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Radical-trapping and preventive antioxidant effects of 2-hydroxymelatonin and 4-hydroxymelatonin: Contributions to the melatonin protection against oxidative stress

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ABSTRACT

Background: Melatonin is well known for its antioxidant capacity, which has been attributed to the combined protective effects of the parent molecule and its metabolites. However, the potential role of 2-hydroxymelatonin (2OHM) and 4-hydroxymelatonin (4OHM) in such protection has not been previously investigated.
Methods: The calculations were performed using the Density Functional Theory, with the M05-2X and M05 functionals, the 6-311 + G(d,p) basis set and the solvation model based on density (SMD).
Results: 4OHM shows excellent antioxidant activity via radical-trapping, reacting with peroxyl radicals faster than Trolox and melatonin. 4OHM can be moderately efficient as a preventing antioxidant by inhibiting Cu(II). This effect would lower the Cu(I) availability, which is the redox state required for the ·OH to be formed, via Fenton-like reactions. 4OHM turns off the oxidant effects of copper-ascorbate mixtures. The presence of a phenolic group was identified as the key structural feature in the antioxidant activity of 4OHM. On the other hand, 2OHM does not present a phenolic group, despite its formal name. Its *keto* tautomer was identified as the most abundant one (~100%). This may explain the relative low antioxidant protection of 2OHM.
Conclusions: 4OHM significantly contributes to the overall antioxidant activity exhibited by melatonin, while the effects of 2OHM in this context are predicted to be only minor. This low reactivity might justify the relatively large abundance of 2OHM in biological systems.
General significance: Hydroxylated melatonin metabolites, such as 4OHM, may play an important role in the protective effects of melatonin against oxidative stress.

1. Introduction

Oxidative stress (OS) is a health threatening phenomenon that arises as a consequence of a chemical imbalance between the production and consumption of oxidants [1]. In biological systems, there are free radicals (FR) such as the hydroxyl (·OH), alkoxyl (RO·) and peroxyl (ROO·) radicals, that can be particularly damaging oxidants. They can trigger chain reactions, resulting in oxidative damage that may self-propagate harming several categories of molecules of high importance including lipids, proteins and DNA. There is compelling evidence supporting the role of OS, and an excess of FR, in the onset and development of a large number of diseases. OS has been associated with renal [2–4], pulmonary [5–7], and ocular [8–10] diseases; rheumatoid arthritis [11–13], fetal growth restriction, preeclampsia, etc. [14–16]. OS is responsible, at least partially, for cancer development [17–19], and serious neurodegenerative disorders including Parkinson's and Alzheimer's diseases, memory loss, multiple sclerosis, and depression [20–26]. There are also abundant data showing that OS is involved in several cardiovascular diseases such as ischemia, atherosclerosis, cardiac hypertrophy, hypertension, cardiomyopathy, and heart failure

Abbreviations: 2OHM, 2-hydroxymelatonin; 4OHM, 4-hydroxymelatonin; OS, oxidative stress; FR, free radicals; OIL, ·OH-inactivating ligand; HOO·, hydroperoxyl radical; ROO·, peroxyl radicals; DFT, Density Functional Theory; SMD, solvation model based on density; HAT, hydrogen atom transfer; RAF, radical adduct formation; SET, single electron transfer; SPLET, sequential proton loss electron transfer; HB, hydrogen bonding; AFMK, N^1-acetyl-N^2-formyl-5-methoxykynuramine; AMK, N^1-acetyl-5-methoxykynuramine; c3OHM, cyclic 3-hydroxymelatonin; NAS, *N*-acetylserotonin; 6OHM, 6-hydroxymelatonin; DCM, direct chelation mechanisms; CDCM, coupled deprotonation-chelation mechanisms

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[27–30].

Molecules that offer protection against OS are of crucial importance in maintaining optimal health. These protectors are frequently referred to as antioxidants and, fortunately, are abundant and of diverse chemical nature. Among them, melatonin and related compounds have been proven to be particularly efficient [31–43]. They can exert their protection against OS through diverse routes, for example by scavenging free radicals [44–53], deactivating other oxidants [54–59], and by inhibiting metal-induced lipid peroxidation [60–65]. In addition, phenolic melatonin-derivatives have been described as better free radical scavengers than melatonin or Trolox, with some of them surpassing the activity of ascorbic acid and resveratrol [66]. However, some of these compounds have been significantly less studied than other melatonin derivatives.

2-Hydroxymelatonin (2OHM) and 4-hydroxymelatonin (4OHM) are both within the group of less investigated antioxidants. They are produced during the UV-induced metabolism of melatonin in keratinocytes and cell-free systems [67]. In plants, 2OHM is the most abundant metabolite of melatonin (99%) followed by 4OHM (0.05%) [68]. In addition, it has been found that 2OHM and 4OHM can be produced during the oxidation of melatonin by different chemical agents. For example, 2OHM is yielded during the oxidation of melatonin by taurine chloramine [69], while both 2OHM and 4OHM were identified as products in the melatonin incubation with a Fenton-type ·OH-generating system, at *p*H 7.4 [70].

To our best knowledge, there are no previous studies regarding the potential role of 2OHM and 4OHM as antioxidants. Thus, the main goal of this work is to explore such a possibility. To that purpose, both their radical-trapping (primary) and preventive (secondary) antioxidant activities were considered. The first case corresponds to the free radical scavenging, while the second may involve diverse chemical routes. The one studied in this work involves metal chelation and the associated ·OH-inactivating ligand (OIL) effects [71–73].

The free radical scavenging activity was investigated using the hydroperoxyl radical (HOO·). This radical was chosen for several reasons. Peroxyl radicals (ROO·) are biologically relevant and – because their half-lives are sufficiently long – they can be successfully scavenged to retard OS [74,75]. Moreover, the low to moderate reactivity of these radicals is a desirable feature for studying trends in free radical scavenging activity [76,77]. HOO· is the smallest of the peroxyl radicals, and plays an essential role in the toxic side effects associated with aerobic respiration [78].

Metal chelation and OIL effects of 2OHM and 4OHM were studied using Cu(II), which is widely distributed in the human body and can induce cellular toxicity [79]. In addition, it has been shown that copper is involved in the pathogenesis of several neurodegenerative disorders [80], which has been attributed to its role in the formation of oxidants [81], in particular the ·OH radical [80]. It has also been reported that, in terms of oxidative damage, the toxicity of Cu(II) is greater than that of Fe(III), under the same experimental conditions [82,83].

Different reaction sites and mechanisms were investigated, and their relative importance was assessed. The influence of the environment, in particular polarity and *p*H, on the antioxidant activity of the target compounds was also explored. Thermochemical and kinetic data are provided, as well as comparisons with other antioxidants. Hopefully, the information provided here may contribute to gain a better understanding of the chemical role of 2OHM and 4OHM under OS conditions.

2. Materials and methods

All the electronic calculations were performed with Gaussian 09 package of programs [84]. Full geometry optimizations and frequency calculations were carried out using the Density Functional Theory (DFT). The M05-2X and M05 functionals [85] were used for the systems without and with Cu, respectively. All the calculations were performed with the 6-311 + G(d,p) basis set and the solvation model based on density (SMD), [86] using water and pentyl ethanoate as solvents to mimic aqueous and lipid solution. Local minima were identified by the absence of imaginary frequencies. Thermodynamic corrections at 298.15 K were included in the calculation of relative energies.

The M05-2X functional was chosen for organic systems because it was recommended for kinetic calculations by its developers [85]. In addition, its reliability has been independently confirmed by other authors [87–90]. It is among the best performing functionals for calculating reaction energies involving free radicals [91], and for kinetic calculations in solution [92]. The M05 functional was chosen for the Cu-involving systems because it was parameterized including both metals and non-metals, while M05-2X has double the amount of non-local exchange (2X) and was parameterized mainly for non-metals. M05 has been recommended for studies involving both metallic and non-metallic elements, and perform well not only for main-group thermochemistry but also for interactions with transition-metals [85]. SMD was chosen for mimicking the solvent effects because it can be consistently used for any charged or uncharged solute in any solvent or liquid medium [86].

Cu(II) ions were modeled coordinated to 4 water molecules, in a near square-planar configuration (Fig. 1S), which has been previously established as the most likely arrangement for this ion [93,94]. Since charged species are expected to be hydrated in the aqueous phase, this model is more appropriate to represent “free” copper under physiological conditions than the bare ion. For consistency purposes, Cu(I) ions were also modeled with 4 explicit water molecules, albeit in this case the linear two-coordinate configuration is preferred [95–97]. Thus, in this case Cu(I) is coordinated to 2 water molecules, and the other 2 are solvating the system.

The kinetic data was obtained by following the quantum mechanism-based test for overall free radical scavenging activity (QM-ORSA) protocol [98]. It has been validated by comparison with experimental results, and its uncertainties have been proven to be no larger than those arising from experiments [98]. The rate constants (k) were calculated using the conventional transition state theory (TST) [99–101] and 1 M standard state, including zero curvature tunneling corrections (ZCT) [102]. For the electron transfer reactions the Gibbs free energy of activation were calculated using the Marcus theory [103]. In addition, since several of the calculated rate constants (k) were found to be close to the diffusion-limit, the apparent rate constant (k_{app}) cannot be directly obtained from TST calculations. The Collins-Kimball theory was used for that purpose [104], in conjunction with the steady-state Smoluchowski [105] rate constant for an irreversible bimolecular diffusion-controlled reaction, and the Stokes–Einstein [106,107] approaches for the diffusion coefficient of the reactants.

3. Results and discussion

3.1. Acid-base equilibria

Acid-base equilibria has a significant influence on the antioxidant behavior of compounds presenting such a chemical feature [108,109]. Therefore, this was the first explored aspect in this work for 2OHM and 4OHM. Albeit from their formal names it may seem that both of them may be involved in acid-base equilibria, due to the presence of a phenolic moiety, this is only the case for 4OHM. On the contrary, 2OHM presents a keto-enol equilibrium (Scheme 1) favoring the keto tautomer by ~13.4 kcal/mol. Thus, probably a better way to refer to 2OHM would be using the proper name of its keto tautomer, i.e., 3-acetamidoethyl-5-methoxyindolin-2-one [110,111].

The above mentioned energy difference is high enough to overcome any uncertainty related to the calculations and allows predicting that the enol tautomer would be present to such a low extent that its contributions to the chemistry of 2OHM can be neglected. Accordingly, in this work only the keto tautomer was used to represent 2OHM.

On the other hand, 4OHM has a conventional phenolic site that may