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Theoretical Studies of Structures and Thermodynamic Parameters of Melatonin and its Metabolites: N1-Acetyl-N2-formyl-5-metoxy kynuramine and N1-Acetyl-5-metoxykynuramine

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Abstract: Melatonin, a neurohormone is well known regulator of a number of physiological processes. In addition, numerous studies both *in vitro* and *in vivo* showed a strong antioxidant and free radical scavenging activity usually considered as a result of direct melatonin reactivity. However, two major metabolites of melatonin, N^1-acetyl-N^2-formyl-5-metoxy kynuramine (AMFK) and N^1-acetyl-5-metoxykyunaramine (AMK) also showed strong free radical scavenging activity, but towards different biological free radicals. Melatonin oxidation mechanism in a complex biological environment has been studied at different conditions but still is partially understood. Reactivity of the molecule is always governed but its electronic properties and kinetic and thermodynamic stability. Thus, we performed theoretical calculations using Density Functional Theory (DFT) at with B3LYP/6-31G* basis set to calculate geometries, atomic charges and thermodynamic parameters for all three molecules. Semi-empirical calculations at PM1 level are also performed and compared with DFT data. Calculated atomic charges showed that nitrogen atoms as the most possible sites for interactions with electrophilic species such as free radicals. Oxygen atom in metoxy group also shows pronounced negative atomic charge. The most stable molecule is AMFK, followed by AMK and melatonin respectively. This trend can partially explain high melatonin reactivity and its fast decomposition in biological systems. Obtained values calculated at semi-empirical and *ab inition* levels are significantly different implying that conclusions based on calculations done at lower levels of theory can not be used as reliable when explaining experimental data.

INTRODUCTION

Melatonin is a neurohormone synthesized from the amino acid tryptophan and secreted by the pineal gland in the brain (Gastel, Roseboom, Rinaldi, 1998). It is involved in a number of biological and physiological regulatory mechanisms including circadian rhythm, ovarian physiology, blood pressure regulation, retinal physiology, seasonal reproduction, and immunity (Claustrat, Brun, Chazot, 2005, Jonas, Garfinkel, Zisapel, *et al*., 2003). Its synthesis and release are stimulated by darkness and suppressed by light (Zeman, Dulkova, Bada, *et al.,* 2005 Brzezinski, 1997, Pangerl, Pangerl, Reiter, 1990). Melatonin is considered to be a potent anti inflammatory reagent in both in vivo and in vitro (Ochoa, Vilchez, Palacios, et al., 2003). Resent data showed the inhibitor activity of melatonin on peroxidases catalyzed formation of hypohalous acids, responsible for host tissue injury. (Galijasevic, Abdulhamid, Abu-Soud, 2008, Lu, Galijasević, Abu-Soud,.2008). Antioxidative ability of melatonin is based on its role as a scavenger of reactive oxygen species including hydroxyl radical, superoxide ion, peroxy radicals, singlet oxygen, nitric oxide, peroxynitrate and its metabolites.(Reiter, Guerrero, Garcia, et al., 1998) Ximenes, Silva, Rodrigues, et al., 2005) It plays an important role in protecting cell membranes from lipid peroxidation, neutralizing hydroxyl radicals and may bind to DNA, promoting further protection beyond antioxidant activity. The oxidized form of melatonin, N^1-acetyl-N^2-formyl-5-methoxynuramine (AFMK), is too free radical scavenger (Ximenes, Silva, Rodrigues, et al.,2005). Besides it beneficiary protective role, recent data showed that melatonin exhibits pro-inflammatory role at early phase of inflammation but switches to an antioxidant activity in a chronic inflammatory phase. (Radogna, F., Diederich,M., Ghibelli, L. 2010).

Number of studies compared reactivity of melatonin, AMFK and AMK showing a different susceptibility towards different free radicals. Despite the known general mechanism of melatonin catabolic pathway, different catabolic products and amounts have been detected in a different organs and tissues. Besides the availability of enzymatic and nonenzymatic reactants involved in formation of AMFK and AMK, usually considered to be major melatonin metabolites, stability and electronic properties of these compounds should play a major role in their activity. There is a possibility that free radical scavenging activity of melatonin molecule is due to the fast and complete formation of AMFK and AMK and their activity rather then the reactivity of the melatonin molecule itself. Thus, we performed theoretical study for all three molecules at the highest level of computational theory calculating energy, enthalpy, and total entropy for most stable structures of melatonin, AMFK, and AMK. Some earlier studies showed several of these parameters for melatonin and AMFK but for different molecule geometries. Also, calculations at semi-empirical and *ab initio* levels are compared in terms of energy trends for all three molecules.

EXPERIMENTAL

The geometry optimization of melatonin, AMFK, and AMK were performed using Density functional theory (DFT) method with B3LYP nonlocal exchange functionals and the 6-31-G(d) basis set as implemented in Spartan Software 08 (Wavefunction Inc. CA) First, seven different optimized melatonin conformational structures computed using RHF level using 6-31G(d) basis set are initially obtained and explored. One with a lowest energy was used for further optimization studies. The optimized structures, atomic charges and thermodynamic properties at DFT level were calculated. Additionally vibrational frequencies were calculated for the optimized structures with a same basis set used for geometry optimizations, in order to confirm structure minima.

RESULTS AND DISCUSSION

Geometries. A large number of melatonin conformers have been initially explored, and the most stable structure was chosen for further studies. Figure 1 shows a plot of energies of six structures with the lowest calculated energie. Only three structures are shown here due to the clarity of the graph. The bond angle C-O in the metoxy group differs for all three proposed structures. The side chain attached to the indole structure does not differ in two structures with the lowest energies being twisted toward the plane of the indole while a third structure, with a higher energy has a side chain being almost perpendicular to the plane of indole ring . In Figure 2, optimized most stable structures for all three molecules are shown with calculated dipole moment and its orientation in a molecule. Twisted geometry of the melatonin molecule visibly forms a globular structure efficiently exposing all the reactive sites towards other reactive molecules.

That conformational structure allows a molecule to get in closer contact with small reactive molecules, but in the same time has the ability to interact with conformationaly restricted sites of larger molecules such as enzymes. Thus, melatonin inhibitory activities towards peroxidases enzymes can be explained in part by this feature.

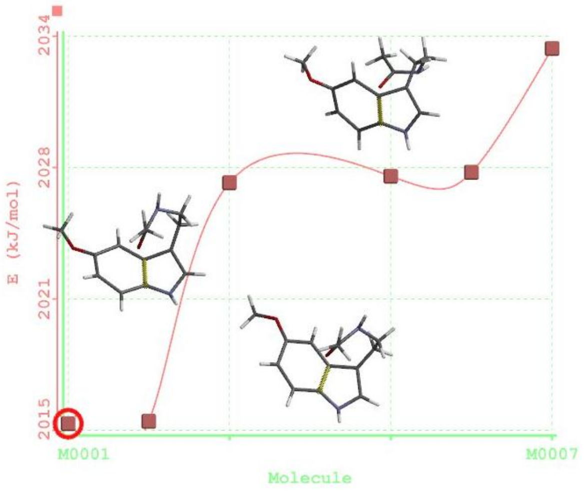
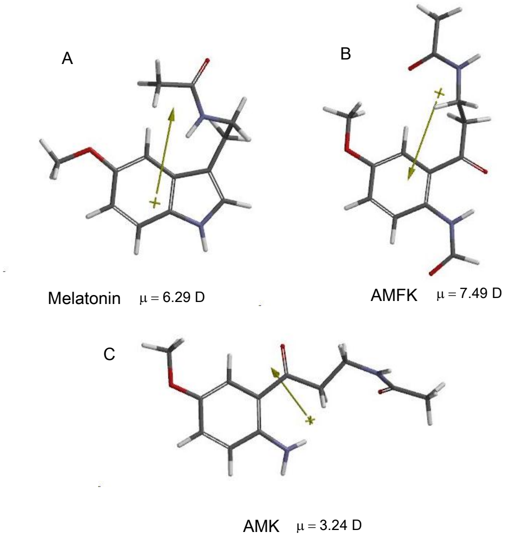


Figure1. Molecule plot of most stable conformers of melatonin molecule. Only three most stable structures has been shown here.



Melatonin $\mu = 6.29$ D

AMFK $\mu = 7.49$ D

AMK $\mu = 3.24$ D

Figure 2. The optimized structures and dipole moment (in debye) of melatonin (A), AMFK (B) and AMK (C). Optimization was performed using Density Functional Theory at B3LYP/6-31G* level. The dipole moment magnitude and vector orientations are shown for each structure.

Atomic charges. Atomic charges using Mulliken theory were calculated and presented in Table 1. Calculations at lower level of theory using basis set HF/6-31G* assigned more negative charges on selected atoms in all structures, namely oxygen and nitrogen. When compared to DTF calculations done using B3LYP/6-31G* basis set it is apparent that lower level of theory will give significantly lower charges on nitrogen atoms, while the difference in atomic charges of oxygen atoms in all structures is much smaller. Collectively, these values indicate possible sites of interactions with electron poor molecules, with nitrogen atoms been the most possible sites for interactions with electrophilic species such as free radicals. This is in accordance with experimental data and proposed mechanism of melatonin oxidations with hydroxyl radical forming indolyl cation radical.

However, this does not exclude interactions of other sites with high negative atomic charges. At higher level of theory the differences between atomic charges on oxygen and nitrogen are much smaller making interactions of electron poor species with oxygen atoms almost equally possible as the interactions with nitrogen atoms. As a result, several of mechanistic pathways of melatonin oxidations should be considered. Calculated atomic charges of AMFK and AMK also show high electron densities and as such are expected to have a high reactivity towards electron poor groups or species once they are formed in standard catabolitic melatonin mechanism. In addition, presented data clearly shows that charge densities are highly dependable on the selected level of theory used for calculations and atoms in question.

Table 1. Atomic charges of nitrogen and oxygen atoms of melatonin, AMFK, and AMK computed at HF/6-31G* and B3LYP/6-31G* levels of theory.

	Melatonin		AFMK		AMK	
Atom	HF/6-31G*	B3LYP/6-31G*	HF/6-31G*	B3LYP/6-31G*	HF/6-31G*	B3LYP/6-31G*
N (amine)					-0.90	-0.82
N (pyrrole)	-0.85	-0.69	-0.90	-0.68		
N (-CONH)	-0.79	-0.59	-0.80	-0.58	-0.81	-0.60
O (metoxy)	-0.66	-0.52	-0.66	-0.52	-0.66	-0.52
O (-CONH)	-0.68	-0.51	-0.63	-0.52	-0.62	-0.52
O (-HCONH)			-0.56	-0.45		
O (C=O)			-0.60	-0.52	-0.57	-0.49

Table 2. Energies of melatonin, AMFK, and AMK computed at semi-empirical (PM1) and *ab initio* (HF/6-31G* and B3LYP/6-31G*) levels of theory.

Energy (kcal/mol)	Base	Structure		
		Melatonin	AFMK	AMK
Semi-empirical				
Total energy	PM1	-62232.40	-75781.25	-66278.29
Core repulsion energy	PM1	349933.96	31628.80	355783.82
Electronic energy	PM1	-412166.36	-75781.25	-66278.29
Ab initio				
Total energy	HF/6-31G*	-477053.53	-571021.78	-500267.03
	B3LYP/6-31G*	-480032.06	-57448.16	-503319.68
ZPE	HF/6-31G*	185.00	191.59	183.64
	B3LYP/6-31G*	172.77	178.38	171.14
Nuclear repulsion energy	HF/6-31G*	750765.413	893163.63	732265.38
	B3LYP/6-31G*	741239.15	890363.54	73270.98

Thermodynamic parameters. Calculated thermodynamic parameters at semi-empirical level using PM1 basis set and *ab inition* calculations using two different basis sets, HF/6-31G* and B3LYP/6-31G* are presented in Table1 and Table 2. Calculations clearly show tenfold difference in calculated total energies at different levels of energy. It is obvious that any calculations done at PM1 level should not be used for a large molecule and can be used only as initial method to save on computing time rather than to give sound conclusions about reactivity of the molecule in question. Thus, we took into account only data calculated at *ab-initio* level. The stability of a molecule is determined by the total energy of the molecule denoting the kinetic energies of all particles forming the molecule and the potential energies of all their interactions. According to our calculations AMFK is the most stable molecule, followed by AMK and finally melatonin molecule. Using B3LYP/6-31G* basis set, changes in total energy are observed, lowering a differences in stabilities of all three molecules but following the same trend.

Zero-point energy is the lowest possible energy that a quantum mechanical system may have. The molecule with the here is highest ZPE is AMFKA, followed with melatonin and AMK. Calculations at the higher level of energy improved values for AMK molecule the most when compared to other two molecules. Calculated values for standard enthalpy and entropy for all three molecules are given in Table 3. Direct comparison of the standard enthalpy values for nonisomeric compounds is not meaningful rather bond dissociation energies are considered better descriptors of a stability of the system. Experimental data for these parameters are not known but several calculated values for melatonin and AMFK have been reported. However authors did not stated basis set used for calculations or choice of conformer, for either compound thus any comparison with mentioned data can be reported. Reported experimental value for the standard molar enthalpy of formation in the gas phase for the indole compounds was 120.0 kJ x mol^{-1} (da Silva MA, Cabral JI, Gomes JR, 2008) being close to the calculated values for melatonin and its metabolites.

Table 3. Calculated changes in total enthalpy and entropy of melatonin, AMFK, and AMK at room temperature computed at *ab initio* (HF/6-31G* and B3LYP/6-31G*) levels of theory.

Parameters	(cal/mol) Base	Structure		
		Melatonin	AFMK	AMK
ΔH°	HF/6-31G*	195520	203647	194877
	B3LYP/6-31G*	183688	190806	182816
Total entropy	HF/6-31G*	132.69	145.13	138.00
	B3LYP/6-31G*	132.81	143.67	139.05

CONCLUSIONS

DFT calculations of melatonin and its metabolites, AMFK and AMK have been performed in order to obtain improved stable geometries and atomic charges and thermodynamic parameters. Calculations at HF level were carried out and compared to data obtained with DFT. Since all three molecules can exist in complex biological environment in the same time showing pronounced reactivity towards free radicals, their stability is an important factor contributing to the overall reactivity. Atomic charges, that can not be determined experimentally, were calculated showing areas of high electron density as possible sites of interactions with electrophilic species such as oxygen free radicals. Some previous molecula mechanics computational studies related to melatonin reactivity produced results in discrepancies with experimental data. *Ab initio* calculations showed significantly different values proving that force filed implemented in MM calculations can not accurately take into account electronic properties and atom interactions for molecular structures like these ones. Thus more accurate computational calculations should be carried out even for mechanistic pathways of melatonin oxidations and subsequent activity of its metabolites.

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Summary/Sažetak

Melatonin, neurohormone je poznat regulator brojnih fizioloških procesa. Osim toga, brojna istraživanja *in vitro* i *in vivo* su pokazala jaku antioksidativnu aktivnost te rekaktivnost prema slobodnim radikalima koja je obično smatrana kao posljedica direktne reaktivnosti molekule melatonina. Međutim, dva glavna metaboliti melatonina, N1-acetil-N2-formil-5-metoksi kinuramine (AMFK) i N1-acetil-5-metoxykinuaramine (AMK) također su pokazali veliku reaktivnost ali prema različitim biološkim slobodnim radikalima u poređenju sa melatoninom . Mehanizam oksidacije melatonina u složenom biološkom okruženju je ispitivan u različitim uslovima, ali jos uvijek nije u potpunosti definisan Elektronska svojstva te kinetička i termodinamička stabilnost molekule uvijek uslovljavaju molekularnu reaktivnost. Iz tog razloga teorijsko izračunavanje koristeći Density Functional teoriju (DFT) sa B3LYP/6-31G * parametrima je urađeno pri čemu su geometrije, atomska naboji i termodinamički parametri izračunati za za sve tri molekule. Podaci dobiveni sa Semi-empirical izračunavanjima koristeći PM1 parametre su upoređeni sa DFT rezultatima . Izračunati atomski naboji pokazuju da atomi nitrogena su moguća mjesta za interakcije sa elektrofilnim vrstama poput slobodnih radikala. Kisikov atom iz metoksi grupe također pokazuje izrazit negativan atomski naboj. Prema termodinamičkim parametrima najstabilnije molekula je AMFK, nakon koje slijedi AMK te melatonin. Ovaj trend djelomično može objasniti visoku reaktivnost melatonina i njegovu brzo razlaganje u biološkim sistemima. Dobivene vrijednosti izračunate koristeći semi-empirical i *ab inition* nivoe su znatno drugačije što znači da zaključci na temelju rezultata izračuna koristeći niže nivoe teorije se ne mogu koristiti kao pouzdani u analiziranju eksperimentalnih podataka.