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Theoretical study of DHEA: comparative HF and DFT calculations of the electronic properties of a complex between DHEA and serotonin

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Abstract Molecular parameters (interatomic distances and angles, total atomic charge, dipole moments) of DHEA (Dehydroepiandrosterone), serotonin and of their putative complex including its heat of formation, have been computed in an ab initio comparative study involving HF and DFT calculations. The 6-31G* basis set and the B3LYP functional were employed. The aim of this study is to emphasize by DFT calculation the possible existence of a complex between DHEA and serotonin that may have the properties of a new drug. A Natural Bond Orbital analysis description offers supplementary details for the structure of the molecular units and their interaction.

Keywords DHEA · Serotonin · Ab initio · Density functional theory · NBO

Introduction

In the last few years, literature references have emphasized the fact that DHEA is the active form of a steroidal hormone, with very desirable physiological and beneficial health properties in animals and humans [1–7].

Recently, several attempts were made to find a new class of antidepressant drugs with a dual activity displayed at the level of the 5-HT_1A serotonin receptors and serotonin transporters [8]. Computational methods also produced data describing a mechanism by which ligands can activate a 5-HT_1A receptor. Together, these data led to a new basis for the rational design of receptor-selective compounds (serotonin) with a predetermined efficiency [9].

Many recent studies on biological systems were carried out to identify the active molecules involved in vivo and to understand their interactions and functions. The advances made in various areas of chemistry with the help of the supramolecular paradigm emphasize the importance of a theoretical analysis of intermolecular interactions in relevant couples of weakly bound biologically active molecules. The supramolecular approach advocated by Lehn provides a universal model to study such interactions [10].

The present paper targets such a goal by characterizing DHEA, generated in the suprarenal glands and in the brain, and the neurotransmitter serotonin, as well as their association in a complex. The electronic structure of DHEA has been described by the authors [11].

Methods

Using the crystallographic data reported in the literature as a starting point [12], the geometry of DHEA was fully optimized. The initial input for serotonin was obtained from a molecular mechanics calculation (MM^+ force field) [13–15]. The molecular geometries were optimized without any constraints. The ab initio calculations were carried out using the Gaussian 98 program [16].

Calculations were carried out with the 3-21G* and 6-31G* basis sets. The geometries of DHEA, serotonin and of their complex were optimized at the 3-21G* level, starting from an INDO guess. A stationary point was found. At this point, a refinement was carried out by a single point at the 6-31G* level (Raffenetti integral calculation was used) and at the B3LYP/6-31G* level [17]. The optimized geometry of the two component molecules and of the complex were also obtained using

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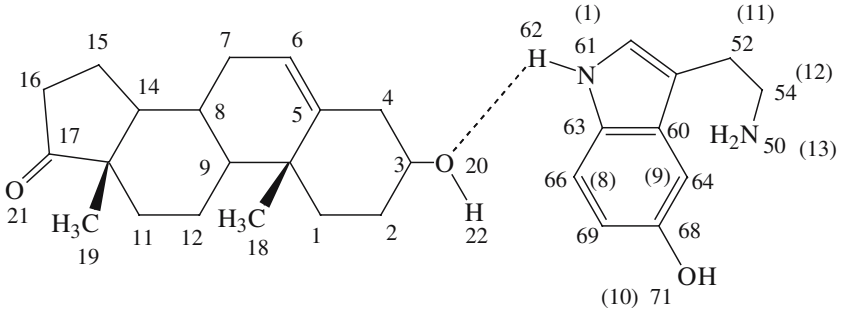
Scheme 1 Numbering of the atoms in the computed DHEA–serotonin complex



density functional theory with Becke's three-parameter exchange functional and the gradient-corrected functional of Lee, Yang and Parr (B3LYP) [17].

In the last part of this paper, an analysis of the molecular wave function performed in terms of localized electron-pair bonding units using the NBO program is given [18, 19]. This analysis is deemed very important to understand the various interactions involving each component of the complex under study.

Computed HOMO and LUMO orbitals were drawn with the MOLEKEL program [20]. The structure and the numbering of selected atoms in the DHEA–serotonin complex are shown in Scheme 1. The numbering related to the computation of the independent serotonin molecule is given in parentheses, while the isolated DHEA has the same labels as in the complex.

Results and discussion

Structure and bonding in the molecular units and their intermolecular complex

The formation of the DHEA–serotonin complex is analyzed in terms of geometry, charge and energy parameters. Finding the absolute minimum for a complex is a nontrivial question, given the subtle balance of the intra- and intermolecular factors. The different nature of the overall molecular constitution of the two biomolecules, DHEA, which has a rigid σ skeleton, and serotonin, which possesses essentially a planar π-conjugated core, practically precludes a significant association of the π–π stacking type. The isolated C=C and C=O bonds in DHEA obviously do not offer enough support for such a cohesion. The strongest association involves hydrogen bonds. There are several possible patterns for hydrogen bonding (O–H...O, O–H...N, involving the various heteroatom combinations). The supramolecular association presented here is the optimal one due to the supplementary stabilization resulting from the alignment of the dipoles on the molecular constituents. The rather floppy $C_2H_4NH_2$ side-chain in serotonin plays an interesting role in balancing this effect.

The bonding association O(22)...H(60) has a regular length for the given type, 1.83 Å. The geometry of association is further characterized by the H62...O20–C3 and N61–H62...O20 angle values (127.1 and 173.2° respectively) and the following dihedral angles, H62...O20–C3–C4 and N61–H62...O20–C3 (−133.7 and −55.0°, respectively).

It should be noted that the atoms of the N–H...O hydrogen bond are almost collinear, which suggests that this interaction is the dominant one in the association. The dipole moment appearing as a result of the formation of the complex, as well as the orientation of the $C_2H_4NH_2$ side-chain are less important.

A systematic insight is gained following the results of the HF and DFT single-point calculations for the geometries optimized at the HF versus DFT levels. A methodological conclusion is that the HF and DFT behave similarly in both the optimized geometry and also in the estimated energy of formation.

Generally, the DFT results can be credited with a higher confidence in the quantitative respects because of their treatment of correlation effects. On the other hand, it is acknowledged that the regular DFT functionals face intrinsic problems in the long-range regime [17]. Therefore, the comparative use of HF and DFT methods in computing weakly bonded systems is a technical necessity. In the present case, the rather small differences computed for the values of the heat of formation and the optimized geometrical parameters validate the results presented as physically acceptable. In our system, the heat of formation being in the range −8 and −10 kcal mol^{-1} is in favor of a strong hydrogen bonding, or in a more detailed way, of a hydrogen bond cumulated with a significant dipolar interaction.

Selected reactivity parameters for DHEA, serotonin and for the complex obtained at the two specified levels are shown in Table 1.

The data in Table 1 show that there are no significant changes in the results obtained in the optimization step at the B3LYP level. The interatomic distances, the valence and the dihedral angles have practically the same values.

In the frame of the interpretation given above, the following details from Table 1 can also be noted:

- A slightly higher stability for the complex is predicted from the DFT data.
- The dipole moment in the complex is higher than the sum of the individual components, suggesting a symbiotic action with the hydrogen bonding, due to some

Table 1 Reactivity parameters calculated at the HF/6-31G* and B3LYP/6-31G* levels

Method	Reactivity parameter	DHEA	Serotonin	DHEA–serotonin	$E_{complex} - (\Sigma E_{complex})$
HF/6-31G* at the HF optimized geometry	Total energy (au)	−885.519	−569.404	−1454.935	−0.012065
	HOMO (au)	−0.33916	−0.26497	−0.26278	
	LUMO (au)	0.15761	0.14169	0.13977	
	μ(D)	2.87	2.33	6.81	
B3LYP/6-31G* at the HF optimized geometry	Total energy (au)	−891.282	−572.982	−1464.281	−0.016847
	HOMO (au)	−0.24098	−0.18745	−0.18437	
	LUMO (au)	−0.02440	−0.00132	−0.02886	
	μ(D)	2.74	2.29	7.25	
B3LYP/6-31G* at the DFT optimized geometry	Total energy (au)	−891.287	−572.9881	−1464.28928	−0.016847
	HOMO (au)	−0.24098	−0.18745	−0.18437	
	LUMO (au)	−0.02440	−0.00132	−0.02886	
	μ(D)	2.74	2.29	7.25	

polarization of the electron density and to conformational changes in the side-chain of serotonin.
- At the HF/6-31G* level, the frontier orbitals in the complex are localized on serotonin.
- Conversely in the B3LYP/6-31G* model, one notes that the frontier orbitals in the complex derive from the HOMO of serotonin and the LUMO of DHEA.

The HOMO orbital (at the HF/6-31G* level) in DHEA is mainly localized on the π_{CC}double bond orbital (C_5–C_6) [11] (Fig. 1a). However, the B3LYP results (Fig. 1b) show that this orbital is localized on the D ring, being mostly a π C=O system involved in a hyperconjugation-like out-of-phase combination with the C–H bonds of the saturated skeleton.

The HOMO–LUMO gap in the DFT calculations is smaller than in the HF case. This is a consequence of systematic positive energy shifts in the occupied MOs and negative energy shifts in the virtual ones, due to the nature of the HF and KS functions. This is a normal situation, considering the physical meaning of each method. A rather different conceptual interpretation characterizes each scheme, the HF energies being assimilated to the ionization potentials (Koopman's theorem), while the KS orbitals correspond to orbital electronegativities (Janak theorem) [17]. Therefore, one may regard the HF–HOMO, in its C=C preponderance, as corresponding to an easily ionized level (involving nonelectronegative atoms), while the KS–HOMO switches the effective status toward a π bond involving the more electronegative group C=O.

In serotonin, the HOMO and LUMO orbitals are seen as pure π orbitals. Because the orbital shape in a π-type system is merely determined by topological reasons, the frontier orbital shapes are similar in the HF and DFT calculations. The frontier orbitals from serotonin lose their almost pure π-nature, and acquire a hybrid character in the complex (Fig. 2). This reveals a subtle influence of the electronegativity factors involved in the donor–acceptor interactions that is accounted for in the frame of the DFT approach.

A detailed description of these interactions is obtained from a comparative analysis of the atomic charges in the isolated molecules and in their association complex (Table 2).

Table 2 shows that atom O20 from DHEA acquires the largest negative charge. A similar trend, but of smaller magnitude, is observed for the other electronegative atom of the hydrogen bridge, namely atom N61 from serotonin. A larger positive increase of the charge is noted for the bridge hydrogen, H62. It is also interesting to note that the inductive effect produces a positive charge increase on atom H22 from the outer O–H

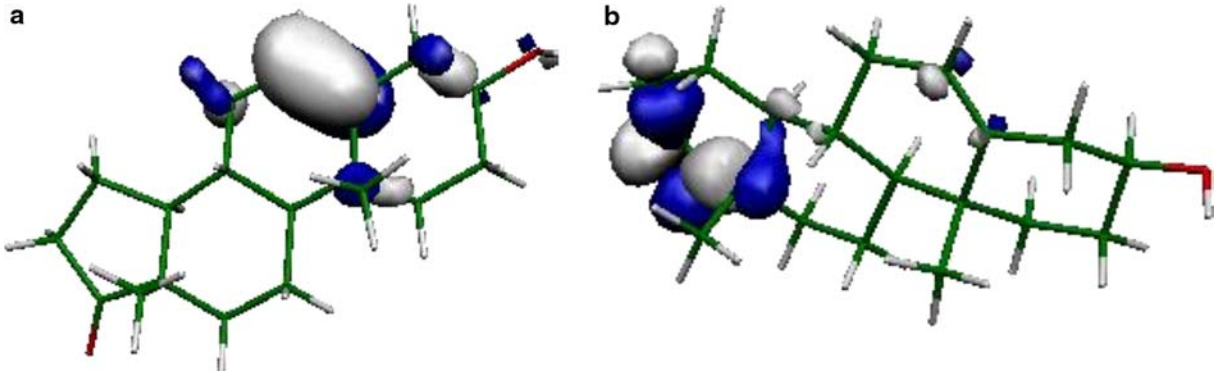


Fig. 1 The HOMO of the DHEA independent molecule in the: **a** HF/6-31G* and **b** B3LYP/6-31G* calculations

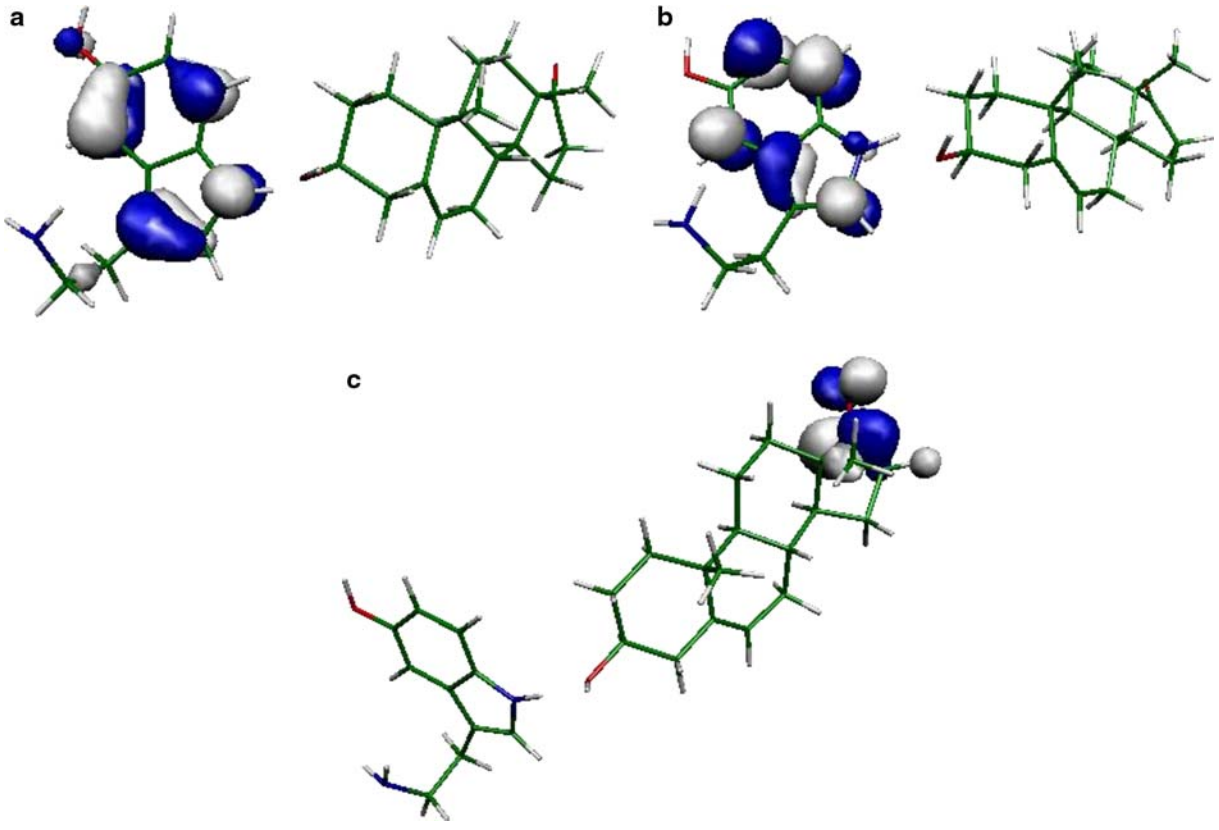


Fig. 2 Frontier orbitals in the complex, from HF and DFT calculations: **a** HOMO for HF/6-31G*, **b** LUMO for HF/6-31G*, **c** LUMO for B3LYP/6-31G*. (Note: the HOMO from the B3LYP calculation is very similar to the HF analogue and is not represented)

bond of the intermolecular association region. In the DHEA skeleton, the charge modifications fade rapidly off with the distance from the interaction site, while in serotonin the conjugation makes the changes, though small, more evenly distributed over the molecular fragment.

Table 2 Total atomic charges on selected atoms in the molecular components and in the association complex, from HF and DFT Mulliken population analysis

	HF			DFT		
	Fragment	Complex	Variation	Fragment	Complex	Variation
ATOM	DHEA	DHEA–serotonin	ΔQ	DHEA	DHEA–serotonin	ΔQ
O_{20}	−0.77	−0.81	−0.04	−0.58	−0.64	−0.06
C_3	0.19	0.19	0.00	0.08	0.10	0.02
H_{22}	0.43	0.45	0.02	0.34	0.37	0.03
H_{45}	0.18	0.19	0.01	0.18	0.19	0.01
H_{46}	0.18	0.19	0.00	0.19	0.20	0.01
	Serotonin			Serotonin		
N_{61}/N_1	−0.86	−0.91	−0.05	−0.81	−0.84	−0.03
H_{62}/H_{14}	0.39	0.49	0.11	0.33	0.39	0.06
H_{67}/H_{17}	0.20	0.20	0.00	0.15	0.16	0.01
N_{50}/N_{13}	−0.88	−0.87	−0.01	−0.71	−0.68	−0.02
C_{52}/C_{12}	−0.12	−0.11	−0.01	−0.19	−0.19	0.00
C_{54}/C_{11}	−0.32	−0.34	−0.02	−0.40	−0.41	−0.01
C_{57}/C_3	−0.02	−0.04	−0.03	0.06	0.04	0.01
C_{58}/C_2	0.04	0.05	0.01	0.07	0.09	0.01
C_{60}/C_9	−0.07	−0.05	−0.02	−0.27	−0.27	−0.01
C_{63}/C_8	0.33	0.31	0.01	0.29	0.28	0.01
C_{64}/C_4	−0.25	−0.26	−0.02	−0.01	−0.07	−0.05
C_{66}/C_7	−0.21	−0.21	0.00	−0.04	−0.03	−0.01
C_{68}/C_5	0.39	0.40	0.01	0.22	0.22	0.00
C_{69}/C_6	−0.28	−0.29	−0.01	−0.18	−0.18	0.00
O_{71}/O_{10}	−0.79	−0.79	0.00	−0.63	−0.63	0.00

The effect of the association with DHEA is then an activation of serotonin induced by this electronic distribution change. The resulting activation mechanism of serotonin would then be due to a possibly significant structural rearrangement.

Comparing the methods, one may see that the DFT-based population analysis gives smaller absolute charge values on each atom. This can be regarded as a better account of the density using DFT, as the HF approach seemingly overestimates the absolute values of the charge separation in covalent polar bonds. However, both HF and DFT account similarly for the density flow associated with molecular association. For such weak donor–acceptor complexes, it is also clear that the outcome of the detailed results depend on the choice of the density function.

NBO analysis of the complex

The natural bond orbital (NBO) method [19, 20] offers supplementary structural information. The simplest analysis consists of checking the composition of the natural hybrid orbitals (NHO), which may reveal details about differential hybridization, i.e. sometimes rather important deviations from the usual sp^2 (s:p = 33:67%) or sp^3 (s:p = 25:75%) compositions.

For example, for the double bond between C5 and C6, one observes that the corresponding hybrid orbitals, with C5 s (38.2%) and p 1.6 (61.9%), C6 s(40.3%) and p 1.4 (59.7%) correspond to a rather higher s percentage than the usual 33%, at the expense of a lower s content in the other C–C bonds connected to C(5) = C(6) [e.g. C5 s (31.8%) and p 2.14 (68.2%) in C(5)–C(10)]. The larger s content can be associated with the strengthening of the bond, noting then the nontrivial consequence that the presence of the double bond in the skeleton slightly weakens the single C–C bonds surrounding it, while giving in turn a supplementary stabilization of the σ-component of the C = C bond. The NBO shows that the π bond is established, as expected with pure p-AOs (C5 s(0.02%) and p 99.9 (99.9%) in BD (2) C5–C6).

Particularly interesting are the hybrid orbitals associated with the intermolecular hydrogen bond formed by the sequence of atoms N(61)–H(62)...O(20). The hybrid orbitals of the N(61) atom are close to the regular sp^2 (s:p = 33:67%) composition. Chemical intuition would suggest that the O(20) atom should have an sp^3 character. However, the NBO analysis shows that the hybrid composition is not so standard, and is in fact better characterized by an sp^2 differential hybridization. A p-like hybrid orbital is oriented perpendicularly to the H(62)...O(20)–H(21) plane. The lone pair devoted to the H(62)...O(20) hydrogen bond has the nonstandard composition s (48.3%) p 1.07 (51.6%) which practically suggests an sp hybrid character (i.e. with the ratio s:p ~1:1 = 50:50%.). The hybrids along the C(3)–O(20) and O(20)–H(22) bonds have the following compositions s(30.9%) p 2.2 (69.0%) and s (20.2%) p 3.9 (79.7%), respectively. The first one is somehow intermediate between sp^3 and sp^2 in character and the last one is poorer in s character than an sp^3 hybrid. The heterogeneous nature of the bond is measured by the 68.9% participation of the oxygen hybrid orbitals in the C–O bond. Similarly in the O–H bond described, the oxygen hybrid orbital percentage is 73.2%.

The NBO analysis automatically identifies two molecular units corresponding to the steroid and serotonin molecules. The perturbation donor–acceptor analysis of the NBO method offers information about intermolecular interactions. A look at the corresponding data shows that the most important intermolecular donor–acceptor contact occurs between an antibonding NBO function (NBO no. 550, BD*(1) N61–H62) of the N–H group and the lone pair of O(20), corresponding to an energy of −11.9 kcal mol^{-1}. The empty antibonding NBO of the N–H group displays a 73.5% character for atomic orbital s of H(62), which corresponds to an unshielded proton pulling electron density from the occupied lone pair hybrid orbitals (NBO no. 120, LP (2) O20). The sum of the donor–acceptor perturbations between the two units amounts to about −14.4 kcal mol^{-1}.

It is interesting to complete the insight with data related to steric exchange energies from the analysis performed with the help of the "steric" keyword from the NBO5 program. A glance at the data for the atoms involved in the interaction shows a repulsion effect between the occupied NBOs of the N(61)–H(62) bond and the O(20) lone pair. Such an exchange energy is estimated to be +11.7 kcal mol^{-1}.

The balance between the donor–acceptor interactions and the steric repulsion amounts to an intermolecular bonding resultant approximately equal to −2.7 kcal mol^{-1}. The complement of the bonding effects is assigned to pure electrostatic effects.

Conclusions

- From these comparative calculations, it appears that the possibility of forming an association complex between DHEA and serotonin is emphasized by the B3LYP model.
- In the B3LYP model, the frontier orbitals in the complex derive from the serotonin (HOMO) and DHEA (LUMO).
- The NBO analysis reveals several nonstandard hybrid compositions and the associated donor–acceptor perturbative schemes support the idea of a moderate-strength hydrogen bond, cumulated with electrostatic effects, leading to a firmly bound molecular complex.
- Particularly interesting are the hybrid orbitals associated with the intermolecular hydrogen bond between N(61)–H(62) and O(20). The NBO analysis shows that the hybrid composition is not so standard, and in fact is better characterized by an sp^2 differential

hybridization. A *p*-like hybrid orbital is oriented perpendicularly to the H(62)...O(20)–H(21) plane. The lone pair devoted to the H(62)...O(20) hydrogen bond has the nonstandard composition *s* (48.3%) and *p* 1.07(51.7%) which practically suggests an *sp* hybrid character (i.e. with the ratio $s{:}p \sim 1{:}1 = 50{:}50\%$).

- The present theoretical study on the electronic changes brought about by complexation leads to the hypothesis that a change in the biological action of serotonin and/or DHEA could result from their interaction. This hypothesis could be reinforced by the experimental observation of an interaction between those two molecules. Such a study is now initiated in our laboratory.
- The possibility for a functional connection between serotonin and DHEA opens up new vistas in the approach to the role of the latter as a new neurohormone, given its presence in the central nervous system, a presence the operational significance of which is still not clear.

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