J Mol Model (2010) 16:193–202
DOI 10.1007/s00894-009-0531-0

ORIGINAL PAPER

Structural analysis and the effect of cyclo(His–Pro) dipeptide on neurotoxins—a dynamics and density functional theory study

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Received: 1 February 2009 / Accepted: 21 April 2009 / Published online: 25 June 2009

Abstract The switching propensity and maximum probability of occurrence of the side chain imidazole group in the dipeptide cyclo(His–Pro) (CHP) were studied by applying molecular dynamics simulations and density functional theory. The atomistic behaviour of CHP with the neurotoxins glutamate (E) and paraquat (Pq) were also explored; E and Pq engage in hydrogen bond formation with the diketopiperazine (DKP) ring of the dipeptide, with which E shows a profound interaction, as confirmed further by NH and CO stretching vibrational frequencies. The effect of CHP was found to be greater on E than on Pq neurotoxin. A ring puckering study indicated a twist boat conformation for the six-membered DKP ring. Molecular electrostatic potential (MESP) mapping was also used to explore the hydrogen bond interactions prevailing between the neurotoxins and the DKP ring. The results of this study reveal that the DKP ring of the dipeptide CHP can be expected to play a significant role in reducing effects such as oxidative stress and cell death caused by neurotoxins.

Keywords Cyclic dipeptides · Glutamate · Paraquat · Molecular dynamics · Density functional theory · Molecular electrostatic potential

Introduction

Cyclic dipeptides [also known as 2,5dioxopiperazines; 2,5-diketopiperazines(DKP); cyclo(dipeptides); or dipeptide anhydrides] are relatively simple compounds and, therefore, are among the most common peptide derivatives found in nature. Though a variety of simple dipeptides have been synthesised for the sole purpose of examining their interesting physicochemical properties [1, 2], many of them were later found to exist in protein and polypeptide hydrolysates as well as in fermentation broths and cultures of yeast, lichens, and fungi [3–7]. Some of these DKPs were found to result from the nonenzymatic cyclisation of dipeptides and their amides, in as much as they are often formed during chemical and thermal manipulations as well as during storage of peptides and proteins [4–7].

Among such dipeptides, histidyl–proline [cyclo(His–Pro), hereafter CHP] is an endogenous cyclic dipeptide produced by primary cleavage of the pyroglutamyl residue of thyrotropin-releasing hormone (TRH) by pyroglutamyl aminopeptidase [8], both in blood and in the central nervous system. Thus, the dipeptide CHP, although present predominantly in mammalian gut and brain (including the hypothalamus [9]), is found ubiquitously. Among several cyclic dipeptides possessing enzyme inhibitory properties, CHP has been reported [10] to be the most potent, e.g. affecting cell separation in *Saccharomyces cerevisiae*, and preventing *Candida albicans* from entering its infectious filamentous form. The ordered histidine side chain of this dipeptide is observed to make several contacts with active site residues, which could explain its profound effects. In addition to facilitating central dopaminergic tone and suppressing food intake [11, 12], this potent dipeptide and some of its derivatives can prevent the neuronal death induced by free radicals, calcium mobilisation, and traumatic injury, thus suggesting their possible use in the treatment of in vivo neuronal degeneration [13].

Underlining its varied significance, the CHP dipeptide has been found to be an endogenous antioxidant abolishing apoptotic cell death [14]. Glutamate and paraquat are

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neurotoxic compounds that trigger cell death through several mechanisms that involve oxidative stress [15, 16]. The interaction of CHP with these neurotoxic compounds reduces oxidation stress thereby retarding cell death. Although the effects of CHP, especially in abolishing cell death, are renowned, the molecular mechanisms underlying the protective actions of CHP remain unknown. The atomic behaviour of this dipeptide with neurotoxic compounds is also yet to be unveiled.

On the other hand, amino acids that contain aromatic side chains are found to be highly receptive to their surrounding environment, and can provide excellent site-specific information in proteins. Earlier experimental studies [17] have confirmed that 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. Moreover, a recent molecular dynamic (MD) study [18] has revealed the existence of an unfolded confirmation of cyclic dipeptides, with the aromatic ring of the side chain facing away from the DKP ring. Such conformational switching of the side chain groups of these dipeptides may influence the conformational transition of secondary structures, and may even play a significant role in changing the conformation of proteins. Hence, the present study investigates the dynamic behaviour of CHP in aqueous medium. The conformational behaviour of the side chain groups and the DKP ring are explored using MD methods. Quantum chemical calculations were carried out in order to acquire a deeper insight into the interaction of CHP with neurotoxic compounds. This study has theoretical and practical importance in exploring the behaviour of CHP with regard to its application in biological\pharmacological processes.

Theoretical background

The initial structure of the CHP dipeptide with the imidazole group facing the DKP ring was constructed using the Chemcraft program package [19]. The duly constructed structure was simulated in aqueous medium and then used for quantum chemical calculations.

Molecular dynamics simulations

The constructed structure was subjected to atomistic MD simulation for a time scale of 3 ns in explicit water with an MD time step of 2 fs. The system was examined at constant pressure with periodic boundary conditions wherein the Langevin piston maintained the pressure of the cell at 1atm. Langevin dynamics were used to control the temperature at 300 K, with a collision frequency of 1.5 ps^{-1}. Bonds involving hydrogen atoms were constrained to their equilibrium value by means of the SHAKE algorithm. A non-bonded cut off distance of 10.0Å was employed for simulation. Hornak et al. [20] compared multiple Amber force fields for protein backbone parameters and found that the ff03 force field fits better with experimental data than other force fields. Hence we used the ff03 force field [21] to extract the significant non-bonded torsions describing the conformational transition of the cyclic dipeptide. As TIP3P water model [22] is found to be well balanced [23] with the Amber force field, it was used here for water modelling. All the simulations presented here were carried out using the program AMBER, version 8.0 [24].

Quantum chemical calculations

The constructed CHP dipeptide was optimised by applying density functional theory (DFT). Becke's three-parameter non-local hybrid exchange potential (functional) with the non-local correlation functional of Lee, Yang, Parr (B3LYP) [25, 26] in concert with 6-311G** basis set [27] was used in this study. Harmonic vibrational frequency analysis suggested that all the optimised geometries belong to minima at their respective potential energy surfaces. Puckering of the six-membered DKP ring due to the interaction of neurotoxins was analysed and compared. For the six-membered ring, the exact definition of three puckering coordinates was reported by Cremer and Pople (CP) [28]. Because of the inconsistency in CP formalism, an alternative conformational analysis of six-membered rings was facilitated by the truncated Fourier (TF) formalism [29], which describes the interdependence of endocyclic torsions (ϕ_j, j=0,…5) in the six-membered ring viz.,

$$\phi_j = \Phi_2 \cos(P_2 + 4\pi j/6) + \Phi_3 \cos(\pi j) \quad (1)$$

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

$$Q = \sqrt{\Phi_2^2 + \Phi_3^2} \quad (2)$$

$$\theta = \arctan(\Phi_2/\Phi_3) \quad (3)$$

where Q is the puckering amplitude with $0 \leq \theta \leq \pi$ [29].

To obtain insight into the binding affinity of neurotoxins with the dipeptide, their interaction energies were calculated using Eq. 4, where these energies are corrected for basis set superposition error (BSSE) applying the counter-poise procedure (CPP) [30, 31] given by Eq. 5.

$$\Delta E_{\text{int}} = (E_{AB} - (E_A + E_B)) + CP \quad (4)$$

$$CPP = \left(E_A(AB) - E_A^{**}(AB)\right) + \left(E_B(AB) - E_B^{**}(AB)\right) \quad (5)$$

In Eq. 5, $E_A(AB)$ and $E_B(AB)$ represent the energy of monomers A and B frozen in the complex geometries, and the terms $E_A^{**}(AB)$ and $E_B^{**}(AB)$ are the energies of A and B in the presence of their respective ghost orbitals. The interaction between dipeptide and the neurotoxins was further explored through molecular electrostatic potential (MESP), which is a tool that explains the approach of a chemical species near a molecule [32]. The electrostatic interaction between a molecule and a test charge of magnitude e (i.e. a proton) placed at a point r, is well represented by the molecular electrostatic potential [V(r)] using Eq. 6,

$$V(r) = \sum_A \frac{Z_A}{|R_A - r|} - \int \frac{\rho\left(r'\right)}{\left|r - r'\right|} dr' \tag{6}$$

where Z_A is the charge on nucleus A, located at R_A, that is considered to be a point charge, and the second term arises from the electron density, $\rho(r')$, of the molecule. The MESP at each atom in the complex was obtained as a standard output from the Gaussian 03 program package [33] at B3LYP/6-311G** level of theory. The graphical program gOpenMol [34] was used to plot MESP data.

Results and discussion

Molecular dynamics

Switching propensity of CHP

A complete conformational analysis of the CHP dipeptide in the presence of explicit water molecules was performed using MD simulation over a 3-ns time scale. Owing to the rigid nature [35, 36] of the side chain pyrrolidine ring, not much dynamicity due to the proline residue is expected in the dipeptide. However, a recent dynamics study [18] on the cyclo (His–His) dipeptide has confirmed the switching behaviour of the imidazole ring, moving towards and away from the DKP. Thus, due to the presence of the histidine residue in CHP, possible conformational changes are expected in aqueous medium. Since earlier studies [18, 37] have confirmed the existence of a folded conformation of histidine-containing cyclic dipeptides, the CHP dipeptide with its folded conformation was considered as the initial structure for MD simulation. During simulation, within a time scale of around 100 ps, the dipeptide picks up an unfolded (away from DKP) conformation. The dipeptide with this conformation continues to be in the same state for a longer time. However, at around 2 ns the imidazole ring tends to move towards the DKP ring but does not lie in the same position for a longer period. Within 100 ps, it again takes up an unfolded conformation and persists in the same position. In general, it was observed that, although the CHP dipeptide changes its state from folded to unfolded and vice versa, the most probable state of its occurrence is that with the imidazole group facing away from the DKP ring. This is, however, at odds with an earlier study [37], which confirmed that the propensity of the imidazole group is to face the DKP ring.

To confirm whether the dipeptide with unfolded conformation has the maximum probability of occurrence, the time evolution of the non bonded angle $\zeta(\angle C(4) - C(6) - C(10))$ between atoms chosen randomly was considered. Variation in this angle will clearly depict whether the imidazole ring faces the DKP or lies away. Figure 1 shows the behaviour of ζ angle of the cyclic dipeptide throughout the simulation, which clearly substantiates that switching from unfolded to

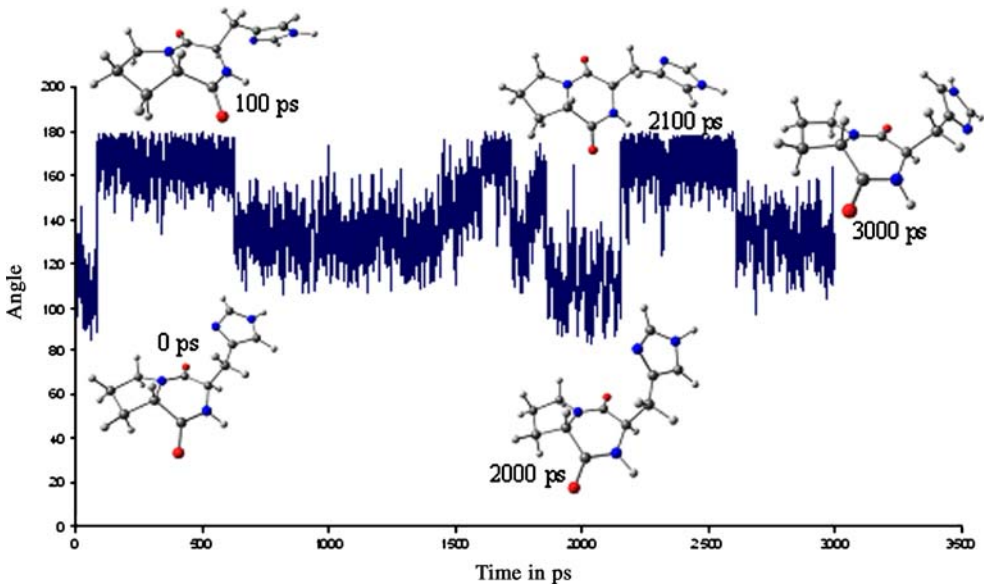

Fig. 1 Time evolution of the angle ζ of the cyclo(His–Pro) (CHP) dipeptide. Switching behaviour of the CHP dipeptide is represented, with the corresponding structures and time of occurrence

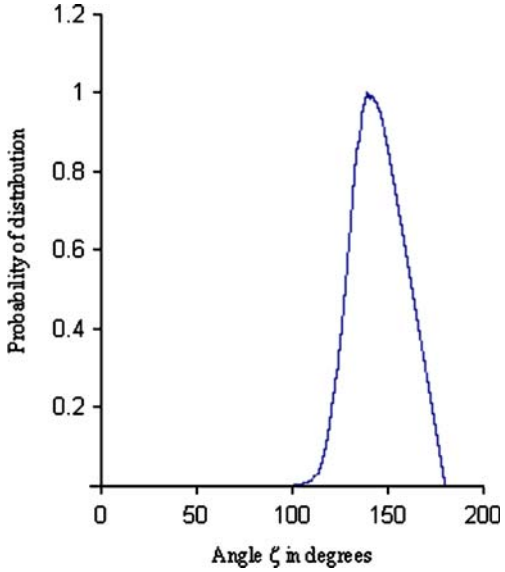

Fig. 2 Probability distribution of the angle ζ of CHP simulated on a 3-ns time scale at 300 K

folded and vice versa as described above does take place. The distribution of ζ angle throughout the run is shown in the graph in Fig. 2. This graph confirms that the maximum probability of occurrence of the angle is around 140°, which clearly corresponds to an unfolded state of the dipeptide. However, in the case of cyclo(His–His) dipeptide, the maximum probability of occurrence is for the folded conformation [18]. One reason may be that there is an interaction between the two imidazole groups of histidine, which is absent in the case of CHP. The foremost reason for the histidine side chain to prevail in the unfolded state may be due to the presence of water molecules. The cyclic dipeptide may have a larger affinity toward water molecules, which in turn might have kept the imidazole group away from the DKP ring for a longer time period. Thus, it is now pertinent to observe the interaction of the dipeptide with water molecules.

Radial distribution function

Hydrogen bond analysis of the structure reveals that the oxygen (O), nitrogen (N) and carbon (C) atoms of the cyclic dipeptide actively interact with water molecules as H-bond donors and thus are the active sites of the CHP dipeptide. The radial distribution function (RDF) shown in Fig. 3 confirms that the coordination shell is characterised by a major peak at around 1.85 Å for O(Peptide)···H(Wat). Following O(Peptide)···H(Wat), the immediate coordination shell is identified as N(Peptide)···H(Wat), featuring a peak at a distance of 1.95 Å. During simulation, although an average of four water molecules constitute the first coordination shell of the structure, it was found that up to seven water molecules actively take part in solvation, which supports the fact that interaction of water molecules keeps the side chains away from the DKP ring. This affinity for water molecules pulled our attention towards looking at the time period of hydrogen bond interaction existing between water molecules and the dipeptide.

Hydrogen bond life time

The origin of the functional preferences of many proteins is due mainly to the interaction of water molecules, i.e. to the formation and breaking of hydrogen bonds. A hydrogen bond is defined as the attractive force (dipole–dipole) between the hydrogen atom bonded to an electronegative atom of one molecule and the electronegative atom of another. In cyclic dipeptides the folding/unfolding of sides chains is due mainly to their interaction with water molecules involved in hydrogen bonds [38]. Hence, exploring the nature of these hydrogen bonds is a significant, and is done usually on the basis of their geometry [39, 40] or energy [41] criteria. Here, we report the structural relaxation of hydrogen bonds of the CHP active site (O, N and C)···H–O(Wat) assemblies using a time correlation function,

$$C(t) = \frac{\langle h(t+\tau).h(\tau)\rangle}{\langle h\rangle} \tag{7}$$

where the hydrogen bond variable $h(t)$ is unity when the active site···H–O(Wat) pair is bonded at time 't' according to the definition used, or zero otherwise. The angular bracket denotes averaging over time, and the correlation function $C(t)$ describes the probability of particular active site···H-O(Wat) hydrogen bond at time t.

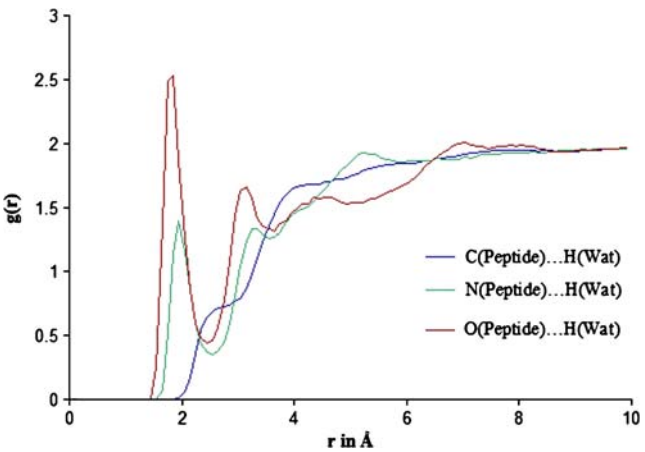

Fig. 3 O(Peptide)···H(Wat), N(Peptide)···H(Wat) and C(Peptide)···H (Wat) radial distribution functions (RDFs) of the CHP dipeptide in water

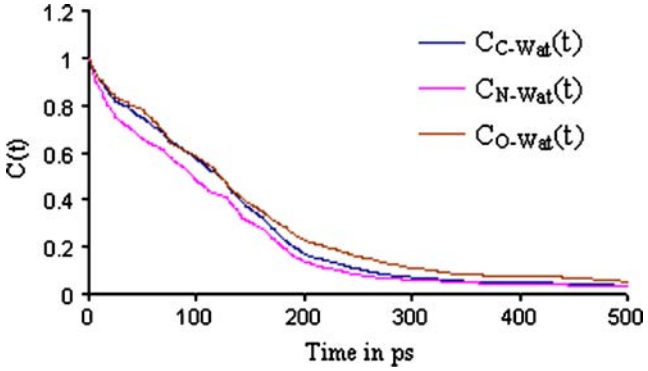

Fig. 4 Time correlation function of the active site···water assemblies

Figure 4 shows the variation of $C(t)$ against time for active site···H–O(Wat) assemblies. It is clearly evident that the structural relaxation of O(Peptide)···H-O(Wat), N (Peptide)···H–O(Wat) and C(Peptide)···H–O(Wat) H-bond interactions in the cyclic dipeptide are much slower. The constant parts of the decay curves stretch to about 500 ps. We used multi exponential fit to obtain the relaxation time (τ) for O, N and C···H–O(Wat) hydrogen bond interactions [42–44] from such slow long time decay curves. Here, we utilised the sum of three exponentials to fit the correlation functions, $C_{O–Wat}(t)$, $C_{N–Wat}(t)$ and $C_{C–Wat}(t)$ of O(Peptide)···H–O(Wat), N(Peptide)···H–O(Wat) and C (Peptide)···H–O(Wat) interactions. The calculated relaxation times for hydrogen bond interactions are listed in Table 1. Note that the relaxation time of hydrogen bonds involving oxygen is much larger than that of carbon and nitrogen atoms. This may be due to the slower mobility of water molecules near the polar/charged peptide active sites [45, 46]. This further validates the finding that the oxygen of the DKP ring has a profound affinity for water molecules as already seen from the RDF. Therefore, the DKP ring is expected to play a key role in regulating the function and properties of the dipeptide. Further, the relaxation time of the N(Peptide)···H–O(Wat) interaction was found to be less than that of the O(Peptide)···H–O(Wat) and C (Peptide)···H–O(Wat) interactions. However, a previous study [47] on all-trans and all-cis cyclo[(1R,3S)-γ-Acc-Gly]$_3$ peptides reported a shorter relaxation time for C (Peptide)···H–O(Wat) interactions, which may be because of the fact that C is less electronegative than O and N atoms. Furthermore, all three O(Peptide)···H–O(Wat), N (Peptide)···H–O(Wat) and C(Peptide)···H–O(Wat) interactions have quite long relaxation times, which is due to fact that water molecules may be bound to the peptide surface for longer times.

Table 1 Active site···water hydrogen bond relaxation time (τ) of the cyclo(His–Pro) (CHP) dipeptide

Active site···water	Relaxation time (τ) of CHP dipeptide (ps)
O(Peptide)···H-O(Wat)	388
C(Peptide)···H-O(Wat)	338
N(Peptide)···H-O(Wat)	276

Quantum chemical study

The most probable state of occurrence of CHP, as confirmed by MD simulation, is that with the imidazole group facing away from the DKP ring. Hence this structure was used in quantum chemical studies, where it was fully optimised using DFT at B3LYP/6-311G** level of theory. In order to check whether the folded structure is more stable than the unfolded structure, the CHP dipeptide with the imidazole facing the DKP ring was also fully optimised at the same level of theory. As expected, the CHP dipeptide with the unfolded conformation was found to be more stable, with an energy barrier of 0.628 kcal mol^{-1} over the folded structure. Hence, in the present case, to study the effect of the cyclic dipeptide on neurotoxic compounds, namely glutamate (E) and paraquat (Pq), the most stable conformation of CHP (unfolded) was considered. The complexes thus formed (hereafter CHP-E and CHP-Pq) were optimised at the same level of theory and are represented along with the CHP dipeptide in Fig. 5.

Geometry

Similar to the MD result, the non bonded angle ζ of the optimised CHP dipeptide is 135.18°, further confirming that the most probable conformation of the dipeptide is with the imidazole group facing away from the DKP. During the complexation of CHP with E, the DKP ring was found to be involved in hydrogen bond formation with the two carbonyl groups of the neurotoxin. The N–H of the DKP ring forms a hydrogen bond with the carbonyl oxygen of E, with an O–H distance of 1.972 Å. Similarly, the C=O group of the DKP ring forms a hydrogen bond with the other carbonyl group of glutamate, with an O–H distance of 1.927 Å. In addition, the imidazole nitrogen was also found to be involved in hydrogen bonding with E as shown in Fig. 5a. As the ionised form of E predominates at physiological pH, we were also interested to see the behaviour of the CHP dipeptide with ionised glutamate (E_I; shown in Fig. 5d). Due to the presence of the two carboxylic groups, E_I has a lower isoelectric point (pI) of 3.22 where it predominates. E_I in complex with the CHP dipeptide (represented as CHP-E_I) was fully optimised at HF/6-31G* level of theory. The optimised structure of CHP-E_I is depicted in Fig. 5d, which shows that, like the CHP-E complex, the CHP dipeptide also involves hydrogen bonding with E_I.

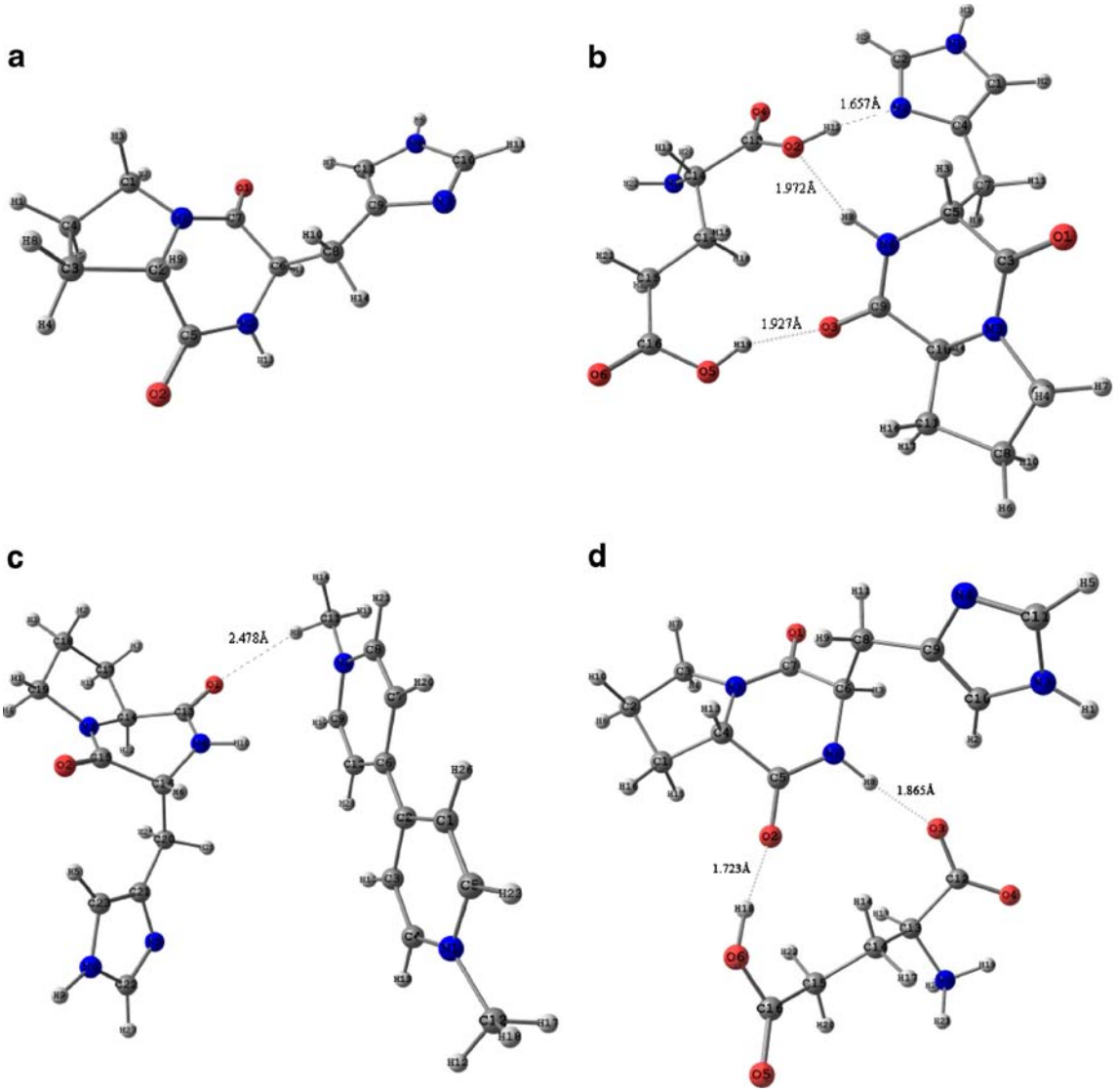

Fig. 5 Three dimensional structure of **a** CHP, **b** CHP-glutamate (E), **c** CHP-paraquat (Pq) optimised at B3LYP/6-311G** level of theory, and **d** CHP-E_I optimised at HF/6-31G* level of theory

In the case of the CHP-Pq complex, the neurotoxin undergoes much more distortion during optimisation than the CHP-E complex. The Pq, initially in a flat position, twists itself (see Fig. 5c) in such a way that its benzene rings faces the CHP dipeptide. The difference in the entropy value is of the order of 19.01 cal mol^{-1} K^{-1} between the complexes, further justifying the larger distortion in CHP-Pq. In CHP-Pq, the DKP ring also takes part in hydrogen bond formation. The C=O of DKP forms a hydrogen bond with the hydrogen of the methyl group in Pq, with a bond distance of 2.48 Å. Thus, from the whole host of involvements between the DKP ring and the neurotoxins, it can be confirmed that, in the CHP dipeptide, the DKP ring plays a major role in reducing the oxidative stress, thereby delaying the process of cell death. Moreover, earlier experimental studies have confirmed the significance of the DKP ring in neuroprotective and nootropic activities [48, 49].

DKP ring conformation

The DKP ring plays a major role in the interaction of the CHP dipeptide with neurotoxins [48, 49]. Hence it is pertinent to explore conformational variation in the ring, both in its isolated form and in the presence of neurotoxins. Previous studies have reported the conformational variation of five-membered rings, namely, the imidazole of histidine and pyrrolidine of proline residues [50–53]. The conformational accessibility of the six-membered DKP ring was analysed using puckering coordinates P_2, θ and Q [29]. Every six-membered ring conformation may be viewed in terms of boat (B), twist-boat (T), chair (C), half chair (H)

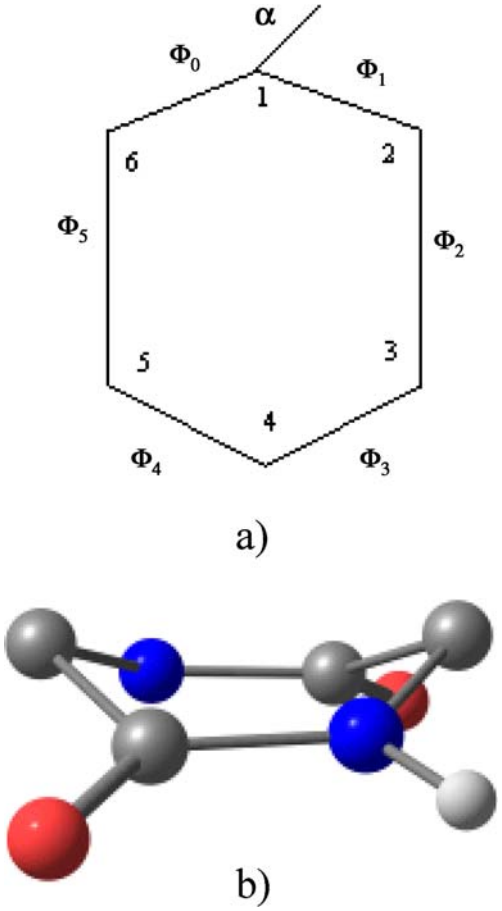

Fig. 6 **a** Torsion angle numbering in six-membered rings. **b** Schematic representation of the six-membered DKP ring of CHP. The DKP ring of neurotoxin interacted dipeptides shows analogy with the isolated dipeptide

and envelope (E) configurations [29]. For any given system, if all six endocyclic angles shown in Fig. 6a are known, then the puckering coordinates can be calculated using the Eqs. 1, 2 and 3. The calculated values of puckering coordinates of all the structures along with their respective endocyclic torsional angles are listed in Table 2. The DKP ring of the CHP dipeptide was found to be characterised by P_2=89.25°, θ=85.35° and Q=24.16°, attributined to a twist-boat (T) conformation (Fig. 6b) [28, 29]. The interaction of the neurotoxin E with CHP varies the pseudorotational parameters such that P_2=84.74°, θ=89.11° and Q=18.50°. In the case of CHP-Pq, the pseudorotational parameters are such that P_2=90.40°, θ=83.34° and Q=24.34°. Although the puckering variation is comparatively greater in the case of CHP-E, the DKP ring of both complexes adopts a twist-boat (T) conformation similar to the isolated CHP dipeptide.

Interaction

As we already know, the interaction of CHP with the neurotoxins E and Pq reduces cell death; it is thus of great interest to study the extent of the interaction of CHP with these neurotoxins. Hence, the interaction energies of the CHP-E and CHP-Pq complexes corrected to BSSE using Eqs. 4 and 5 were calculated and are listed in Table 3. The calculated interaction energy was found to be greater (−13.19 kcal mol^{-1}) for the CHP-E complex, as can be seen clearly in Fig. 5; the CHP dipeptide is involved in various hydrogen bond formation with E rather than Pq. As the interaction of neurotoxins with CHP is predominantly via the N–H and C=O groups of the DKP ring, the complexation of these groups is expected to cause discrepancies in NH and CO stretching vibrational frequencies. Table 3 lists the vibrational frequencies of all the structures. Significant red shift in the NH vibration of about 105 and 97 cm^{-1}, respectively, were identified in the complexes CHP-E and CHP-Pq. Similarly, the CO vibrations are also red shifted from the isolated dipeptide by about 36 and 26 cm^{-1}, respectively, in CHP-E and CHP-Pq complexes. Comparing the NH and CO vibrations, the CHP-E complex was found to have a considerable red shift in its frequency i.e., an order of 10 cm^{-1} greater than the CHP-Pq complex, confirming the larger interaction prevailing in this complex.

Furthermore, the larger interaction energy of CHP-E (−13.19 kcal mol^{-1}) compared to CHP-Pq confirms the strong affinity of CHP towards glutamate, which in turn may favour greater reduction of oxidation stress. The binding energies of the CHP-E and CHP-Pq complexes were also checked against values obtained through single point calculations at HF/6-311G**//B3LYP/6-311G**, MP2/6-311G**//B3LYP/6-311G** and B3LYP/6-311++G**//B3LYP/6-311G** levels of theory (see Table 3). The values obtained showed inconsistencies of the order of 1 to 4 kcal mol^{-1} compared to the binding energy of fully optimised complexes at B3LYP/6-311+G* level of theory.

Table 2 Endocyclic torsion angles and pseudorotation parameters (P, Q and θ) (units of degrees) of all the structures calculated at B3LYP/6-311G** level of theory. *E* Glutamate, *Pq* paraquat

Structure	Pseudorotational parameters								
	Φ_0	Φ_1	Φ_2	Φ_3	Φ_4	Φ_5	P_2	Q	θ
CHP	1.61	34.16	-34.32	-2.98	37.49	-38.17	89.25	24.16	85.35
CHP-E	1.82	26.41	-28.53	1.02	26.74	-29.12	84.74	18.50	89.11
CHP-Pq	2.63	33.37	-33.49	-3.67	38.30	-39.21	90.40	24.34	83.34

Table 3 Basis set superposition error (BSSE) corrected interaction energies, ΔE_{int} (in kcal mol^{-1}), of the complexes (level of theory: *A* B3LYP/6-311G**, *B* B3LYP/6-311++G**, *C* HF/6-311G**, *D* MP2/6-311G**) along with computed NH and CO stretching vibrational infrared frequencies (in cm^{-1}) and entropy S (cal mol^{-1} K^{-1}) of isolated and neurotoxin-interacted CHP dipeptides calculated at B3LYP/6-311G** level of theory

Structures	ΔE_{int}				N-H	C-O	S
	A	B	C	D			
CHP	–	–	–	–	3,599	1,779	120
CHP-E	-13.19	-14.25	-9.62	-12.32	3,494	1,743	188
CHP-Pq	-3.50	-3.63	-3.14	-6.45	3,502	1,753	207

Such inconsistencies in binding energies were also noted earlier for various other complexes obtained using DFT and ab initio theories [53, 54]. However, in all cases, a larger interaction energy was obtained for the CHP-E complex, confirming the profound affinity of CHP for the glutamate neurotoxin.

Molecular electrostatic potential

MESP is a well-established tool for exploring molecular reactivities, intermolecular interactions, and a variety of other chemical phenomena including bonding [55, 56]. MESP represents the electrostatic interactions that exist in a molecule [57]. As already observed, the interaction of neurotoxins forms various hydrogen bonds with the DKP ring of the CHP dipeptide. Illustration of these electrostatic interactions by means of MESP maps will thus provide defined information on the different regions of the complexes involved in hydrogen bonding. The 3D electrostatic potential of the CHP-E and CHP-Pq complexes was mapped on the molecular surface as shown in Fig. 7, with an isodensity surface value of 0.01 a.u, wherein regions with higher negative potential [V(r)] values are richer in electron density [58]. The maps clearly justify our geometrical findings that it is the DKP ring of the dipeptide that takes part actively in hydrogen bonding with the neurotoxins. In addition, the electrostatic interaction (with a bond length of 1.675 Å) prevailing between the carbonyl hydrogen of the glutamate and the imidazole nitrogen of histidine in CHP is also clearly seen from the MESP map. Although our earlier findings did not predict the interaction of the N–H of DKP with the carbon of the aromatic ring in paraquat, the presence of such an interaction is clearly seen from the molecular mapping. Thus, the twist of the paraquat molecule towards the dipeptide as discussed earlier is due solely to the aromatic interaction of the former with the DKP ring. On the whole, it is the DKP ring of CHP that plays a major part, during the interaction with neurotoxins, in reducing oxidation stress and cell death.

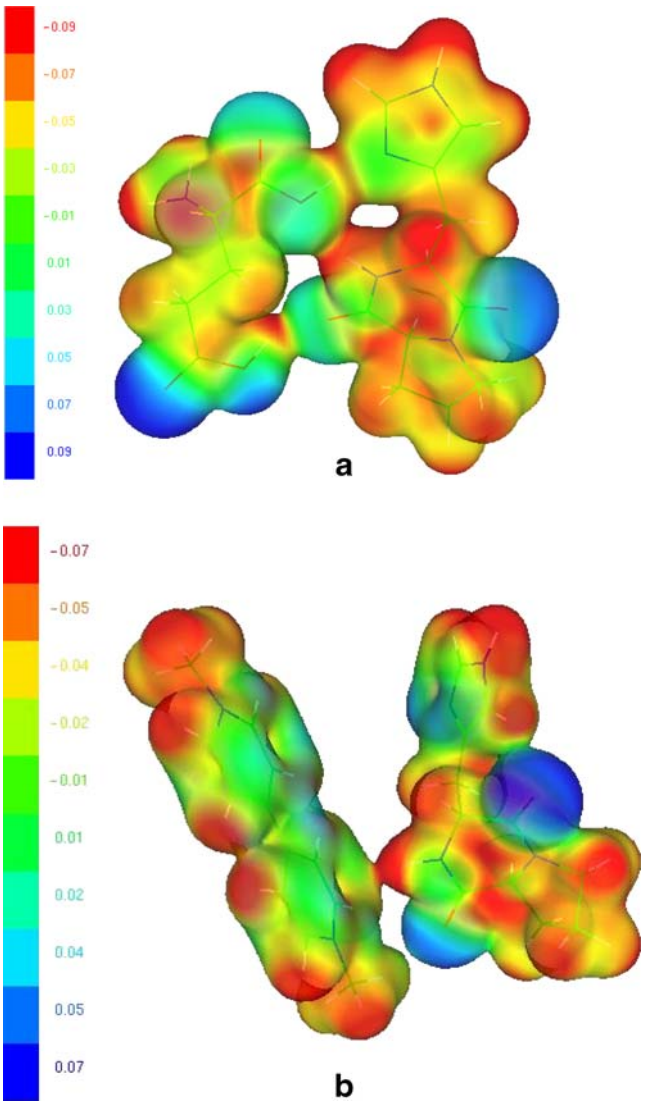

Fig. 7 The electrostatic potential for **a** CHP-E and **b** CHP-Pq complexes mapped on the molecular isodensity surface (ρ=0.01 a.u). The colour scale ranges from *red* (0.09 a.u) via *green* (zero) to *blue* (0.09 a.u)

Conclusions

The structural behaviour of the CHP dipeptide was studied by applying both molecular dynamics simulations and quantum chemical calculations. Simulation of CHP in aqueous solution on a 3-ns time scale confirmed the switching propensity of the histidine imidazole ring, and predicted the unfolded conformation to have the maximum probability of occurrence. The DKP ring of CHP was found to be involved in hydrogen bonding when interacting with the neurotoxins glutamate and paraquat in the gas phase. The profound interaction of CHP with glutamate was seen from the interaction energy and confirmed further from NH and CO stretching vibration energies. The interaction of neurotoxins with the dipeptide causes a red shift in the NH and CO stretching vibrations of the DKP ring. The effect of CHP on glutamate was found to be considerably greater than the effect on paraquat. Hydrogen bond interactions between the neurotoxins and the DKP ring of the dipeptide were also depicted through MESP. The interaction between the DKP ring and the neurotoxins explains why they play a major role in reducing oxidation stress, thereby delaying the processes of cell death. This study is of theoretical and practical importance in exploring the behaviour of the CHP dipeptide during its application in biological\pharmacological processes.

Acknowledgment A.A. is grateful to the Council for Scientific and Industrial Research (CSIR) New Delhi for the award of a Senior Research Fellowship.

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