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REGULAR ARTICLE

The key role of the sequential proton loss electron transfer mechanism on the free radical scavenging activity of some melatonin-related compounds

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Abstract The role of the acid–base equilibria and the sequential proton loss electron transfer mechanism (SPLET) on the free radical scavenging activity of six melatonin-related compounds was investigated using the density functional theory. It was found that this chemical route is particularly important for about half of the studied compounds. Some of their pKa values are reported here for the first time. In addition, our results also indicate that anionic species, presenting the phenolate moiety, may be crucial to scavenge peroxyl radicals albeit their populations are relatively low at physiological pH. The key number to consider in this context should be the product of the molar fraction of the reacting compound, at the pH of interest, by the corresponding rate constant.

Keywords pKa · Reaction mechanisms · Kinetics · Antioxidant activity · SPLET · DFT

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1 Introduction

Antioxidants are currently considered a chemical alternative to the enzymatic defense systems against oxidative stress (OS). There is compelling evidence that melatonin—and related compounds—is very efficient for that purpose [1–3]. It has been proposed that they exhibit a rather unique feature that makes them particularly efficient against OS. They jointly act as a combined antioxidant, through both their free radical scavenging activities and their metal chelation ability, behaving as $^{\bullet}$OH-inactivating ligands. The antioxidant capabilities of melatonin metabolites are responsible for this collective defense, frequently referred to as a cascade-like protection [4–6]. In addition, the melatonin's family of compound exhibits a "task-division" behavior in their antioxidant protection. Some members of the family have been identified as particularly efficient free radical scavengers (FRS), while others are mainly metal chelators (MC). For example, *N*-acetylserotonin (NAS) and 6-hydroxymelatonin (6OHM) belong to the FRS group, while melatonin itself and N^1-acetyl-N^2-formyl-5-methoxykynuramine (AFMK) act mainly as MC. On the other hand, it has been proposed that one of the metabolites—cyclic 3-hydroxymelatonin (c3OHM)—can be efficient in both ways [7].

Several reaction mechanisms have been previously investigated regarding the free radical scavenging activity (FRSA) of these compounds including radical adduct formation (RAF), hydrogen transfer (HT), and single electron transfer (SET). However, to our best knowledge, the role of the sequential proton loss electron transfer (SPLET) mechanism on the FRSA of these compounds has not been assessed yet. This is probably because there is no information about the pKa values of most of them. The SPLET mechanism was first proposed by Litwinienko and Ingold

for the reactions of substituted phenols with the DPPH radical [8–11] and comprises two consecutive steps, namely (1) the deprotonation of the antioxidant and (2) an electron transfer from the deprotonated antioxidant to the free radical [12]. Accordingly, knowing the pKa value, or values, of the antioxidant is crucial to assess the relative importance of this mechanism. This is because pKa values rule the proportion of the deprotonated species in aqueous solution at any pH of interest, for example, at pH = 7.4 under physiological conditions. In other words, the pKa values would determine the extension of step (1). At the same time, SPLET is currently known to be crucial for the antioxidant protection exerted by numerous chemical compounds including curcumin [9], esculetin [13], piceatannol [14], resveratrol [15, 16], hydroxybenzoic and dihydroxybenzoic acids [17, 18], xanthones [19], hydroxychalcones [20], procyanidins [21], kaempferol [22], gallic acid [23], isoflavonoids [24], fraxetin [25], and genistein [26]. In addition, it has been demonstrated that theoretical chemistry-based approaches can provide reliable and valuable information on this subject [27, 28]. Accordingly, it is the main goal of the present work to assess the relative importance of the SPLET mechanism in the FRSA activity of several members of the melatonin's family and to estimate their pKa values.

2 Computational details

Geometry optimizations and frequency calculations have been carried out using the M05-2X functional [29] and the 6-31 + G(*d*,*p*) basis set, in conjunction with the solvation model based on density (SMD) [30] using water as solvent. The M05-2X functional has been recommended for kinetic calculations by their developers [29], and it has been also successfully used by independent authors for that purpose [31–33]. It is also among the best performing functionals for calculating reaction energies involving free radicals [34]. SMD is considered a universal solvation model, due to its applicability to any charged or uncharged solute in any solvent or liquid medium for which a few key descriptors are known [30].

Unrestricted calculations were used for open-shell systems, and local minima were identified by the absence of imaginary frequencies. All the electronic calculations were performed with the Gaussian 09 package of programs [35]. Thermodynamic corrections at 298.15 K were included in the calculation of relative energies. The rate constants (k) were calculated using the conventional transition state theory (TST) [36–38] and 1 M standard state as:

$$k = \frac{k_B T}{h} e^{-(\Delta G^{\neq})/RT}$$

where k_B and h are the Boltzmann and Planck constants and $\Delta G^{\neq}$ is the Gibbs free energy of activation that were calculated using the Marcus theory [39, 40].

In addition, since several of the calculated rate constants (k) are close to the diffusion limit, the apparent rate constant (k_{app}) cannot be directly obtained from TST calculations. The Collins–Kimball theory is used to that purpose [41], in conjunction with the steady-state Smoluchowski [42] rate constant for an irreversible bimolecular diffusion-controlled reaction and the Stokes–Einstein [43, 44] approaches for the diffusion coefficient of the reactants.

These computational details are in line with the quantum mechanics-based test for overall free radical scavenging activity (QM-ORSA) protocol [45]. It was validated by comparison with experimental results and proven to produce uncertainties no larger than those arising from experiments.

3 Results and discussion

To estimate the pKa values of the investigated compounds, we have used the isodesmic method, also known as the proton exchange method, or the relative method [46]. It is based on the following reaction scheme:

$$HA + Ref^{-} \leftrightarrow A^{-} + HRef$$

where *HRef*/*Ref*$^-$ is the acid–base pair of a reference compound. Within this approach, the *p*Ka is calculated as:

$$p\mathrm{Ka(HA)} = \frac{\Delta G_s}{RT \ln(10)} + p\mathrm{Ka}(HRef)$$

Albeit this method has been proven to produce accurate pKa values, there are two key factors when using it that need to be taken into account to achieve the desired accuracy. *HRef* should be as structurally similar as possible to the system of interest, and its experimental pKa should be known. In the present case, we have chosen *HRef* = melatonin (pKa = 12.3) [47] for calculating the pKas of AMK and AFMK and *HRef* = serotonin for c3OHM, 6OHM, and NAS. At this point, it is important to note that, albeit serotonin has two pKa values, the relevant in this context is the second one, which corresponds to the deprotonation of the neutral species from its phenolic site. The value used for this pKa corresponds to the average of all the previously reported ones (pKa = 10.82). The individual values from which this average was estimated are provided in Table 1. The structures of the investigated compounds, and those of the molecules used as *HRef*, as well as the most likely deprotonation site for each of them, are shown in Scheme 1. The corresponding Cartesian coordinates are provided as Electronic Supplementary Material (ESM).

Table 1 Experimental values for the pKa of serotonin, corresponding to the deprotonation of the phenolic OH

Ref.	pKa
11.26	Ref. [48]
10.73	Ref. [49]
10.4	Ref. [50]
10.89	Ref. [51]
10.82	Average

The pKas used for step (1) in the SPLET mechanism are reported in Table 2, together with the molar fractions (^{M}f) of the anions at pH = 7.4. The latter are crucial to assess the relative importance of the SPLET mechanism. It was found that for melatonin itself, AMK, and c3OHM, the $^{M}f_{\text{anion}}$ are so low that it seems unlikely that the SPLET mechanism significantly contributes to the overall reactivity of these compounds. On the contrary, albeit the populations of the anions of NAS, AFMK, and 6OHM are rather low at physiological pH (0.1, 4.8, and 1.0 %, respectively), they might still be enough to make the SPLET route relevant. This is because the contributions of this mechanism to the overall reactivity of the studied compounds toward free radicals can be estimated as:

$$\%\text{SPLET} = 100 \times \frac{^{M}f_{\text{anion}}\, k^{\text{SPLET}}}{k_{\text{overall}}}$$

where k^{SPLET} and k_{overall} represent the rate constant of the SPLET reaction and the overall rate coefficient, respectively. Therefore, the number that really matters is the product $^{M}f_{\text{anion}}k^{\text{SPLET}}$. The SPLET contributions, at physiological pH, are reported in Table 3, while the influence of the pH on the relative importance of the SPLET mechanism is shown in Fig. 1. The data in both Table 3 and Fig. 1 correspond to the reactions between the studied compounds and the hydroperoxyl radical (HOO$^{\bullet}$).

Table 2 pKa values and molar fraction of the anion ($^{M}f_{\text{anion}}$) at pH = 7.4

	pKa	Ref.	$^{M}f_{\text{anion}}$
Melatonin	12.3	[47]	$<10^{-4}$
NAS	10.7	This work	0.001
AMK	16.8	[52]	$<10^{-9}$
AFMK	8.7	[52]	0.048
c3OHM	15.1	This work	$<10^{-7}$
6OHM	9.4	This work	0.010

It was found that the SPLET route is rather fast for AFMK and c3OHM, while it is near to or within the diffusion-limited regime for NAS and 6OHM. However, after considering the corresponding $^{M}f_{\text{anion}}$, it became evident that only for NAS, AFMK, and 6OHM, the SPLET mechanism is the key one, regarding the HOO$^{\bullet}$ scavenging activity at physiological pH (Table 3). Moreover, for melatonin, AMK, and c3OHM, the SPLET route is predicted to be only of minor importance, compared to other reaction mechanisms such as HT and RAF, in the whole range of pH (from 0 to 14). On the contrary, SPLET becomes the

Scheme 1 Structures of the investigated compounds and the *HRef*. The circles highlight the most likely deprotonation site

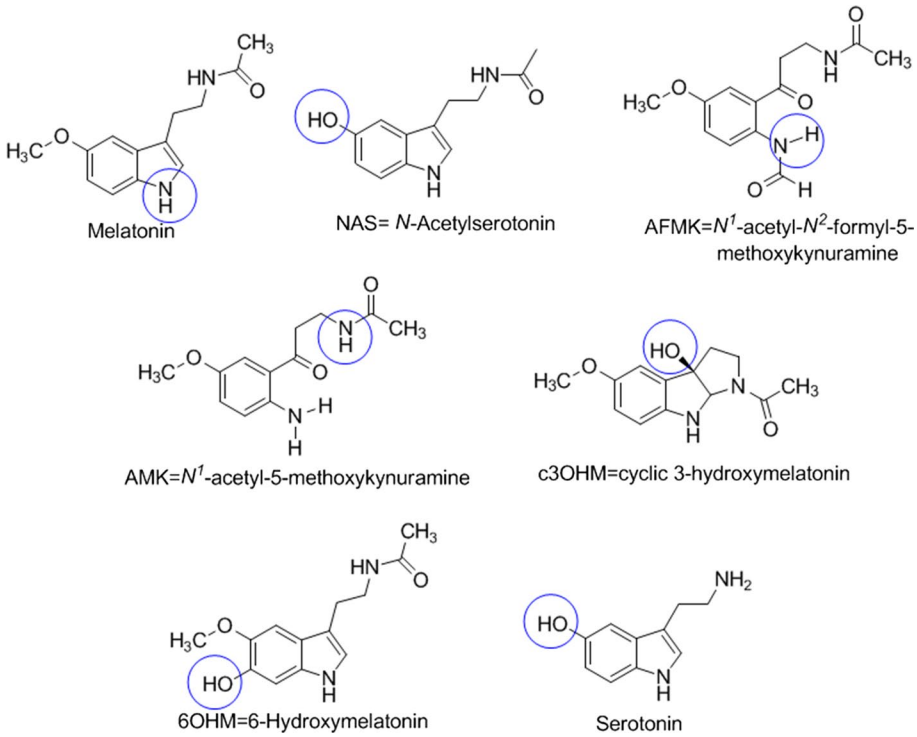

Table 3 Rate constants of the SPLET reaction (k^{SPLET}, M^{-1} s^{-1}), overall rate coefficient ($k_{overall}$, M^{-1} s^{-1}) at pH = 7.4, and SPLET contributions to the overall reactivity (%SPLET) at the same pH

	k^{SPLET*}	$k^{overall}$	%SPLET
Melatonin	8.28E−04	1.90E+01	~0.0
NAS	7.95E+09	5.50E+06	78.8
AMK	4.77E+01	1.35E+02	~0.0
AFMK	3.38E+04	1.62E+03	99.7
c3OHM	4.57E+06	2.84E+06	~0.0
6OHM	8.23E+09	8.39E+07	95.7

* Before including the molar fractions

route contributing the most to the peroxyl scavenging activities of NAS, AFMK, and 6OHM at pH values above 7.8, 5.9, and 7.0, respectively (Fig. 1). SPLET is particularly important for the phenolic compounds (NAS and 6OHM), and such importance increases with the pH.

Accordingly, it is recommended to include the SPLET mechanism in future studies of melatonin-related compounds. Additionally, it is crucial to fully characterize the acid–base equilibria of these compounds, since such equilibria may significantly influence their activity and mechanisms of action. This is particularly important when analyzing reactions that occur in biological systems, since the pH of the environment could vary depending on the investigated region of the system. For example, in the case of melatonin-related compounds, they can be present in different organs of the human body.

4 Conclusions

The results from the theoretical investigation presented in here indicate that the SPLET mechanism is likely to play a key role on the free radical scavenging activity of phenolic

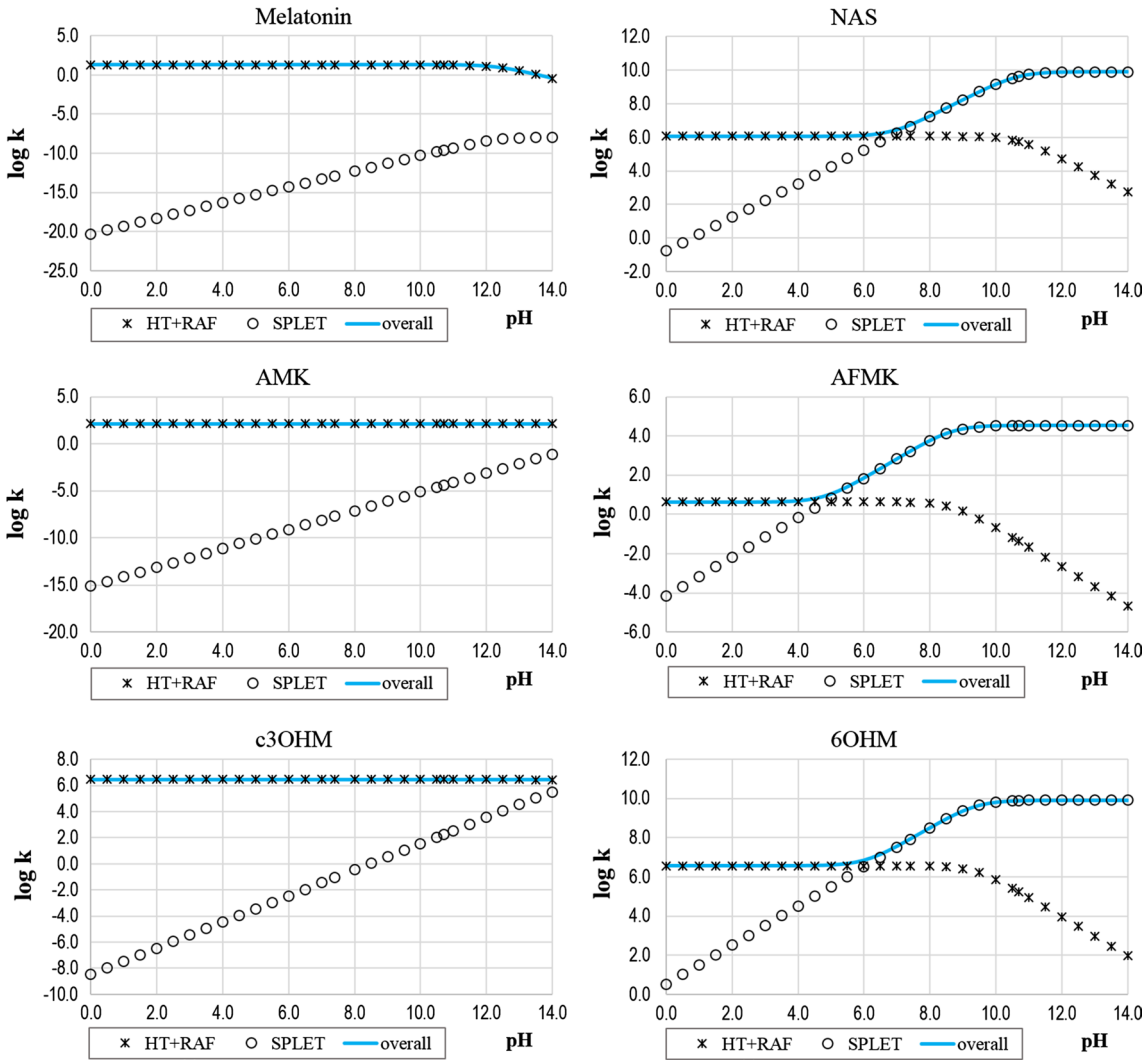

Fig. 1 Influence of the pH on the relative importance of the SPLET mechanism in the •OOH scavenging activity of the studied compounds

melatonin-related compounds. Moreover, they support the importance of fully characterizing the physicochemical properties of chemical compounds with antioxidant properties, in particular acid–base equilibria, since they may significantly influence other properties and biological effects. It seems also relevant to call attention to the fact that identifying relatively low populations of anionic species, presenting the phenolate moiety, is not enough to rule out their role in the scavenging activity of chemical compounds. The key number to consider should be the product of the molar fraction by the corresponding rate constant, at the pH of interest.

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