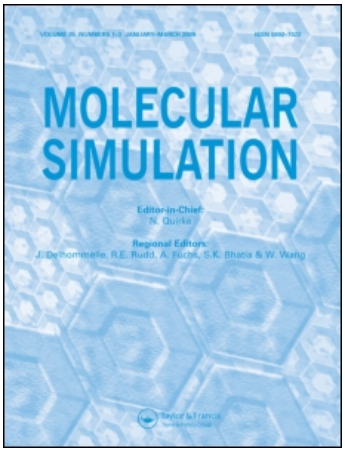

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Switching conformation of cyclo(histidyl-histidyl) dipeptide: dynamics and density functional theory study

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The backbone–side-chain interaction and switching propensity of cyclo(histidyl-histidyl) dipeptide from a folded–folded to unfolded–unfolded state and *vice versa* have been studied, combining molecular dynamics and density functional theory. Dynamic simulation for a time scale of 3 ns confirms the switching behaviour of the dipeptide, with a folded–folded structure having the maximum probability of occurrence. The geometry optimisation of the folded–folded (U-shaped) and unfolded–unfolded (linear-shaped) structure in the gas phase predicts the latter to be more stable. The ring-puckering study indicates a boat conformation for the six-membered diketopiperazine (DKP) ring. All the above studies are found to correlate well with the earlier experimental results. The interaction of the water molecules in the first solvation shell of cyclo(histidyl-histidyl) dipeptide lowers the energy barrier more for the folded–folded (U-shaped) structure than for its counterpart. Water molecules are found to act as a bridge between the side-chain imidazole and the DKP ring, thus deciding the conformation of the dipeptide. The structural propensities are found to be in good agreement with the obtained electronic effects. This study would be helpful in understanding the conformational preferences of cyclic dipeptides in the aqueous medium.

Keywords: cyclic dipeptide; histidine; molecular dynamics; conformations; density functional theory

1. Introduction

Diketopiperazines (DKP) or 3,6-disubstituted-2,5-piperazinediones are the simplest cyclic peptides of biological interest. Due to their simplicity and structural rigidity, they have been an attractive model system for characterising the interactions between two amide groups [1–4]. DKP have been used as intermediates in the asymmetric synthesis of amino acid derivatives and related natural products [5]. The acyclic dipeptide–DKP reaction plays a key role in the kinetics of the decomposition of proteins in fossils [6]. Although earlier studies [7–9] and reviews were concerned predominantly with the natural occurrence of DKPs [10–13], as well as their structure [14–16] and reactivity [17], recently, they have gained importance in drug discovery as inhibitors of various enzymes [18]. Cyclic dipeptides, referred to as DKP, are found to inhibit cancer cell growth and induce apoptosis in colon cancer cells [19], and have also been identified as involved in antifungal [20] and antibacterial activities [21]. Several experimental and theoretical studies have been performed to explore the conformational flexibility of the DKP ring [2,3,7,15,22–25].

Amino acids with aromatic side chains are relatively rare but sensitive to their immediate environment. They are hence excellent probes for protein structure and can provide site-specific information. The NMR, proton magnetic resonance and X-ray measurements [1,26] confirm that those cyclic dipeptides containing histidine, phenylalanine and tyrosine residues tend to have a folded conformation with the aromatic ring of the side chain facing the DKP ring. Kopple and Marr [27] have demonstrated that the preferred conformation about the α—β bond of the aromatic ring in cyclic dipeptides is the one bringing them into closest proximity to the DKP ring. Experimental studies on these kinds of cyclic dipeptides, in solution, have elucidated that the aromatic rings mostly prefer a folded conformation but not an unfolded one [1,26–29]. An interaction between these aromatic and DKP rings (probably a dipole-induced dipole one) appears to have a great influence on the overall conformation of cyclic dipeptides [29,30].

Kojima et al. [31] have determined the X-ray crystallographic structure of cyclo(histidyl-histidyl) dipeptide in solution and described three possible conformations, namely folded–folded (both imidazole rings facing the DKP ring), unfolded–unfolded (both imidazole rings kept away from the ring) and folded–unfolded conformations. The ^{1}H NMR measurement shows a more or less equivalent preferential ratio (49:51) for the folded and unfolded conformations. However, it is reported [32] that His-Tyr cyclic dipeptide in aqueous solutions, although favouring a folded conformation for the tyrosyl side chain, keeps the histidyl imidazole group away from the DKP ring (unfolded state). Cotrait et al. [32] have explained that the presence of a water bridge that links Thr and His via

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hydrogen bonds is the reason to keep the side chain of histidine away from the DKP ring. Moreover, preliminary molecular mechanics calculations [33] have shown that in an environment unrestricted by crystal forces, the preferred conformation of cyclo(histidyl-histidyl) dipeptide is actually those where the side chains are bent outwards. Exclusive interaction studies of this dipeptide and other histidine containing cyclic dipeptides with various metals have been carried out in solution. The interaction of Cu^{2+}[34] and Ag^{+}[31] ions with the cyclo(histidyl-histidyl) dipeptide causes the structure to take an unfolded–unfolded conformation. In the case of the Zn^{2+} ion complex [31], the dipeptide has both folded and unfolded the imidazole ring conformations. Therefore, it is very clear that cyclo(histidyl-histidyl) dipeptide basically exists in two different forms: folded–folded (U-shaped) and unfolded–unfolded (linear-shaped). Due to such conformational existence, the interacting nature of the cyclo(histidyl-histidyl) dipeptide with the surrounding environment may vary. It is thus significant to know which conformation of this dipeptide (linear- or U-shaped) has the maximum possibility of occurrence. However, the molecular dynamics simulation is found to be a well-known tool to identify the conformational states of various systems possessing switchable properties [35,36]. Previously [35,36], the picosecond conformational switching of (4-aminomethyl)phenylazobenzoic acid (AMPB) and thereby its influence on the conformational transition of the secondary structure have been studied.

Hence, we planned to apply the molecular dynamics method to study the conformational transition of cyclo(histidyl-histidyl) dipeptide in the water environment. To the best of our knowledge, no such attempt has been made to explore the switching behaviour of cyclo(histidyl-histidyl) dipeptide in the aqueous medium. This study would be of theoretical and practical importance to identify the time scale of structural switching (U- to linear-shaped and *vice versa*) of the dipeptide. The number of the water molecules in the first solvation shell of the dipeptide has been identified, and the dominance of the water bridge in deciding the position of the side-chain group has also been examined through the H-bond analysis. Following the dynamic simulations, quantum chemical calculations have also been carried out on the two forms of cyclo(histidyl-histidyl) dipeptide (linear- and U-shaped). The number of the water molecules in the first solvation shell obtained from molecular dynamics is made to interact with the U- and linear-shaped structures for quantum chemical studies. The structural properties of isolated and water complexes have been explored through hydrogen-bond distances and a non-bond torsional parameter (ζ). The electronic effects of the isolated and water complex structures have also been explored using Frontier molecular orbitals. This study as a whole would provide an insight into the behaviour of this dipeptide while involving in various biological processes.

2. Theoretical background

The constructed cyclo(histidyl-histidyl) dipeptide using Molden program was first simulated in the aqueous medium and then taken for the quantum chemical calculation.

2.1 Molecular dynamics simulations

The constructed structure is subject to an atomistic MD simulation for a time scale of 3 ns in explicit water with a MD time step of 2 fs. The system was examined at constant pressure with a periodic boundary condition, wherein the Langevin piston maintained the pressure of the cell at 1 atm. The information of the system prepared for the simulation is given in Table 1. The Langevin dynamics were used to control the temperature at 300 K, with the collision frequency of 1.5 ps^{-1}. Bonds involving hydrogen atoms were constrained to their equilibrium value by means of the SHAKE algorithm. The non-bonded cut-off distance of 10.0 Å is employed for the simulation. Hornak et al. [37] have compared the multiple Amber force fields for the protein backbone parameters and found that the ff03 force field fits better with the experimental data than other force fields. Hence, we have used the ff03 force field [38] to extract the significant non-bonded torsions describing the conformational transition of the cyclic dipeptide. As the TIP3P water model [39] is found to be well balanced [40] with the Amber force field, here, it is used for water modelling. All the simulations presented here were carried out using the program AMBER version 8.0 [41].

2.2 Quantum chemical calculation

The two forms of cyclo(histidyl-histidyl) dipeptide, namely U- and linear-shaped, were optimised using the density functional theory and *ab initio* methods. Becke's three-parameter non-local hybrid exchange potential (functional) with the non-local correlation functional of Lee, Yang and Parr (B3LYP) [42,43] in concert with the 6-311++G** basis set [44] have been used for the DFT study. For the *ab initio* optimisation, the Møller–Plesset theory (MP2) [45] with the 6-311++G** basis set has been employed. The harmonic vibrational frequency analysis suggested that all the optimised geometries belong to minima at the respective potential energy surfaces. The geometries of these structures in the presence of the water molecules were fully optimised using the density functional theory. The puckering of the six-membered DKP rings was analysed and compared. For the six-membered ring, the exact definition of the three puckering coordinates was reported by Cremer and Pople (CP) [46]. Because of the inconsistency in the CP formalism [47], an alternative conformational analysis of the six-membered rings was facilitated by the truncated

Table 1. Simulation set-up prepared to run molecular dynamics for U- and linear-shaped cyclo(histidyl-histidyl) dipeptides.

Solute cyclo(histidyl-histidyl) dipeptide	Force field	No. of water molecules	Volume of the simulation box (Å^3)	Solvent	Simulation length (ns)	Temperature (K)	Dimension of the PBC box (Å^3)
U-shaped	Leaprcff03	718	33884.8	H_2O	3	300	39.6 × 40.3 × 29.6
Linear-shaped		735	34649.4				39.6 × 37.8 × 31.6

Fourier (TF) formalism [48], which describes the interdependence of endocyclic torsions (ϕ_j, $j = 0, \ldots, 5$) in the six-membered ring, viz.

$$\phi_j = \Phi_2 \cos(P_2 + 4\pi j/6) + \Phi_3 \cos(\pi j), \tag{1}$$

where Φ_2, Φ_3 and P_2 are the puckering coordinates that were replaced by a spherical polar set (P_2,θ,Q) as

$$Q = \sqrt{\Phi_2^2 + \Phi_3^2}, \tag{2}$$

$$\theta = \arctan\left(\Phi_2/\Phi_3\right), \tag{3}$$

where Q is the puckering amplitude, with $0 \leq \theta \leq \pi$ [48].

All computations were performed using the Gaussian 03W program package [49], and all graphical representations were prepared with the aid of the Molden program [50].

3. Results and discussion

3.1 Molecular dynamics

3.1.1 Switching propensities of the structure

A complete conformational analysis of the cyclo(histidyl-histidyl) dipeptide in the presence of explicit water molecules was performed using the MD simulation for over a time scale of 3 ns. During the simulation, cyclo(histidyl-histidyl) dipeptide initially in the linear form switches to the U-shape with its imidazole rings facing the DKP ring within a time scale of around 500 ps. This structure is expected to be in this folded state for a longer time, as suggested earlier by Kopple and Marr [27] that these kinds of cyclic dipeptides prefer to have a folded conformation. Although it prevailed in the same state for a longer period, at around 1.5 ns, it again switched back to the linear form. To determine whether these conformational changes persist, the simulation was extended to another 1.5 ns, and it was observed that the structure fluctuates between the U and linear forms.

To obtain a clear picture of the switching propensity and to identify which structure has the maximum probability of occurrence, time evolution of the non-bonded torsion ζ ($\angle$C(5)–C(7)–C(10)–C(17)) between atoms that are chosen randomly from the imidazole and DKP rings is noted. The variation of this dihedral angle will clearly depict whether the imidazole ring faces the DKP or lies away. Figure 1 shows the behaviour of the ζ angle of the cyclic dipeptide throughout the simulation, which clearly substantiates that the switching of linear- to U-shaped and *vice versa* does take place as explained above. The distribution of the ζ angle throughout the run is shown by means of a graph (Figure 2), which illustrates that the maximum probability of occurrence is around -30° that corresponds to the U-shaped structure.

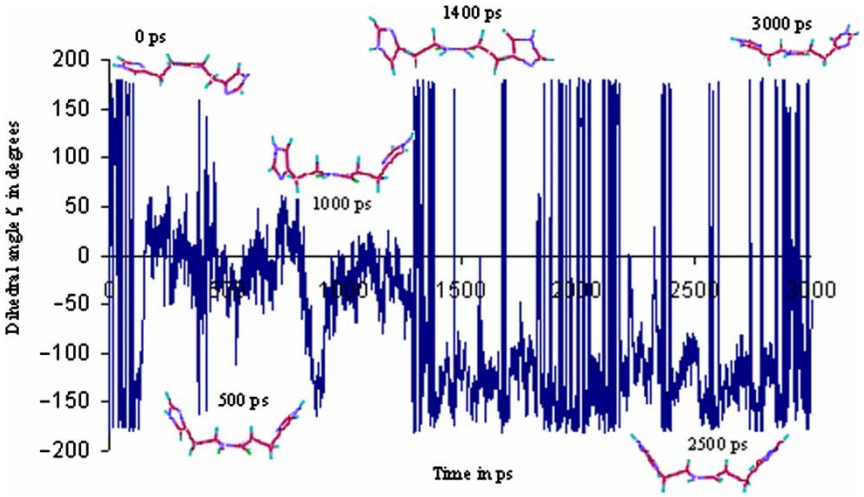

Figure 1. Time evolution of the dihedral angle ζ of cyclo(histidyl-histidyl) dipeptide. Switching behaviour of the dipeptide is represented by the corresponding structures and time of occurrence.

Thus, it could be concluded that the switching of the dipeptide from one form to another takes place with the maximum preference of existence for the U-shaped structure. This switching of the structure will certainly play a significant part in changing the conformation of its surrounding. The reason for the dipeptide to fluctuate between the two forms is due to the presence of the water molecules. During the simulation, the interaction of the water molecules with the side-chain group of the cyclic dipeptide might have kept the imidazole group away from the DKP ring. It is thus significant to analyse hydrogen-bond interactions characterising the solvated states of the cyclic dipeptide and to look into their distribution functions in order to identify the number of the water molecules that fall within the first solvation shell.

3.1.2 *Radial distribution functions*

The H-bond analysis reveals that oxygen (O), nitrogen (N) and carbon (C) atoms of the cyclic dipeptide actively interact with water molecules as H-bond donors. The radial distribution function (RDF; Figure 3) confirms that the coordination shell is characterised with a major peak at around 2.05 Å for O(peptide)···H(wat). Figure 4 represents the graph drawn between the duration of the simulation and the number of the water molecules that constitute the first coordination shell of the structure. An average of four water molecules is found to constitute the first coordination shell during the simulation. From Figure 4, it is clearly understood that water molecules never fail to interact with the cyclic peptide and thus actively taking part in solvation, which also supports that

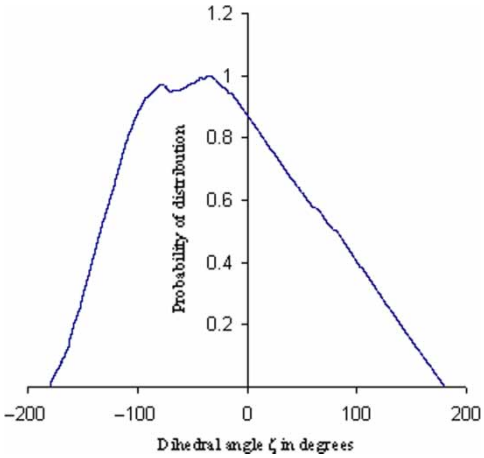

Figure 2. Probability distribution of the dihedral angle ζ of cyclo(histidyl-histidyl) dipeptide simulated for a time scale of 3 ns at 300 K.

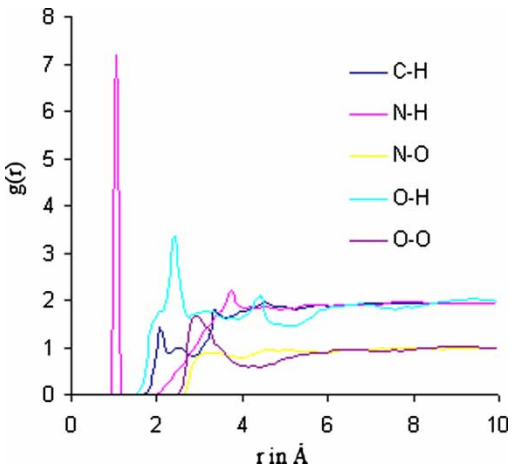

Figure 3. Radial distribution function of cyclo(histidyl-histidyl) dipeptide in water.

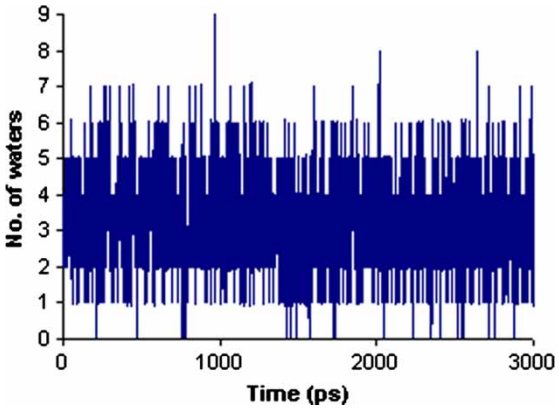

Figure 4. Time progression of the number of the water molecules in the 2.05 Å shell of cyclo(histidyl-histidyl) dipeptide.

the interaction of the water molecules keeps the side chains away from the DKP ring.

The overall dynamical analysis of the cyclic dipeptide confirms its switching propensity and reveals that the interaction of the water molecules with the imidazole group of the cyclic dipeptide is the cause for the fluctuation of side chains. To understand the atomistic behaviour of the two forms of the cyclo(histidyl-histidyl) dipeptide, namely U- and linear-shaped, and also to know their interacting nature with water molecules, quantum chemical calculations were performed.

3.2 Quantum chemical study

3.2.1 Energy

The U- and linear-shaped cyclo(histidyl-histidyl) dipeptides were fully optimised using the density functional theory at the B3LYP/6-311++G** level and are represented in Figure 5. Table 2 lists the relative energies of the two structures, where one can find the linear-shaped structure to be more stable than U-shape with an energy barrier of about 2.51 kcal/mol. On the other hand, optimisation at the MP2/6-311G** level of theory suggests the U-shaped structure to be 1.26 kcal/mol lower in energy than its counterpart. Due to these controversial results, energy calculations at HF/6-311G**//MP2/6-311G**, B3PW91/6-311++G**//B3LYP/6-311++G**, PBE1PBE/

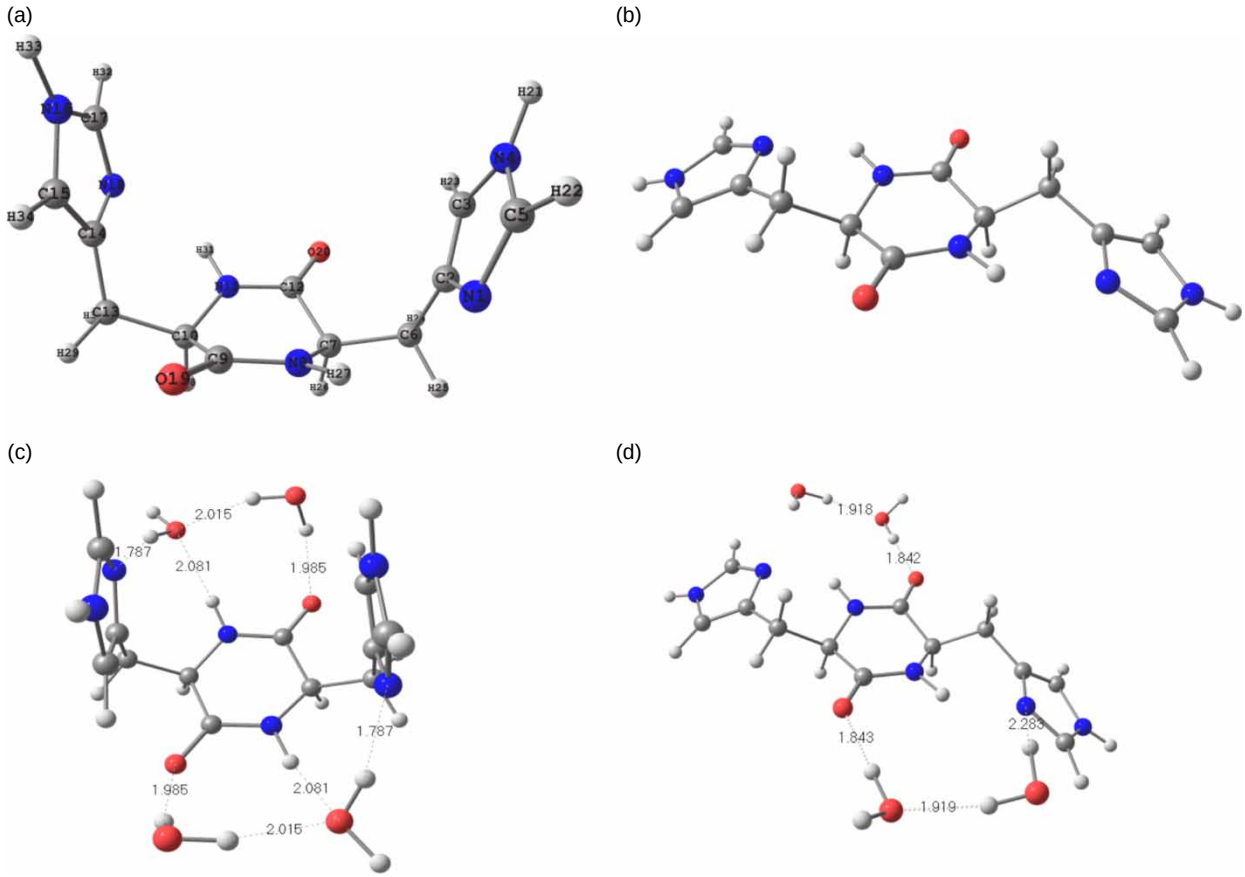

Figure 5. Three-dimensional structure of (a) U-shape, (b) linear, (c) U-shape + wat and (d) linear + wat cyclo(histidyl-histidyl) dipeptide optimised at the B3LYP/6-311++G** level of theory where the bond lengths are in Å units.

Table 2. Relative energies ΔE (in kcal/mol) of U- and linear-shaped cyclo(histidyl-histidyl) dipeptides.

	ΔE						
Structures	B3LYP/6-311++G**	MP2/6-311G**	HF//6-311G**	B3PW91//6-311++G**	PBE1PBE//6-311++G**	B3LYP//Aug-CC-PVTZ
U-shaped	2.51	0.0	0.0	3.14	2.51	3.77
Linear-shaped	0.0	1.26	0.63	0.0	0.0	0.0

6-311++G2mu**//B3LYP/6-311++G** and B3LYP/Aug-cc-PvTZ//B3LYP/6-311++G** levels of theory were carried out and their relative energies are shown in Table 2. Analogous to the MP2/6-311G** calculations, the HF/6-311G**//MP2/6-311G** level of theory also predicts the U-shaped structure to be more stable. However, all the density functional theory calculations suggest the linear-shaped cyclo(histidyl-histidyl) dipeptide to be the most stable one. Among these, the energy difference of 2.51 kcal/mol between the two structures calculated at the PBE1PBE/6-311++G**//B3LYP/6-311++G** level coincides well with our B3LYP/6-311++G** level calculation. Although the B3PW91/6-311++G**//B3LYP/6-311++G** and B3LYP/Aug-cc-PvTZ//B3LYP/6-311++G** levels of theory show a larger energy difference of 3.14 and 3.77 kcal/mol, respectively, they still substantiate to the prediction of the B3LYP/6-311++G** level of theory. Furthermore, the minimum energy conformers of the cyclo(histidyl-histidyl) dipeptide were also identified by the potential energy surface scan by varying θ_1 (C(7)–C(6)–C(2)–C(3)) and θ_2 (C(10)–C(13)–C(14)–C(15)) dihedral angles, connecting the side-chain imidazole group and DKP ring of the dipeptide, from 0° to 360°. The 3D plot shown in Figure 6 illustrates the minimum energy conformers with their respective energies. The lowest minimum energy conformer of the U-shaped structure has θ_1 as 240.0° and θ_2 as 240.0°, with an energy of −944.915 a.u. For the linear-shaped structure, the lowest energy conformer has θ_1 as 120.0° and θ_2 as 120.0°, with an energy of −944.967 a.u. Consequently, the linear-shaped structure is 32.63 kcal/mol lower in energy than the U-shaped structure, substantiating its stability in the gas phase.

Moreover, earlier studies [51,52] have accredited the reliability and accuracy of density functional theory methods for a wide range of biomolecules. Hence, according to the DFT optimisation in the gas phase, the linear form of the cyclo(histidyl-histidyl) dipeptide is found to be more stable. But as seen from dynamics, the presence of water interaction with the imidazole groups of the dipeptide may disturb the stability of the conformation. Therefore, finding the energetics of the conformations in the presence of the water molecules is also an important factor to be analysed. Based on the dynamical statistics, four water molecules were made to interact at the predominant positions of the linear- and U-shaped conformations. The water complexes thus formed are optimised at the B3LYP/6-311++G** level of theory and are shown in Figure 5(c),(d). The optimisation of the U- and linear-shaped water complexes suggests the former to be the most stable one, varying from the linear complex with an energy barrier of about 5.65 kcal/mol. It is clearly seen from Figure 5(c),(d) that water molecules interact with the imidazole group of the U-shaped conformation, whereas it is absent in the case of linear, thereby

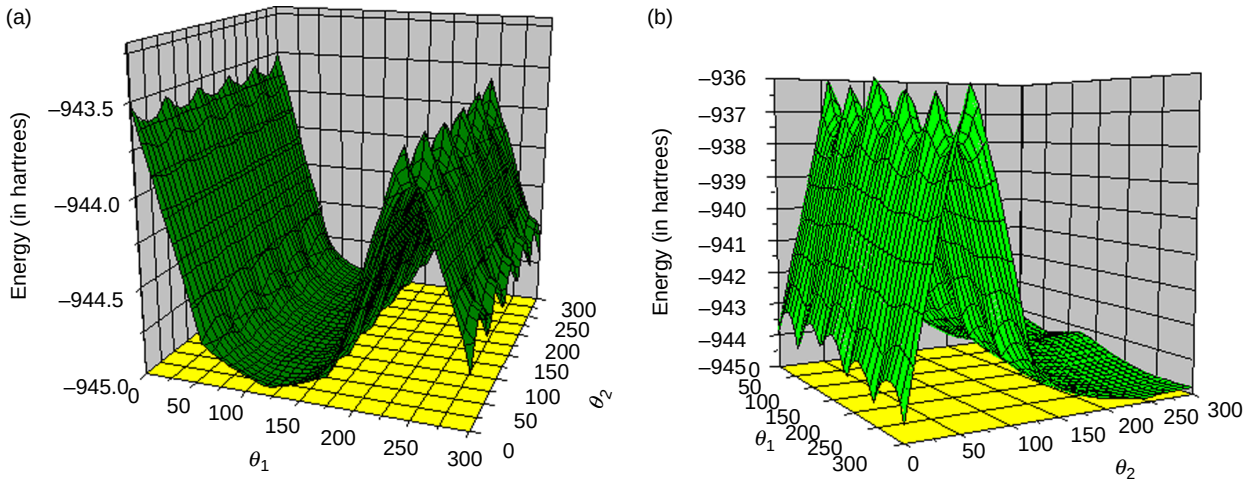

Figure 6. A 3D plot representing the minimum energy conformers due to the variation of θ_1 and θ_2 angles of (a) linear-shaped and (b) U-shaped cyclo(histidyl-histidyl) dipeptide at the B3LYP/6-311++G** level of theory.

systematically lowering the energy barrier for the former structure than its counterpart. This further validates the maximum probability of occurrence of the U-shaped structure during the simulation.

3.2.2 *Geometrical parameters and electronic effects*

The geometrical parameters of all the optimised isolated and water complexes of U- and linear-shaped are listed in Table 3. Similar to the radial distribution function

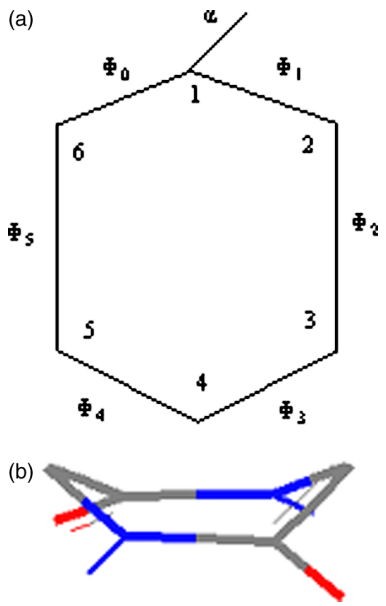

Figure 7. (a) Torsion angle numbering in the six-membered rings. (b) A schematic of the boat-shaped conformation of the six-membered ring of U-shaped cyclo(histidyl-histidyl) dipeptide. The six-membered ring of all other structures shows an analogy to the U-shaped structure in their ring conformation.

of molecular dynamics, the oxygen atom of the DKP ring forms hydrogen bonding with water molecule in the U-shaped and linear forms, at a bond distance of 1.98 and 1.84 Å, respectively. Water molecules in the U-shaped structure interact with the nitrogen atom of the imidazole group at a distance of 1.79 Å. Although hydrogen bonding between the imidazole nitrogen and water molecule is absent in the linear-shaped structure, a significant change in the bond distance of N(8)–H(27), N(8)–C(9) and N(8)–C(7) from the isolated structure is observed. The non-bonded torsional angle ζ considered in the dynamic study depicting the switching propensity of the side-chain group is also calculated quantum mechanically for the U- and linear-shaped structures and their values are found to be $-49.70°$ and $-178.59°$, respectively. The interaction of the water molecules does cause a significant variation in the ζ angle of both the structures altering their values to 17.74° and 178.37°, respectively. This further elucidates that the presence of the water molecules distorts the conformation of the cyclic dipeptides.

However, the cause for the interaction of the water molecules with the imidazole group of the U-shaped structure and not in the linear could also be clearly understood through the electronic effects prevailing in the conformations. Figure 8 shows the 3D representation of the molecular orbitals of the U- and linear-shaped conformations of the cyclo(histidyl-histidyl) dipeptides. It is exciting to see that in addition to the DKP ring, the HOMOs of the U-shaped structure (Figure 8(a)) are also accumulated on the imidazole rings, whereas it is completely absent in the linear-shaped conformation. The presence of such HOMOs evidently confirms its interaction with the DKP ring. In fact, such additional HOMOs on the imidazole ring substantiate the profound interaction of the water molecules with the U-shape, thus

Table 3. Geometrical parameters (the asterisk denotes non-bonded) of all structures optimised at the B3LYP/6-311++G** level of theory.

Parameters	U-shaped	U-shaped+water	Linear-shaped	Linear-shaped + water
Bond length (Å)				
C(12)–N(11)	1.355	1.347	1.353	1.344
C(12)–O(20)	1.222	1.229	1.223	1.231
N(11)–C(10)	1.465	1.461	1.462	1.464
C(9)–C(10)	1.541	1.531	1.535	1.530
C(9)–N(8)	1.355	1.347	1.353	1.344
N(8)–H(27)	1.015	1.021	1.012	1.021
N(8)–C(7)	1.464	1.461	1.462	1.464
C(7)–C(12)	1.541	1.531	1.535	1.530
C(7)–C(6)	1.538	1.546	1.539	1.541
C(6)–C(2)	1.497	1.501	1.498	1.498
C(2)–C(3)	1.371	1.373	1.372	1.371
C(3)–N(4)	1.380	1.378	1.381	1.382
N(4)–C(5)	1.364	1.358	1.362	1.359
C(5)–N(1)	1.313	1.317	1.313	1.316
N(1)–C(2)	1.384	1.386	1.384	1.385
Bond angle (°)				
∠C(7)–C(12)–N(11)	112.90	117.21	115.74	116.03
∠C(7)–C(6)–C(2)	114.47	115.54	113.50	112.86
∠C(6)–C(7)–C(12)	112.43	110.24	110.44	111.24
∠C(6)–C(7)–N(8)	111.35	111.57	110.47	110.34
∠C(6)–C(2)–C(3)	129.50	129.01	128.71	129.26
∠C(6)–C(2)–N(1)	120.65	121.65	121.78	121.31
∠C(5)–C(10)–C(17)*	87.50	56.24	161.17	160.18
Dihedral angle (°)				
∠C(7)–C(6)–C(2)–C(3)	−99.57	−80.56	124.16	117.72
∠C(7)–C(6)–C(2)–N(1)	79.50	100.09	−56.25	−61.19
∠C(12)–C(7)–C(6)–C(2)	75.27	64.44	−168.84	−171.41
∠N(8)–C(7)–C(6)–C(2)	−49.03	−61.91	66.25	62.81
∠C(5)–C(7)–C(10)–C(17)*	49.70	17.74	−178.59	178.37

lowering its energy than the linear-shaped conformation. This corroborates to the results of molecular dynamics, suggesting the U-shaped conformation to have a maximum probability of existence in water than its linear counterpart. The overall analysis suggests the dominance of the water molecules in deciding the position of the imidazole either away or close to the DKP group and further the stability of the conformations.

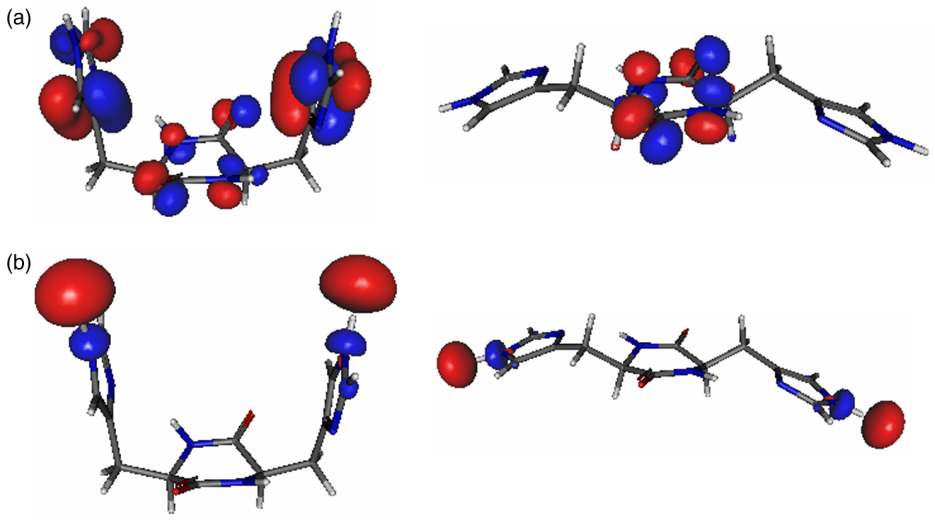

Figure 8. Graphical representation of the molecular orbitals (a) HOMO and (b) LUMO of U- and linear-shaped cyclo(histidyl-histidyl) dipeptide calculated at the B3LYP/6-311++G** level of theory.

Table 4. Endocyclic torsion angles and pseudorotation parameters (P, Q and θ, in units of degrees) of all the structures optimised at the B3LYP/6-311++G** level of theory.

	Pseudorotational parameters								
Structures	Φ_0	Φ_1	Φ_2	Φ_3	Φ_4	Φ_5	P	Q	θ
U-shaped	0.40	−39.23	39.84	0.45	−39.28	39.89	89.83	26.38	90.05
Linear-shaped	−8.79	−21.20	30.96	−8.76	−21.23	30.98	120.05	18.17	90.04
U-shaped + water	−10.55	−14.16	25.32	−10.54	−14.17	25.33	137.63	14.55	90.02
Linear-shaped + water	−6.94	−23.06	30.96	−6.92	−23.07	30.97	113.28	18.35	90.02

3.2.3 DKP ring conformation

The conformational accessibility of the six-membered DKP ring is analysed using the puckering coordinates P_2, θ and Q [48]. Every six-membered ring conformation may be viewed in terms of boat (B), twist-boat (T), chair (C), half-chair (H) and envelope (E) [48]. If for a given system all the six endocyclic angles shown in Figure 7 are known, then the puckering coordinates can be calculated using Equations (1)–(3). The calculated values of the puckering coordinates of all the structures along with their respective endocyclic torsional angles are listed in Table 4. The DKP ring of the linear-shaped structure is found to be characterised by $P_2 = 120.05°$, $\theta = 90.04°$ and $Q = 18.17°$, attributing them to the twist-boat (T) conformation (Figure 7) [48]. In the case of the U-shaped structure, the puckering amplitude increases by 8.24 due to the folded imidazole groups. The interaction of the imidazole side chain with the DKP ring has led to an increase in the distortion of the six-membered ring and thus increasing the amplitude. In both the structures, the DKP ring attributes to a boat conformation that is identical to that of the earlier experimental and theoretical studies [7,53]. The presence of the water molecules in the U-shaped structure, however, reduces the amplitude of puckering to 14.55°, which, in turn, varies the phase angle.

4. Conclusion

The structural behaviour of the cyclo(histidyl-histidyl) dipeptide has been studied by applying both the molecular dynamics simulation and the quantum chemical calculation. The simulation of the cyclo(histidyl-histidyl) dipeptide in the aqueous solution for a time scale of 3 ns confirms the switching propensity of the structure from the U-shaped (folded–folded) to the linear-shaped (unfolded–unfolded) conformation and *vice versa*. Although earlier study suggests equal preferential existence for both the folded–folded and unfolded–unfolded conformations of the cyclo(histidyl-histidyl) dipeptide, the dynamic simulation for a time scale of 3 ns predicts the folded–folded shape to have the maximum probability of existence. However, the geometry optimisation of the two conformations in the gas phase suggests the unfolded–unfolded cyclo(histidyl-histidyl) dipeptide (linear-shaped) to be more stable. The interaction of the water molecules in the gas phase lowers the energy barrier more for the folded–folded cyclo(histidyl-histidyl) dipeptide (U-shaped) structure than its counterpart. The structural propensities are found to be in good agreement with the obtained electronic effects. The profound interaction of the water molecules with the U-shaped conformation was substantiated through frontier orbitals and supports the maximum probability of occurrence confirmed by the dynamic simulation. In general, it is noted that the folded–folded conformations of cyclo(histidyl-histidyl) dipeptides would have a more pronounced contribution to its surrounding environment than the unfolded–unfolded one. This study as a whole emphasises that due to the conformational switching property, cyclo(histidyl-histidyl) dipeptides would have considerable effects on the surrounding environment, thereby being suitable for various interesting physiological and pharmacological processes.

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