7,8-dihydro-2′-deoxyguanosine; DHPN, *N*,*N*′-di(2,3-dihydroxypropyl)-1,4-naphthalenedipropanamide; L, linear; OC, open circular; SC, supercoiled; LEDs, light-emitting diodes.

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illustrated in Fig. 1(A) [8–10]. Both 8-oxodGuo and dSp pair preferentially with adenines, and this erroneous pairing may cause permanent DNA mutations [4,11]. Moreover, 8-oxodGTP (8-oxodGuo triphosphate) can be recognized by DNA polymerase and incorporated into the new strand of DNA during replication [12,13]. However, it remains unknown whether dSp generated in the pool of deoxyribonucleotides can also be used by DNA polymerase during DNA synthesis.

Whereas $^1O_2$ is very selective for guanine among DNA constituents, ·OH can react with all DNA components. When ·OH reacts with dGuo, it can generate 2 main products, 8-oxodGuo under oxidant conditions and 2,6-diamine-4-hydroxy-5-formamidopyrimidine-2′-deoxyguanosine (Fapy-dG) under reductive conditions (Fig. 1(B)) [14–17]. Fapy-dG, like 8-oxodGuo, can be misrecognized by DNA polymerase during replication because these lesions pair preferentially with adenine [18,19]. Thus, the two products generated from dGuo oxidation by ·OH are potentially mutagenic.

In addition to the oxidation of nucleotides, base-pairing properties can also be perturbed by the interaction of DNA with toxic exogenous or endogenous agents [20–22]. Thus, the recognition and elimination of these lesions by DNA repair pathways is crucial for the preservation of genome information [23]. Genomic integrity is also protected by compounds that prevent the damage, such as tocopherols, carotenoids and bilirubin, which can behave as ROS scavengers [24]. In this context, the skin pigment eumelanin has been considered protective [25,26]. This pigment is a heterogeneous polymer composed mainly of monomers of 5,6-dihydroxyindole (DHI) and 5,6-dihydroxyindole-2-carboxylic acid (DHICA) (Fig. 2).

However, some studies have shown that this polymer does not always exert a protective function. The eumelanin precursor DHICA can induce DNA damage under UVA or UVB radiation, most likely due to the formation of ROS [27,28]. The deleterious effects of the monomer units of eumelanin have also been described. For

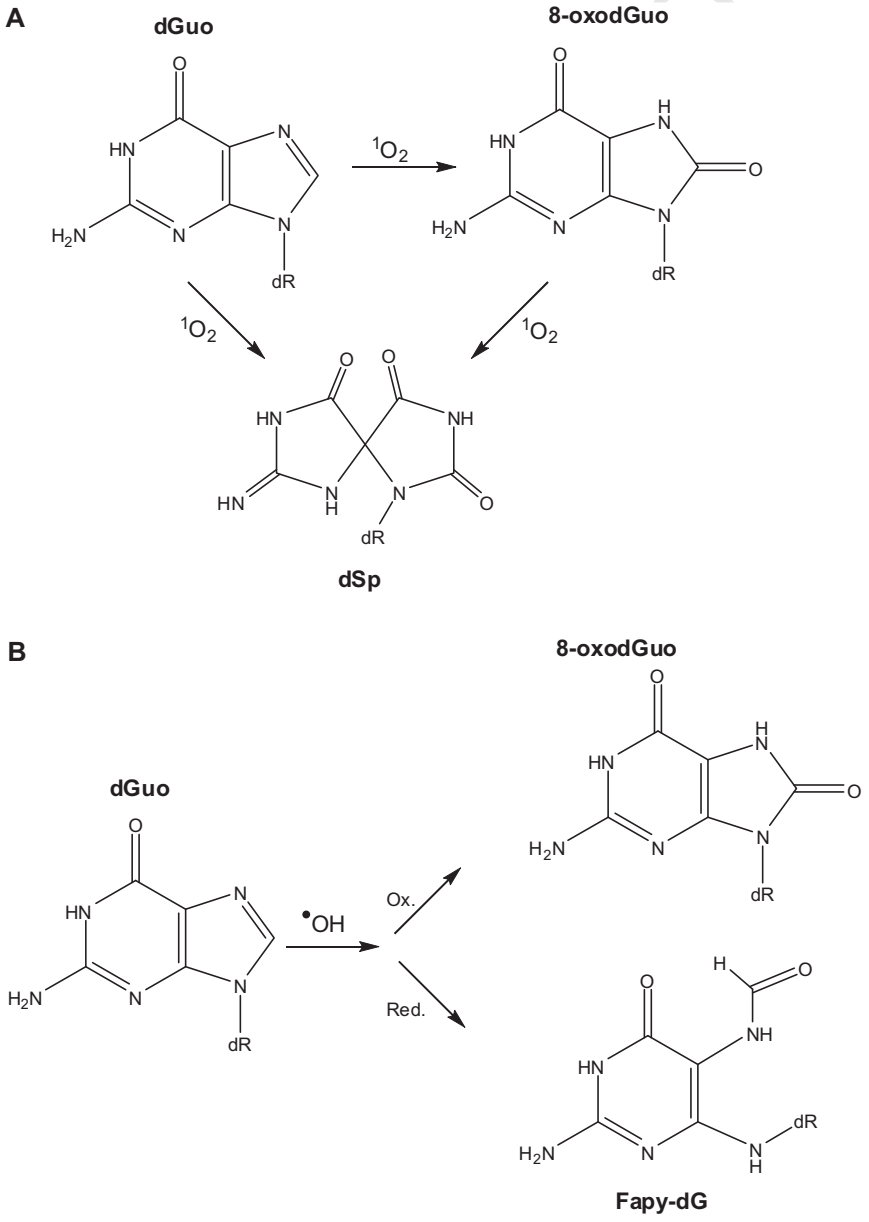


**Fig. 1.** Molecular structure of the main products generated by the reaction of $^1O_2$ (A) or ·OH (B) with dGuo. (A) dGuo oxidation products by $^1O_2$—8-oxodGuo and dSp. The product dSp can be generated directly by oxidation of dGuo or by oxidation of 8-oxodGuo. (B) dGuo oxidation products by ·OH. 8-oxodGuo is generated under oxidant conditions, whereas Fapy-dG is formed under reductive conditions. dR = deoxyribose.

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