

Site specific interaction between ZnO nanoparticles and tryptophan: a first principles quantum mechanical study

Prachi Joshi,^a Vasundhara Shewale,^a Ravindra Pandey,^{*a} Virendra Shanker,^b Saber Hussain^c and Shashi P. Karna^{*d}

Received 10th August 2010, Accepted 27th September 2010

DOI: 10.1039/c0cp01466d

First principles density functional theory calculations are performed on tryptophan–ZnO nanoparticles complex in order to study site specific interactions between tryptophan and ZnO. The calculated results find the salt bridge structure involving the –COOH group and ZnO cluster to be energetically more favorable than other interacting sites, such as indole and amine groups in tryptophan. The interaction between tryptophan and ZnO appears to be mediated by both ionic and hydrogen bonds. The calculated molecular orbital energy levels and charge distributions suggest non-radiative energy transfer from an excited state of tryptophan to states associated with ZnO, which may lead to a reduction in the emission intensity assigned to the π – π^* transition of the indole functional group of tryptophan.

1. Introduction

In recent years, there has been an increased interest in understanding the interaction between inorganic nanoparticles and biological molecules at the atomic level due to their potential applications in biomedical nanotechnologies. Of particular interest has been the interaction between various biological molecules, such as nucleic and amino acids, and ZnO nanoparticles, which have emerged as the material of choice for biomedical applications due to their biocompatibility and high excitonic band gap.^{1,2}

Among the 20 protein-forming amino acids, tryptophan (Trp) is of particular interest due to its role as an essential human diet amino acid and a protein fluorophore. Trp is known to form hydrogen bonds together with weak ionic bonds in a conjugated system. The fluorescence response of Trp is associated with the π – π^* transition of its indole functional group with the absorption and fluorescence wavelengths of about 280 and 348 nm, respectively.^{3,4} In a recent experimental study of interaction between ZnO and Trp,⁵ a static mode of fluorescence quenching was observed, indicating the formation of ground state complex, with the number of binding sites, n , being ~ 1 . Furthermore, this study also reported a decrease in the binding constant, K , with increase in temperature, suggesting the ZnO–Trp system to be a weakly bound complex, most probably formed by hydrogen bonding and van der Waals interactions. While the experimental observations provide important features of the ZnO–Trp interactions, they also raise some fundamental questions. For example, Trp molecule (Fig. 1) can offer at least three major sites for

interaction: (1) salt-bridge interaction involving the carboxylic group, $-\text{COO}^-$, (2) charge–solvent structure interaction, involving the indole ring, and (3) amine site interaction involving the $-\text{NH}_2$ group.⁶ Therefore, a natural question arises: what is the most stable binding site on Trp for a stable ZnO–Trp complex formation? It is well known that site selectivity and specificity play a critical role in the functionality of biomolecules. A related and equally important question that needs to be answered is, “what is the nature of interaction (binding) leading to the ground-state complex formation? In order to answer these questions and to also understand the possible mechanism of the observed fluorescence quenching, we have performed first-principles quantum mechanical study of Trp–ZnO nanoparticle complex. A cage-like cluster of ZnO, $(\text{ZnO})_{12}$, which has been shown to be a symmetric, highly stable, and potential candidate for cluster assembly of materials,^{7–9} is used to represent ZnO nanoparticles. Quantum mechanical calculations based on density functional theory (DFT) are performed to obtain stable geometries, site specific interaction energy, molecular orbitals, and charge density of Trp– $(\text{ZnO})_{12}$ complex.

2. Methodology: computational details

All calculations were performed within the framework of density functional theory (DFT) as implemented in SIESTA code.¹⁰ The exchange–correlation functional form proposed by Perdew, Burke and Ernzerhof (PBE) was used.¹¹ The norm-conserving pseudopotentials were constructed for each chemical species in Trp and ZnO nanocluster using the Troullier and Martins scheme.¹² The valence configurations of H ($1s^1$), C ($2s^2 2p^2$), N ($2s^2 2p^3$), O ($2s^2 2p^4$), and Zn ($3d^{10} 4s^2$) were represented by double-zeta basis sets with polarization functions (DZP).¹³ The numerical integrals were performed on a real space grid with an equivalent energy cutoff of 3400 eV. The calculations were considered to be converged when the force on each ion was less than $0.001 \text{ eV } \text{\AA}^{-1}$. Also, total energy convergence criterion of 10^{-5} eV was used.

^a Department of Physics, Michigan Technological University, Houghton, Michigan, 49931, USA. E-mail: pandey@mtu.edu

^b National Physical Laboratory (CSIR), Dr. K.S. Krishnan Marg, New Delhi-110012, India

^c US Air Force Research Laboratory/HEPB, Wright Patterson Air Force Base, Dayton, OH 45433-5707, USA

^d US Army Research Laboratory, Weapons and Materials Research Directorate, ATTN: RDRL-WM, Aberdeen Proving Ground, MD 21005-5069, USA. E-mail: shashi.karna@us.army.mil

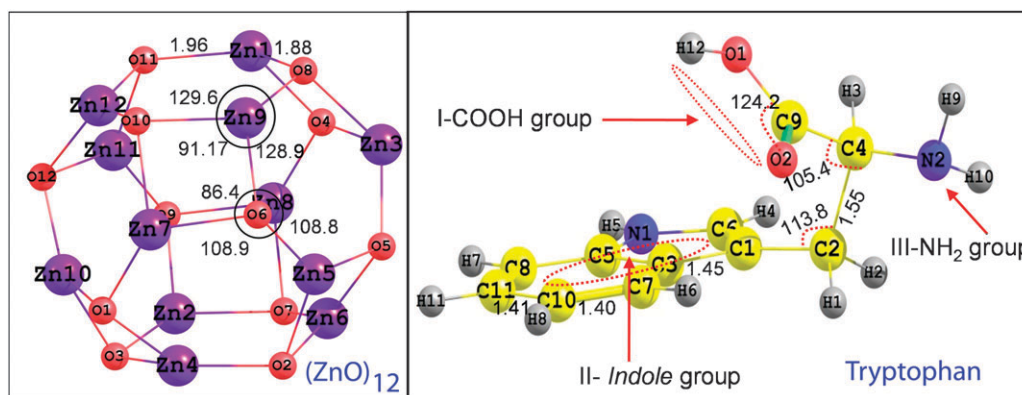


Fig. 1 The calculated ground state configurations of $(\text{ZnO})_{12}$ and tryptophan (Trp). The interaction sites of tryptophan with ZnO are also shown.

3. Results and discussion

3.1 $(\text{ZnO})_{12}$

The calculated ground state configuration of $(\text{ZnO})_{12}$ has a T_h symmetry with a cage-like structure as shown in Fig. 1. It is based on six $(\text{ZnO})_2$ and eight $(\text{ZnO})_3$ rings forming a truncated octahedron in which all Zn and O vertices remain equivalent. The calculated structural properties of $(\text{ZnO})_{12}$ are in good agreement with the previously reported values based on density functional theory.¹⁴ For example, the bond lengths, $R_{(\text{Zn}-\text{O})}$, of 1.89 and 1.97 Å compare well with the earlier reported values of 1.87 and 1.97 Å, respectively.¹⁴ The Zn–O bond is primarily ionic, with charge transfer from Zn to O atoms. The gap between the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO) is calculated to be 2.5 eV.

3.2 Tryptophan

The distinguishing structural characteristic of the Trp residue is the presence of an indole functional group with an amine. The carboxyl group is oriented perpendicular to the indole ring (Fig. 1). In the optimized configuration, the indole ring is 113.77° out-of-plane with respect to the plane containing –COOH and –NH₂ functional groups. The C–C and C=C bond lengths in the hexagonal ring of indole are 1.42 and 1.40 Å, respectively. The C–C and C=C bond lengths in the pentagonal ring of indole are 1.45 and 1.39 Å, respectively. The C–C bond distinguishing a plane of the indole ring and –COOH/–NH₂ functional groups is 1.56 Å making an angle of 105.45° with the –COOH group directed towards the indole ring. The O–C–O angle of the –COOH group is 123.23° . It appears that an oxygen atom of the –COOH group is also involved at the –NH₂ site and one of the H atoms of the NH₂ group forms a bond with the cluster O atom (Fig. 2). The calculated HOMO–LUMO gap and dipole moments are 3.66 eV and 4.01 debye (D), respectively. The calculated values of bond lengths and bond angles of Trp are in excellent agreement with the previously reported values also obtained using DFT calculations.¹⁵

3.3 Trp– $(\text{ZnO})_{12}$ bioconjugated complex

Fig. 2 shows the optimized isomeric configurations of the Trp– $(\text{ZnO})_{12}$ complex representing the interaction of the

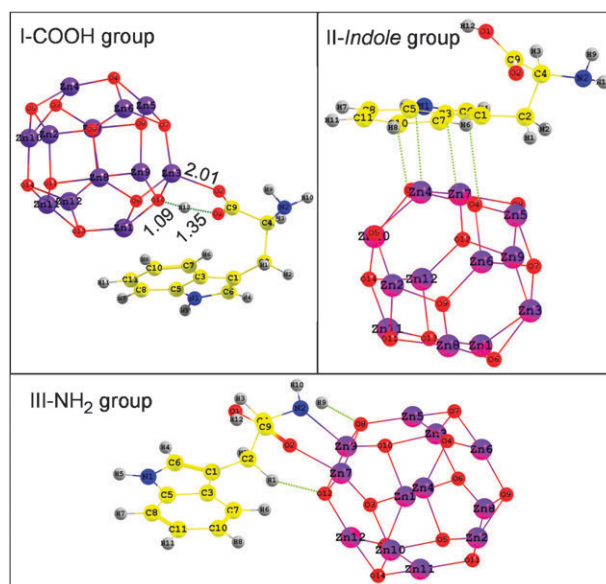


Fig. 2 The ground state configurations of Trp–ZnO complex showing the site specific interaction of Trp with ZnO.

cluster with Trp molecular segments containing (i) –COOH, (ii) indole, and (iii) amine functional groups. No geometrical constraints were imposed to obtain the optimal structures of these isomeric configurations of Trp– $(\text{ZnO})_{12}$. The bond-lengths, binding energy, dipole moment and HOMO–LUMO gap of the optimized isomeric configurations are listed in Table 1.

The binding energy of the Trp–cluster conjugate is calculated as the difference of the energy associated with the equilibrium configuration and the energies associated with the isolated cluster and Trp. As noted from Table 1, the salt bridge structure involving the –COOH group appears to be energetically more favorable than other interacting sites.

A larger binding energy of the COOH with ZnO appears to be the result of the formation of $\text{Zn}_{(\text{cluster})}-\text{O}_{(\text{Trp})}$ bond at the –COOH site. Fig. 2 also shows the formation of a stronger $\text{O}_{(\text{cluster})}-\text{H}_{\text{Trp}}$ bond, where H_{Trp} moves away from Trp to ZnO. Such bonds are not formed at the indole or –NH₂ group binding sites where relatively large cluster–Trp bond lengths are found (Table 1). It should be noted that the H-passivated

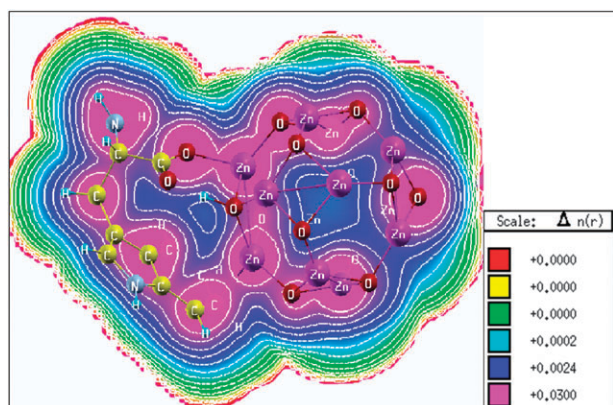
Table 1 The calculated structural and electronic properties of the isomeric configurations of the Trp-(ZnO)₁₂ complex

| Isomers | ZnO-COOH group | ZnO-indole group | ZnO-NH ₂ group |
|--|----------------|------------------|---------------------------|
| Binding energy/eV | 3.40 | 1.64 | 2.36 |
| $R_{(\text{cluster-Trp})}/\text{\AA}$ | — | — | — |
| $O_{\text{cluster}}-\text{H}_{\text{Trp}}$ | 1.09 | 2.60 | 1.66 |
| $Zn_{\text{cluster}}-O_{\text{Trp}}$ | 2.01 | — | 2.12 |
| $Zn_{\text{cluster}}-N_{\text{Trp}}$ | — | — | 2.14 |
| $Zn_{\text{cluster}}-C_{\text{Trp}}$ | 2.57 | 2.39 | — |
| $O_{\text{cluster}}-C_{\text{Trp}}$ | 2.78 | 2.64 | — |
| Dipole moment/D | 5.43 | 7.66 | 9.49 |
| HOMO-LUMO gap/eV | 2.38 | 2.43 | 2.60 |

(ZnO)₁₂H₂₄ cluster does not form a bound complex with Trp (not shown here). Due to H-passivation, the surface Zn and O atoms do not participate in further interactions with Trp.

In protein chemistry, salt bridge structures involve interactions with the -COO⁻ group of an amino acid whereas interactions involving the indole ring are represented in charge-solvent structures.⁶ Following this nomenclature, the isomeric configuration involving the -COOH group can be classified as a salt bridge structure. Likewise, the isomeric configuration involving the indole group can be considered as a charge solvation structure. Thus the calculated results clearly show a single-site ground state complex formation with a preference for the salt bridge type bonding over the charge solvation bonding, supporting the experimental observation.⁵

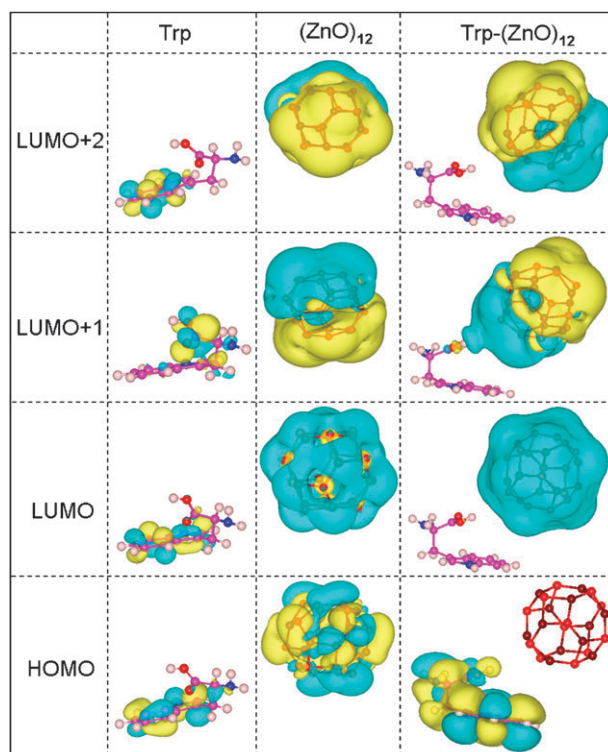
Fig. 3 shows the charge density plot projected along (001) plane of the salt bridge configuration where direct bonding of the -COOH group can clearly be seen with the cluster Zn and O atoms. A formation of both Zn_(cluster)-O_(Trp) ionic bond and O_(cluster)-H_(Trp)-O_(Trp) hydrogen bond is seen with the bond lengths of 2.01 and 1.09 Å, respectively. This is further confirmed by the Mulliken charge analysis of the complex which reveals a small charge transfer (~0.27e) from Zn_(cluster) to O_(Trp) and the charge redistribution in O_(cluster)-H_(Trp)-O_(Trp) bond. Interestingly, the calculated dipole moment of the complex is relatively larger than that of Trp suggesting the rearrangement of electron density upon formation of the complex. Furthermore, the calculated results find the charge transfer to be much smaller for both indole and -NH₂ binding sites. Thus, while our calculation does support a single site ($n = 1$)

**Fig. 3** Total charge density plot of the salt bridge configuration of Trp-(ZnO)₁₂ projected along (001) plane. A superimposed ball and stick model identifies the atoms in the complex.

complex formation, the nature of the bonding is predicted to be ionic and H-bonding, excluding van der Waals interactions, as suggested in ref. 5.

The experimental paper⁵ also reported that mixing of Trp with ZnO nanoparticles appeared to quench its fluorescence.⁵ No spectral shift in the absorption peak at 280 nm was observed with the increase in concentration of ZnO nanoparticles. Since the spectral signatures of Trp-ZnO complex are dominated by the $\pi-\pi^*$ transition of Trp's indole functional group, one could argue that the formation of the complex facilitates non-radiative energy transfer to states associated with ZnO, thus reducing the Trp fluorescence intensity. In order to examine this, we plot the HOMO and low-lying excited states of pristine Trp, (ZnO)₁₂ and Trp-(ZnO)₁₂ complex in Fig. 4.

As seen from the figure, the HOMO and LUMO in the case of pristine Trp are localized at the indole functional group. However, significant modification in the nature of molecular orbitals, and hence the charge distribution, happens upon

**Fig. 4** The contour plots of molecular orbitals of Trp, (ZnO)₁₂ and Trp-(ZnO)₁₂—salt bridge configuration.

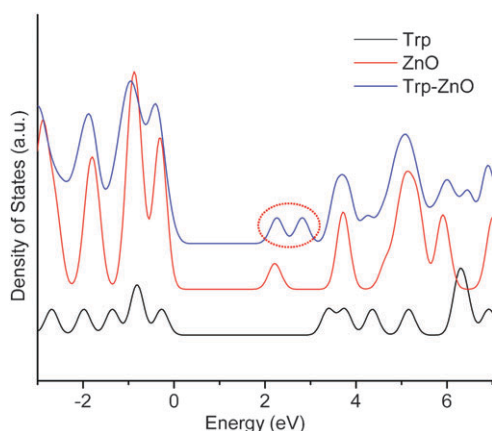


Fig. 5 Density of states of Trp–ZnO complex, Trp and ZnO. The zero of the energy is aligned to Fermi energy.

Trp–ZnO complex formation. The orbitals associated with Zn and O atoms appear as low-lying excited states in the energy spectrum. These states may potentially act as acceptor from the excited fluorescence state of Trp in the complex and, thus reducing the fluorescence intensity. We note that the excited state associated with Trp appears as LUMO + 5 state in the energy spectrum.

The appearance of states associated with ZnO in the Trp–ZnO complex is further confirmed by density of states (DOS) shown in Fig. 5. In the vicinity of Fermi energy, the peak in the Trp–ZnO complex is seen to be present in DOS of ZnO but not in DOS of Trp. The calculated results therefore suggest that ZnO nanoparticles with appropriate dimensions form site-specific, stable complex with Trp and introduce low-lying excited states which most likely cause fluorescence quenching as reported in experiments.⁵

4. Conclusion

Calculations based on density functional theory (DFT) are performed on Trp, (ZnO)₁₂ and Trp–(ZnO)₁₂ in order to study site specific interaction between tryptophan and ZnO. We find that preference of the interaction involving both ionic and hydrogen bonds yielding the salt bridge structure to be energetically more favorable than indole and amine interacting sites. The present study supports the experimental finding⁵ of a single-site ground-state Trp–ZnO complex formation.

However, the present study does not predict van der Waals interaction between Trp and ZnO nanoparticles. Formation of a stable Trp–ZnO complex is predicted to modify the nature of molecular orbitals by introducing states associated with ZnO as low lying excited states. The non-radiative energy transfer to these states is likely to cause the observed fluorescence quenching.⁵

Acknowledgements

We acknowledge the support from the Henry M. Jackson Foundation for the Advancement of Military Medicine, Inc. (611-243-2AFWP-00-104583). Helpful discussions with Saikat Mukhopadhyay and Prof. Mrinalini Deshpande are acknowledged. PJ and VS also acknowledge the support received during their stay at Michigan Technological University.

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