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Regular Article

Ab-initio study of pyrrole ring deformation in the indole group of 5-HT interacting with water molecules

Kei Odai[1], Tohru Sugimoto[2,4] and Etsuro Ito[3]

[1]*School of Science and Engineering, Kokushikan University, Setagaya-ku, Tokyo 154-8515, Japan*
[2]*College of Science and Engineering, Kanto Gakuin University, Yokohama, Kanagawa 236-0037, Japan*
[3]*Department of Biology, Waseda University, Shinjuku-ku, Tokyo 162-8480, Japan*
[4]*Deceased*

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5-Hydroxytryptamine (5-HT; serotonin) regulates metabolism and various homeostatic mechanisms in the body, and is involved in depression. These complicated functions of 5-HT are supported by several 5-HT receptor and 5-HT transporter subtypes. The development of agonists/antagonists and activators/inhibitors of 5-HT receptors and transporters is a strong target for drug studies. Toward this purpose, we calculated the conformations and electrical states of ionized 5-HT in aqueous solution using *ab-initio* methods. When we assumed an ionized 5-HT molecule and three surrounding water molecules, the hydrogen bond network for these four molecules formed a ring shape, resulting in deformation of the pyrrole ring in the indole group of 5-HT. To our knowledge, this is the first finding demonstrating deformation of the indole skeleton. The findings suggest that the direct involvement of water in the binding between 5-HT and its receptors and transporters should be taken account when designing candidate 5-HT active compounds.

Corresponding authors: Kei Odai, Department of Electronics and Informatics, School of Science and Engineering, Kokushikan University, 4-28-1 Setagaya, Setagaya-ku, Tokyo 154-8515, Japan. e-mail: odaikei@kokushikan.ac.jp; Etsuro Ito, Department of Biology, Waseda University, TWIns, 2-2 Wakamatsucho, Shinjuku-ku, Tokyo 162-8480, Japan. e-mail: eito@waseda.jp

Key words: 5-hydroxytryptamine, *ab-initio* calculation, optimization, self-consistent reaction field method

5-Hydroxytryptamine (5-HT; serotonin) is an indoleamine and a monoamine (Fig. 1) synthesized from the amino acid L-tryptophan by the enzymes (tryptophan hydroxylase and 5-hydroxytryptophan decarboxylase), and is metabolized mainly by monoamine oxidase A [1]. Discovery of the brain 5-HT system prompted the theory that biochemical abnormalities in the 5-HT system underlie various forms of mental illness. This theory gained traction when depletion of brain 5-HT by the alkaloid reserpine was found to result in profound behavioral depression [1,2]. Since then, the role of 5-HT in depression has been vigorously discussed from various viewpoints, such as energy regulation [3]. In peripheral tissues, 5-HT is synthesized in enterochromaffin cells and then circulated throughout the body, where it regulates glucose homeostasis, lipid metabolism, bone density, and diseases of obesity and diabetes mellitus [4].

The many actions of 5-HT in the brain and peripheral tissues are achieved through the activation of various 5-HT receptor subtypes [5]. These receptor subtypes include G-protein coupled receptors and ligand-gated channels, and

◀ ***Significance*** ▶

The physiologic actions of 5-hydroxytryptamine (5-HT, serotonin) are multifaceted, and include control of feelings of well-being. To exert these various actions, the body has many 5-HT receptor subtypes and specific 5-HT transporters. Our *ab-initio* calculations revealed that 5-HT in aqueous solution is ionized and its indole group is deformed. These findings will be useful for designing 5-HT receptor and transporter agonists, antagonists, activators, and inhibitors.

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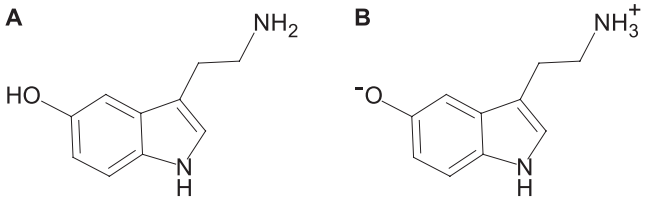

Figure 1 5-Hydroxytryptamine (5-HT; serotonin). A. structural formula. B. ionized form. The molecular formula is $C_{10}H_{12}N_2O$. This is an indoleamine, including an indole skeleton, and an amino group. 5-HT is referred to as a monoamine neurotransmitter. We used an ionized form for the calculations in aqueous solution.

5-HT produces both excitatory and inhibitory effects on cells containing 5-HT receptors. Further, the function of 5-HT transporters allows for complicated pharmacological actions of 5-HT [6]. Therefore, the design of novel 5-HT receptor and transporter agonists, antagonists, activators, and inhibitors is strongly expected to advance medical treatments [7-9]. Toward this aim, the optimized conformation of 5-HT in aqueous solution should be carefully elucidated.

In the present study, we investigated the optimized conformation of 5-HT (particularly its ionized structure) in aqueous solution using *ab-initio* calculations. Our results suggest that the direct effects of water on the conformation of 5-HT should be taken into consideration by studying 5-HT surrounded by water molecules.

Materials and Methods

Quantum chemical calculation

We used Gaussian03 software [10] and the second-order Møller-Plesset perturbation (MP2) method [11]. The basis set was 6-311++G(d,p), which included not only the polarization function, but also the diffuse function for the calculation of O^- in the OH group and that of NH_3^+ in the NH_2 group of 5-HT. Figures were drawn using GaussView 4.1 (Gaussian Inc., Wallingford, CT, USA).

Optimization calculation for 5-HT in gas phase

Although the proton in the OH group of 5-HT was considered to have two possible directions, only one direction was calculated (Fig. 2). The validity of this selection was confirmed on the basis of previous work by Wilke *et al.* [12], in which our selected conformation was called *anti*-serotonin, and the other conformation was called *syn*-serotonin. They concluded that the *anti*-conformers are always more stable in the ground state [12]. In the present study, we prepared 12 molecular structures as the initial condition in gas phase.

Optimization calculation for 5-HT in aqueous solution

We prepared an ionized model of 5-HT in aqueous solution, assuming O^- for the OH group and NH_3^+ for the NH_2 group. The solvent effects surrounding 5-HT were considered using the self-consistent reaction field (SCRF) method [13]. For the solvation model, we used a conductor-like polarizable continuum model (CPCM) [14,15] or a polarizable continuum model (PCM) [16].

Optimization calculation for 5-HT with three surrounding water molecules

An ionized model for 5-HT, as described above, was also used in this case. Three water molecules were arranged surrounding 5-HT, and a solvent effect was not considered.

Results

Molecular conformations of 5-HT in gas phase

We used 12 initial conformations of 5-HT and calculated them in gas phase. Their optimized conformations are shown in Figure 3, and the total energy of each conformation is shown in Figure 4. The numbers (I to XII) in these figures were assigned in ascending order of the total energy. The indole group of 5-HT was planar in all conformations. Differences among the conformations were detected in the direction of the side chain CH_2-CH_2-NH_2, with a maximum difference in energy between the conformations I and XII of 3.3 kcal/mol.

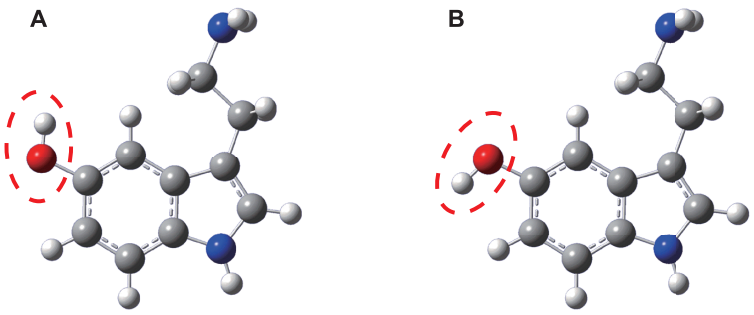

Figure 2 Direction of the OH group in 5-HT. A and B. There are two conformations. Gray balls are carbon atoms, blue balls are nitrogen atoms, red balls are oxygen atoms, and white balls are hydrogen atoms. The following figures are drawn in the same manner. Conformation (A) is called *anti*-serotonin, and conformation (B) is called *syn*-serotonin. Conformation (A) has lower total energy than conformation (B) [12].

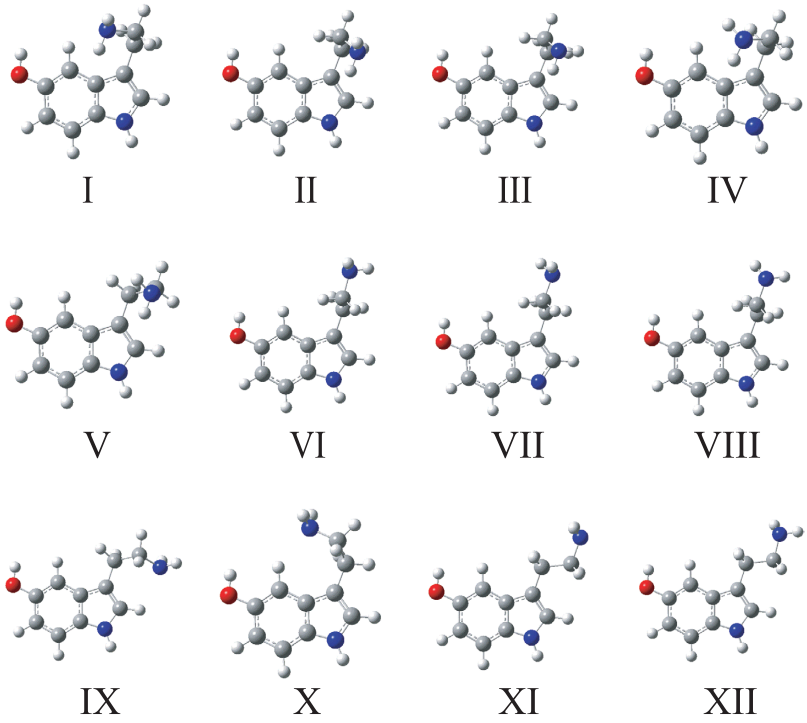

Figure 3 Optimized conformations of 5-HT in gas phase. As the initial condition, 12 conformations were selected. The numbers I to XII were assigned in ascending order on the basis of the total energy.

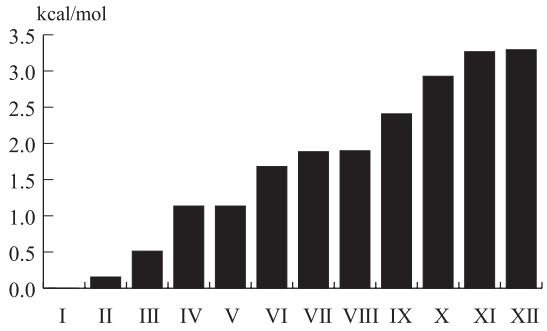

Figure 4 Total energy in each optimized conformation of 5-HT in gas phase. The numbers (I to XII) are the same as in Figure 3. The energy values are relative compared with that of the most stable conformation, Conformation I.

Molecular conformations of 5-HT in aqueous solution

We used the 12 conformations of 5-HT obtained in Figure 3 as the initial condition, ionized the OH group and the NH_2 group in these 5-HT molecules, and then calculated them by SCRF with a solvation model (CPCM or PCM). The optimization calculation resulted in only three conformations in aqueous solution (Fig. 5), which we called Type A, B, and C, assigned in ascending order of energy. Type A conformation resembled conformations II and III in the gas phase; Type B conformation resembled conformations VI, VII, and VIII in the gas phase; and Type C conformation resembled conformations XI and XII in the gas phase. When CPCM was used as the solvation model, we failed to obtain the optimization conformation for Type C. The total energy and dipole moment of each of these types are compared in Table 1.

The indole group of 5-HT was planar in all conformations. Differences among these three types in aqueous solution were observed only in the direction of the side chain CH_2-CH_2-NH_3^+. These differences were the same as those in the gas phase. The maximum difference in the total energy between Type A and Type C was 3.8 kcal/mol.

Molecular conformations of 5-HT with three surrounding water molecules

To examine the interactions between 5-HT and water, we arranged three water molecules surrounding an ionized 5-HT. In other words, we did not apply SCRF to this calculation. We initiated the calculation using the PCM-model Type A conformation obtained in the calculation above for an aqueous solution, and observed the hydrogen bonds between O^-/NH_3^+ and water molecules (Fig. 6). This hydrogen bond network comprised the following five bonds: (i) bond between O^- of 5-HT and a water molecule (referred to as Water 1), whose bond length was 1.47 Å; (ii) bond between Water 1 and another water molecule (referred to as Water 2), whose bond length was 1.62 Å; (iii) bond between Water 2 and NH_3^+ of 5-HT, whose bond length was 1.71 Å; (iv) bond between NH_3^+ of 5-HT and another water molecule (referred to as Water 3), whose bond length was 2.00 Å; and (v) bond between Water 3 and N in the pyrrole ring of 5-HT,

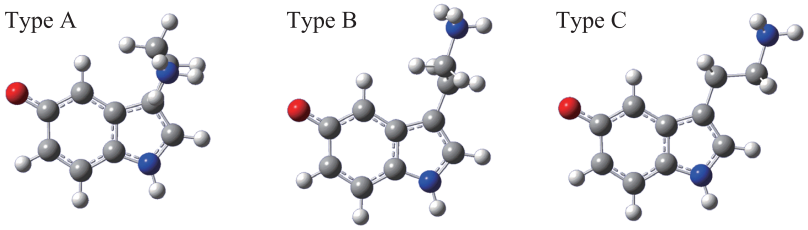

Figure 5 Optimized conformations of 5-HT in aqueous solution calculated by the SCRF method. When we used the 12 initial conformations obtained in gas phase (Fig. 3) and ionized them, 3 optimized conformations were obtained. Types A to C were assigned in ascending order of the total energy.

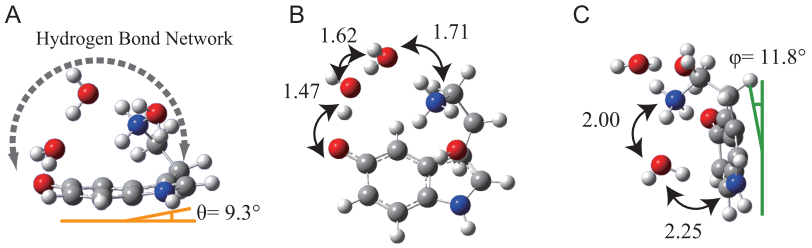

Figure 6 Optimized conformations of an ionized 5-HT and three water molecules. The hydrogen bond network formed a ring shape, resulting in pyrrole ring deformation in the indole group of 5-HT. A-C. To visualize this hydrogen bond network, three views from different directions are depicted. Numbers indicate the hydrogen bond lengths in angstroms. Deformation of the pyrrole ring can be observed. The angle between the plane of the benzene ring and that of the pyrrole ring is 9.3°, and the angle between the plane of the pyrrole ring and the carbon atom of the side chain is 11.8°.

Table 1 Relative total energy and values of dipole moment for 5-HT conformations in aqueous solution calculated by the SCRF method with a solvation model (CPCM or PCM)

Conformation type	Total energy (kcal/mol)		Dipole moment (debye)	
	CPCM	PCM	CPCM	PCM
Type A	0.00	0.00	28.5	28.2
Type B	1.77	1.77	29.7	29.7
Type C	–	3.76	–	35.6

Total energy of Type A shown in Figure 5 was always the most stable. The differences in total energy between Type A and other types are indicated.

whose bond length was 2.25 Å. This hydrogen bond network formed a ring shape, resulting in pyrrole ring deformation in the indole group of 5-HT (Fig. 6). The angle between the plane of the benzene ring and that of the pyrrole ring was 9.3°, and the angle between the plane of the pyrrole ring and the carbon atom of the side chain was 11.8°. In addition, we found that the hydrogen bond network among the ionized 5-HT and three water molecules has a stabilization energy of 12.4 kcal/mol.

Discussion

The gas phase calculations showed a maximum difference of 3.3 kcal/mol among the 12 conformations evaluated. This value is similar to the energy of a hydrogen bond in aqueous solution; i.e., all 12 of these conformations (I to XII) seem to form in the gas phase. Even in aqueous solution, the maximum energy difference was 3.8 kcal/mol among the three optimized conformations. This value is as low as the energy of a hydrogen bond in aqueous solution. That is, the three optimized conformations (Types A, B, and C) can be taken on in aqueous solution. The most important finding of the present study is that the hydrogen bond network among an ionized 5-HT and its surrounding water molecules deforms the pyrrole ring in the indole group of 5-HT, and stabilizes the conformation. This effect could not be mimicked by SCRF calculation, because of the following reasons.

SCRF is a method of accounting for the effect of a polarizable solvent on the quantum system. One of the SCRF methods is PCM using the integral equation formalism variant. This method creates the solute cavity via a set of overlapping spheres. It was initially devised by the Tomasi group and Pascual-Ahuir *et al.* [17–19]. Further, the Cossi group reformulated PCM and set it as CPCM [14,15]. In CPCM, particular attention was paid to large systems requiring suitable iterative algorithms to compute the solvation charges. Therefore, although the SCRF method, including PCM and CPCM, accounts for the effect of a polarizable solvent, the interaction between the target molecule (i.e., 5-HT in the present study) and individual water molecules is ignored. That is, there is a large difference in calculations by the SCRF method and those by the method with due regard to the direct interaction between a target molecule and its surrounding water molecules. In the present study, when we

applied two water molecules into the system, no remarkable change was observed. However, when we applied three water molecules into the system, a remarkable change was observed.

Some experimental and theoretical attempts have been made to clarify 5-HT conformations [12,20–25]. Of these studies, LeGreve's work [23] has reached the results close to ours. They considered the solvent effects on 5-HT conformation, and demonstrated that a water molecule forms a hydrogen bond with the NH_2 group. Even though interactions between 5-HT and the surrounding water molecules have been reported, to our knowledge there has been no descriptions of deformation of the indole skeleton. The current results suggest that the conformation of pyrrole ring in 5-HT should not be fixed in a flat form for drug design calculations. The experimental conformations suggesting deformation of the pyrrole ring in the indole group of 5-HT are expected to improve our understanding of the actions of 5-HT.

Some data in the present paper were published in Transactions of the Kokushikan University, Faculty of Engineering, with the copyright belonging to the authors. Thus, the authors have been permitted to present the same data in this journal.

Conflicts of Interest

All authors declare that they have no conflicts of interest.

Author Contribution

K. O. and T. S. performed the calculations. K. O., T. S., and E. I. analyzed the data. K. O. and E. I. wrote the manuscript.

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