Computational Study of Peptide Plane Stacking with Polar and Ionizable Amino Acid Side Chains

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Supporting Information

ABSTRACT: Parallel and T-shaped stacking interactions of the peptide plane with polar and ionizable amino acid side chains (including aspartic/glutamic acid, asparagine/glutamine, and arginine) are investigated using the quantum mechanical MP2 and CCSD computational methods. It is found that the electrostatic interaction plays an essential role in determining the optimal stacking configurations for all investigated stacking models. For certain complexes, the dispersion interaction also contributes considerably to stacking. In the gas phase, the stacking interaction of the charged system is stronger than that of the neutral system, and T-shaped stacking is generally more preferred than parallel stacking, with the stacking energy in the range of -4 to -18 kcal/mol. The solvation effect overall weakens stacking, especially for the charged system and the T-shaped stacking configurations. In water, the interaction energies of different stacking models are comparable.

INTRODUCTION

π interactions are an important type of noncovalent interactions in molecules and essential in chemistry and biology.^{1–6} π interactions, involving π electrons, can be divided into different subtypes, for example, π – π ,¹ anion– π ,² cation– π ,^{7–9} and XH – π ^{10–12} depending on the components of the system. Accurate binding energies can be retrieved from high-level quantum mechanical calculations, for example, the CCSD(T)/complete basis set (CBS) limit,^{4,13} which allows a direct comparison of different types of π interactions. The binding energy can be decomposed to four terms, electrostatic, exchange repulsion, polarization, and dispersion.^{14–16} The electrostatic term describes the interaction of electric multipoles; the polarization term is the interaction between the permanent multipoles and the induced multipoles; the dispersion term describes the interaction between the instantaneous multipoles and the induced multipoles. Taking benzene dimer as an example, the dispersion and electrostatic terms contribute the most to the interaction energy.^{17–20} Compared with the σ -electron system, the π -electron system has a high polarizability due to the delocalization property of the π electrons.²¹ Thus, the polarization energy can be significant in the cation– π and anion– π systems,^{2,22,23} whereas the dispersion energy is important when the complex is neutral.

For proteins, interactions with aromatic amino acid side chains, including phenylalanine, tyrosine, and tryptophan, can be very important for their stability and function.^{24–30} Molecular dynamics (MD) simulations showed that the miniprotein Trp-cage³¹ with a tryptophan (W5) in the hydrophobic core cannot fold properly if the dispersion term is turned off,²⁸ and the protein refolds if the dispersion term is restored. The stabilization from the aromatic residues to the

protein structure comes from two aspects, the entropic (hydrophobic) effect and the enthalpic effect (direct interactions). Quantum mechanical (QM) calculations suggest that the enthalpic effect plays a more important role for the protein rubredoxin stability.^{24,25}

Most studies of π interactions in proteins focus on the aromatic side chains. Recently, stacking with the protein backbone amide has been investigated as well.^{32,33} The intramolecular peptide plane stacking is competitive with the hydrogen bonding.^{33,34} Compared with a benzene ring, the backbone amide plane has a large dipole moment, underlining the importance of electrostatics in the amide stacking interactions. QM calculations showed that the interaction energy between *N*-methylacetamide (NMA) and the aromatic ring can be as large as -5 kcal/mol, depending on the dipole moment of the aromatic ring.³² This binding energy is more negative than that of the benzene dimer, -2.7 kcal/mol.³⁵ Our previous study demonstrated that stacking of the E305 carboxylate, a buried side chain, with two peptide plane amides is important for the stability of *Trichoderma reesei* CelSA.³⁶ A survey of the protein structures in the pdb databank suggests that the parallel peptide backbone amide stacking with aspartate/glutamate has an occurrence frequency of 28 per 100 structures.³⁶ Ionizable residues inside proteins play an important role in protein functions, such as ligand binding, protein stability, and enzyme catalysis. It has been shown that electrostatic interactions are critical for the stabilization of the ionizable residues;^{37–42} however, stacking with the peptide

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plane, which can be important for the ionizable side-chain stability as well, has not been thoroughly investigated.

In this study, we focus on peptide amide stacking with aspartate/glutamate (both protonated (DE) and deprotonated (DE[−])), asparagine/glutamine (NQ), and arginine side chains (R⁺) using QM calculations. We characterize the peptide plane stacking preferences with different groups, the role of the charge distribution, and the solvation effect. The optimal stacking configurations are identified through scanning the interaction energy at the MP2/6-31+G(d,p) level.⁴³ Then, these interaction energies are decomposed by the localized molecular orbital energy decomposition analysis (LMO-EDA),¹⁴ a very efficient method that provides more insights into the property of stacking. The results show that the electrostatic interaction plays a dominant role in the stacking orientation and position especially for the charged system. For the neutral system, the dispersion interaction contributes considerably to stacking. For some T-shaped complexes, the hydrogen bond can be formed. In the gas phase, stacking of the charged system is much stronger than that of the neutral system. The solvation effect generally weakens stacking for both systems, with the charged system interaction affected more significantly. As a result, the interaction energies are similar for the charged and the neutral systems in water.

METHODS AND MATERIALS

The truncated molecular fragments were used to model the π – π interactions between the peptide amide plane and the amino acid side chains. Specifically, the peptide plane was modeled using NMA, while aspartate/glutamate (DE[−]), aspartic/glutamic acid (DE), asparagine/glutamine (NQ), or arginine (R⁺) was truncated to a functional group saturated with a methyl (Figure 1). With the geometries of the stacking

Figure 1. Diagram of DE[−], DE, NQ, and R⁺–NMA dimers. The xy plane is defined by the three atoms, N1', C1', and O1' of NMA, with the y axis along the bond vector O1'–C1'. d is defined as the distance between the atom C1 and its projection on the xy . The dihedral ω is defined as $\angle O_1-C_1-C_1'-O_1'$ for DE[−], DE, and NQ or $\angle N_1-C_1-C_1'-O_1'$ for R⁺.

components optimized at the MP2/6-31+G(d,p) level,^{43,44} the parallel and T-shaped NMA–amino acid side-chain models were constructed by arranging the two planes of the monomers parallelly or perpendicularly. The plane was defined by three atoms, that is, O1'–C1'–N1' for NMA, O1–C1–O2 for DE and DE[−], O1–C1–N1 for NQ, and N1–C1–N2 for R⁺ (Figure 1). For parallel stacking with DE, NQ, and R⁺, two mirror structures resulted in two different configurations, which were denoted as P₁ and P₂. A 3-D potential energy surface (PES) was

constructed at the MP2/6-31+G(d,p) level for each complex as follows. By defining N1'–C1'–O1' of NMA as the xy plane (with C1' as origin) and restricting the distance between C1 and the xy plane (d) to 4.0 Å, the dihedral ω (defined as $\angle O_1-C_1-C_1'-O_1'$ for DE[−], DE, and NQ or $\angle N_1-C_1-C_1'-O_1'$ for R⁺) was rotated around the Z axis by 30.0° increments (Figure 1). Simultaneously, the projection of C1 on the xy plane (with the coordinate (x, y)) was scanned by sampling grid points every 0.5 Å (from −5 to +5 Å) where the + y direction was along the O1'–C1' bond vector. After the optimal configuration was obtained, the d distance was scanned in 0.1 Å increments. The starting configuration of each complex and the geometric variables are shown in Figure 1. All of the interaction energies (ΔE) were counterpoise-corrected for the basis set superposition error (BSSE).⁴⁵

The binding energies of the optimal configurations were also calculated by MP2/CBS^{43,44} (complete basis set) and CCSD(T)/CBS to validate the obtained PESs. The MP2/CBS energies were determined with the use of aug-cc-pVnZ ($n = D, T$)^{46,47} basis sets and the Helgaker's extrapolation scheme.⁴⁸ The CCSD(T)/CBS energies were obtained by adding the difference between the MP2/6-31+G(d,p) and CCSD(T)/6-31+G(d,p) energies to the MP2/CBS values.³⁰ The total interaction energies were decomposed to electrostatic, exchange-repulsion, polarization, and dispersion components by the LMO-EDA analysis to reveal the contributions of various intermolecular interactions.¹⁴ The polarizable continuum model (PCM)⁴⁹ with different dielectric constants (4.0 and 80.0) was adopted to investigate the solvation effect on binding energies. All calculations were performed using the Gaussian 09⁵⁰ and Gamess^{43,51–53} software packages.

A total of 6338 high-quality protein structures were selected from the pdb databank (resolution <2.0 Å, $R < 0.3$, chain length >100) using the program PISCES.⁵⁴ The sequence identity between pair structures was limited to <25%. The amide (of glutamine and asparagine) peptide plane stacking as well as the guanidino group (of arginine) peptide plane stacking from the selected structures were identified using an in-house VMD⁵⁵ script.

RESULTS AND DISCUSSION

PES Scan. The 3-D (x, y, ω) ΔE binding PES at the MP2/6-31+G(d,p) level was constructed for DE[−], DE, NQ, and R⁺–NMA with the fragment distance d fixed at 4.0 Å. (The geometric parameters are defined in Figure 1.) After the (x, y, ω) values of the energy minimum state were identified, the distance d was then scanned with (x, y, ω) fixed to determine the optimal stacking configuration (Table 1). The CCSD(T)/CBS binding energy values were also calculated for the optimal configurations of different complexes and correlated well with the MP2/6-31+G(d,p) data (Figure 2A). The CCSD(T)/CBS calculations validate the PES scan at the MP2/6-31+G(d,p) level. The LMO-EDA energy decomposition analysis was also performed at MP2/aug-cc-pVnZ ($n = D, T$) levels for optimal configurations (Figure 2B). As can be seen, different energy terms at MP2/6-31+G(d,p) agree quite well with the MP2/aug-cc-pVnZ ($n = D, T$) results and those extrapolated at MP2/CBS and CCSD(T)/CBS, except for the fact that the dispersion term is underestimated by ~1 kcal/mol, which explains the intercept of −1.2 kcal/mol in Figure 2A.

Optimal Configurations of the Parallel Stacking. Figure 3 and Figure S1 in the Supporting Information (SI) shows the 2-D (x, y) PES contour plot with the ω dihedral

Table 1. Components of the MP2/6-31+G(d,p) Interaction Energy for Optimal Configurations in the Gas Phase (kcal/mol)

	(<i>x</i> , <i>y</i> , <i>ω</i> , <i>d</i>) (Å, Å, deg, Å)	ΔE_{elec}	ΔE_{exre}	ΔE_{pola}	ΔE_{disp}	ΔE_{total}
DE_P ₁	(1.0, 0.0, 120, 3.4)	−3.7	3.6	−0.7	−2.3	−3.1
DE_P ₂	(0.5, 0.0, 210, 3.5)	−3.4	2.6	−0.6	−1.7	−3.1
NQ_P ₁	(−0.5, 0.5, 180, 3.6)	−3.7	2.8	−0.6	−2.0	−3.6
NQ_P ₂	(1.5, 0.0, 150, 3.6)	−3.2	2.7	−0.6	−2.2	−3.3
DE [−] _P	(1.5, 2.0, 120, 3.4)	−6.5	3.9	−1.8	−1.9	−6.3
R ⁺ _P ₁	(−0.5, −4.0, 240, 2.8)	−10.9	5.9	−2.9	−1.7	−9.6
R ⁺ _P ₂	(−0.5, −4.0, 90, 3.5)	−7.8	3.5	−1.9	−1.2	−7.5
DE [−] _T	(0.0, −0.5, 180, 3.6)	−10.5	8.1	−2.7	−1.0	−6.1
NQ [−] _T	(0.0, −0.5, 180, 3.7)	−9.5	6.9	−2.5	−1.1	−6.2
DE [−] _T	(1.0, 3.0, 90, 2.2)	−21.2	15.5	−8.0	−2.0	−15.6
R ⁺ _T	(−0.5, −2.5, 120, 3.4)	−19.1	11.9	−7.6	−1.2	−15.9

Figure 2. (A) Correlation between the MP2/6-31+G(d,p) binding energy and the CCSD(T)/CBS binding energy for the optimal configurations. (B) LMO-EDA energy decomposition using different methods: MP2/6-31+G(d, p) (M1), CCSD(T)/6-31+G(d, p) (M2), MP2/aug-cc-pVDZ (M3), MP2/aug-cc-pVTZ (M4), MP2/CBS (M5), and CCSD(T)/CBS (M6).

angle value the same as that of the optimal configuration in the searched space and $d = 4.0$ Å. The optimal configuration for each parallel stacking model is shown in Figure 4. Considering that all stacking components are charged or polar, we attempt to rationalize the optimal stacking configurations in terms of electrostatics. The electrostatic potentials (ESPs) and dipole moment vectors of all components are shown in Figure 5. Taking the DE[−] as an example, the ESPs around all atoms are negative, and the most negative areas are centered on O₁ and

Figure 3. MP2/6-31+G(d,p) (*x*, *y*) PES that contains the binding energy minimum of the parallel dimer, with each contour corresponding to 1 kcal/mol. The minimum energy site was marked with a white square.

Figure 4. Optimal parallel complexes obtained at the MP2/6-31+G(d,p) level ($d = 4.0$ Å).

O₂. The DE[−] carboxyl group tends to locate above the most positive ESP area of NMA (around N₁H, Figures 1, 4, and 5) with the corresponding (*x*, *y*) coordinates of (1.5 Å, 2.0 Å)

Figure 5. Molecular electrostatic potentials (EPs) and the dipole moment vectors for all monomers optimized at MP2/6-31+G(d,p).

(Table 1). As shown in Figure 5, the dipole of NMA is approximately along the $C_1'-O_1'$ vector (tilted by 12°), while the dipole of DE^- points along the bisector of the angle $\angle O_1-C_1-O_2$. In the optimal DE^- -NMA parallel stacking (DE^-_P , $\omega = 120^\circ$) (Table 1), the two dipoles are aligned antiparallelly, suggesting that the orientation is determined by the dipole-dipole interaction (Figure 4). To confirm this, we extracted the interaction energy at each ω (with (x, y) fixed at $(1.5 \text{ \AA}, 2.0 \text{ \AA})$) from the 3-D PES (Figure 6 and Figure S2 in the SI). As can be seen, the rotation of the DE^- dipole leads to $\sim 1.7 \text{ kcal/mol}$ variation of the interaction energy. The LMO-EDA analysis

Figure 6. Energy components as a function of ω with (x, y) coordinates fixed at the optimal values and $d = 4.0 \text{ \AA}$ (Table 1). The curves were obtained from the MP2/6-31+G(d,p) calculations.

shows that the electrostatic energy profile is similar to that of the total binding energy, whereas the other three terms have much smaller variations (Figure 6 and Figure S2 in the SI). As reported in Table 1, the electrostatic term dominates the total interaction energy for the DE^-_P optimal complex configuration ((x, y, ω, d) of $(1.5 \text{ \AA}, 2.0 \text{ \AA}, 120^\circ, 3.4 \text{ \AA})$).

For the DE -NMA complex DE_P1 and DE_P2 (Figure 1), the absolute values of the binding energy are considerably smaller than that of DE^- -NMA (Figure 3). The ESPs of DE have both negative and positive values (Figure 5). Specifically, the areas around O_1 and O_2 atoms are negative, while the other areas are positive. The LMO-EDA analysis of the optimal DE -NMA configuration ($(1.0 \text{ \AA}, 0.0 \text{ \AA}, 120^\circ, 3.4 \text{ \AA})$ for DE_P1 and $(0.5 \text{ \AA}, 0.0 \text{ \AA}, 210^\circ, 3.5 \text{ \AA})$ for DE_P2) shows that the dispersion term, about 75 or 56% of the total binding energy, may play an important role in the DE -NMA parallel stacking (Table 1). To probe what determines the relative position of the two stacking components, we show in the 2-D (x, y) PES, plotted with ω fixed at 120° for DE_P1 (or at 210° for DE_P2), that both the dispersion and the electrostatic terms have an energy minimum site near the total energy minimum site (Figure 7 and Figure S3 in the SI) but with the electrostatic

Figure 7. (x, y) PESs of the electrostatic and dispersion components for the DE -NMA and NQ -NMA parallel stacking dimers with each contour corresponding to 0.25 kcal/mol . ($d = 4.0 \text{ \AA}$ and ω was fixed at its optimal value (Table 1).) The energy minimum site of the electrostatic PESs (left) or the dispersion PESs (right) was marked with a filled white square, while the total energy minimum site was marked with a filled white triangle

minimum site closer. Therefore, the optimal position is determined by both terms, but the electrostatic term appears to be more important. To see which term determines the relative orientation of the two stacking monomers, different energy components were plotted as a function of ω for DE_P1 and DE_P2 with (x, y, d) fixed (Figure 6 and Figure S2 in the SI). The shape of the electrostatic curve is almost the same as the total energy curve with two minima at 120 and 240° for DE_P1 (60 and 210° for DE_P2), suggesting that the orientation is determined primarily by the electrostatic

interaction. It is found that the dipole of DE, roughly along the direction of C_1-O_1 , aligns not antiparallely to that of NMA but tilts away by $\sim 40^\circ$ (Figure 4).

The electrostatic properties of asparagine/glutamine (NQ) are similar to the neutral DE. The ESPs are negative around O_1 and N_1 atoms but positive around other areas. The dipole of NQ is approximately along the direction of C_1-O_1 , with a tilt angle of 7° (Figure 5). Compared with DE, the weaker electron-attractive ability of nitrogen in NQ would lead to less π delocalization, indicating a more negative ESP on O_1 and a larger molecular dipole along C_1-O_1 bond (4.5 D for NQ and 2.0 D for DE). The LMO-EDA analysis suggests that the electrostatic term and the dispersion term contribute the most to the total binding energy for the optimal NQ–NMA configuration ((x, y, ω, d) of $(-0.5 \text{ \AA}, 0.5 \text{ \AA}, 180^\circ, 3.6 \text{ \AA})$ for NQ P_1 and $(1.5 \text{ \AA}, 0.0 \text{ \AA}, 150^\circ, 3.6 \text{ \AA})$ for NQ P_2) (Table 1). Similar to DE–NMA complex, the 2-D (x, y) PES, plotted with ω fixed at 180° for NQ P_1 (or at 150° for NQ P_2), shows that both the dispersion and the electrostatic terms have a minimum near the total energy minimum site but with the electrostatic term closer (Figures 7 and Figure S3 in the SI). By fixing the (x, y) coordinates, the four LMO-EDA energy terms are plotted as a function of ω for NQ P_1 or P_2 models (Figure 6 and Figure S2 in the SI). It is found that the shape of the total energy curve is almost the same as the electrostatic curve, with the total energy change up to 4.1 kcal/mol for NQ P_1 or 3.9 kcal/mol for NQ P_2 (Figure 6 and Figure S2 in the SI). Different from the DE–NMA complex, the monomeric dipoles of NQ–NMA are aligned antiparallely in both NQ P_1 and NQ P_2 optimal stacking. Thus, the optimal position of the parallel NQ–NMA is mainly determined by both electrostatic and dispersion, whereas the optimal orientation is determined by the electrostatic interaction.

For the R^+ –NMA parallel stacking model, the electrostatic term dominates the total interactions for both P_1 and P_2 optimal configurations ((x, y, ω, d) of $(-0.5 \text{ \AA}, -4.0 \text{ \AA}, 240^\circ, 2.8 \text{ \AA})$ and $(-0.5 \text{ \AA}, -4.0 \text{ \AA}, 90^\circ, 3.5 \text{ \AA})$, respectively) (Table 1), where the arginine side chain with positive ESP prefers to locate above the most negative ESP area of NMA (O_1') (Figure 4). The much lower d value of $R^+_{P_1}$ is attributed to the stacking configuration. As shown in Figures 3 and 4, in the optimal configuration, the R^+ model moved $\sim 4 \text{ \AA}$ away from the initial position, and only the CH_3 group of R^+ is on top of NMA. Thus, the steric hindrance effect is small, mainly from the CH_3 . In addition, the methyl carbon C is not on the $N_1-C_1-N_2$ plane of the R^+ monomer but points away from NMA with the dihedral $\angle N_1-C_1-N_2-C$ of $\sim 12^\circ$. In the $R^+_{P_1}$ complex, the distance between the methyl carbon C of R^+ and NMA plane is $\sim 3.1 \text{ \AA}$, which is $0.3 \text{ \AA}$ larger than the interplanar distance, d . The dipole of R^+ model is perpendicular to C_1-N_1 bond, roughly along the C_1-N_3 bond vector (Figure 5). As shown in Figure 4, the direction of the R^+ dipole is approximately antiparallel to that of NMA in the optimal configurations. The total binding energy and the different energy components were plotted versus the ω angle ((x, y) fixed at $(-0.5 \text{ \AA}, -4.0 \text{ \AA})$) (Figure 6 and Figure S2 in the SI). As the orientation of the R^+ dipole varies relative to that of NMA, the interaction energy can change by 0.6 kcal/mol for $R^+_{P_1}$ or 1.0 kcal/mol for $R^+_{P_2}$. The small variation indicates that the electrostatic energy is mainly from the charge–dipole interaction.

Optimal Configurations of the T-Shaped Stacking. The (x, y) 2-D PES slice is shown for the DE^- –, DE^- –, NQ^- –,

and R^+ –NMA complexes (Figure 8, $d = 4.0 \text{ \AA}$ and ω equals the optimal value (Table 1)). For the DE^- –NMA complex, the

Figure 8. (x, y) PES of the T-shaped dimers, each contour corresponding to 1 kcal/mol. The distance d was fixed at $4.0 \text{ \AA}$ and the ω angle was fixed to its optimal value (Table 1). The minimum energy site was marked with a filled white square.

optimal configuration is that the DE^- carboxyl group with negative ESPs locates above the most positive ESP area of NMA (H of N_1'), with the N_1' –H bond aligning in between the two oxygen atoms O_1 and O_2 (Figure 9). For the DE^- –

Figure 9. Optimal T-shaped stacking configurations obtained from the MP2/6-31+G(d,p) scan.

NMA optimal configuration ((x, y, ω, d) of $(0.0 \text{ \AA}, -0.5 \text{ \AA}, 180^\circ, 3.6 \text{ \AA})$), the positive ESP area (H of O_2) of DE is located above the negative ESP area of NMA (O_1') and a $O_2-H\cdots O_1'$ hydrogen bond is formed, with the O_2-O_1' distance of $3.2 \text{ \AA}$ and the angle $\angle O_2-H-O_1'$ of 173° . In addition, the dipole of DE aligns approximately antiparallely to that of NMA. As for the NQ–NMA complex, the optimal configuration is very similar to that of the DE–NMA dimer. A $N_1-H\cdots O_1'$ hydrogen bond is formed, with the N_1-O_1' distance of $3.2 \text{ \AA}$ and the $\angle N_1-H-O_1'$ angle of 162° , while the monomer dipoles are also aligned roughly antiparallely. For the R^+ –NMA complex with the optimal configuration at $(-0.5 \text{ \AA}, -2.5 \text{ \AA}, 120^\circ, 3.4 \text{ \AA})$, R^+ with the positive ESP sits near the negative ESP area of NMA (O_1'), whereas a hydrogen bond is formed between N_1 and O_1' , with the N_1-O_1' distance of $2.8 \text{ \AA}$ and $\angle N_1-H-O_1'$ of 172° . The shorter distance between the heavy atoms of the hydrogen bond for R^+_T compared with that from DE^-_T is likely due to the smaller steric hindrance effect (Figure

9). As reported in Table 1, the total interaction energies of the optimal configurations have the largest contributions from the electrostatic and polarization terms for the T-shaped stacking models. Therefore, all of the optimal T-shaped configurations are determined primarily by the electrostatic interaction. In addition, the DE^-_T complex has an optimal d value (2.2 Å) much smaller than others (Table 1) due to the strong electrostatic attraction (Figure 9).

Binding Energy and the Solvation Effect. The CCSD(T)/CBS interaction energies for optimal configurations are reported (Table 2). For the parallel stacking, the absolute

Table 2. Solvation Effect on the Interaction Energies at CCSD(T)/CBS for Optimal Configurations (kcal/mol)

	ΔE_{total}		
	$\epsilon = 1.0$	$\epsilon = 4.0$	$\epsilon = 80.0$
DE_P1	-4.6	-3.2	-2.7
DE_P2	-4.5	-3.1	-2.5
NQ_P1	-4.8	-3.4	-2.5
NQ_P2	-4.6	-3.2	-2.4
DE^-_P	-7.8	-3.1	-1.9
R^+_P1	-11.0	-3.9	-1.7
R^+_P2	-8.6	-3.1	-1.4
DE_T	-8.0	-5.4	-4.2
NQ_T	-8.0	-4.8	-3.3
DE^-_T	-18.1	-5.7	-1.5
R^+_T	-18.0	-8.7	-5.5

interaction energies are in the order of $\text{R}^+ > \text{DE}^- > \text{DE} \approx \text{NQ}$. It has been reported that the interaction energy of benzene/NMA parallel stacking is about -2.5 kcal/mol,³⁰ smaller than that of the complexes reported in this work, where a large portion of the interaction energy comes from the electrostatics, for example, the charge-dipole and the dipole-dipole interactions (Table 1). As for the T-shaped stacking models, the interaction is in the order of $\text{R}^+_T \approx \text{DE}^-_T > \text{DE}_T \approx \text{NQ}_T$, with the absolute interaction energies much larger than those of the parallel stacking models (Table 2).

Because the stacking occurs within a protein or on a protein surface, the PCM calculations with different dielectric constants (4.0 and 80.0) were carried out to mimic the solvation effect for the optimal configurations without further reoptimization (Table 2). It is worth noting that interaction energy at the PCM/CCSD(T)/CBS was extrapolated in a similar way as in the gas-phase calculations for the purpose of direct comparison with the gas-phase results. The interaction energy in PCM is not very sensitive to the used basis set (aug-cc-pVDZ and aug-

cc-pVTZ), consistent with the gas-phase calculations (Figure 2). The extrapolated interaction energy at PCM/CCSD(T)/CBS is very similar to that at PCM/MP2/aug-cc-pVTZ. For the stacking at the low dielectric constant environment ($\epsilon = 4.0$), all of the interactions are weakened. Taking the parallel stacking as an example, the binding energy increases (becomes less negative) by 4.7, 7.1 (5.5), 1.4, and 1.4 kcal/mol for DE^-_P , R^+_P1 (P_2), DE_P , and NQ_P , respectively, meaning that the interactions of the charged models are weakened much more than those of the neutral ones. As a result, the interaction energies of the neutral complexes are nearly equivalent to those of the charged ones at $\epsilon = 4.0$, with the values ranging from approximately -3.1 to -3.9 kcal/mol for the parallel stacking models. For the T-shaped configurations, the charged systems are also affected more than the neutral ones. The interaction energy of neutral DE_T (-5.4 kcal/mol) or NQ_T (-4.8 kcal/mol) is nearly equal to that of DE^-_T (-5.7 kcal/mol) but less negative than R^+_T (-8.7 kcal/mol), which has a combination of the charge-dipole interaction and the intermolecular hydrogen bond. The energy difference between the parallel and the T-shaped stacking models also becomes smaller (Table 2). As the dielectric constant increases to 80.0, the interactions are further weakened for both the parallel stacking and the T-shaped stacking models. For the neutral complexes, the parallel stacking interaction energies change to about -2.5 kcal/mol, whereas the T-shaped stacking interaction energies change to -3 to -4 kcal/mol. The charged complexes generally have interactions weaker than the corresponding neutral ones, except for R^+_T , which has the strongest interaction energy of -5.5 kcal/mol.

Survey of the Parallel Amide- and Guanidino-Peptide Plane Stacking in Proteins. The stacking of carboxyl-peptide plane has been surveyed in the pdb databank previously, and it shows that the parallel stacking is more common (0.28 interactions per protein structure) than the T-shaped stacking. In this work, we performed a similar search for the parallel amide-peptide plane and guanidino-peptide plane stacking interactions. Three parameters are used to describe the stacking, namely, the distance, d , the angle, θ , between the two planes (amide or guanidino group versus the peptide plane), and the distance, r , between the projection of C_1 on the peptide plane and the origin C_1' (Figure 1). The cutoff values are $d < 5.0$ Å, $\theta < 20^\circ$, and $r < 1.5$ Å for the parallel stacking. A total of 2577 and 3129 parallel stacking interactions are identified from a database of 6338 high-resolution X-ray structures, yielding an average of 0.41 and 0.49 interactions per structure for the amide- and guanidino-peptide plane stacking, respectively. An analysis of the distribution of the distance, d , shows a maximum

Figure 10. Histogram of the distance, d , (Figure 1) for the parallel amide-peptide plane (A) and guanidino-peptide plane stacking (B). The probability was corrected for the increase of volume at larger distance.

occurrence at $d = 3.4$ Å for the amide–peptide plane stacking and $d = 3.5$ Å for the guanidino–peptide plane stacking (Figure 10), consistent with the QM calculations (Table 1).

CONCLUSIONS

Parallel and T-shaped stacking interactions of DE^- , DE^- , NQ^- , and R^+-NMA were investigated using the QM computational methods. For all stacking models, the electrostatic interaction plays an essential role in determining the optimal stacking position and the relative orientation. For the neutral complexes, the dispersion also contributes considerably to the stacking. In the gas phase, the stacking of the charged system is stronger than that of the neutral system, and the T-shaped stacking is generally stronger than the parallel stacking, but the solvation has a large effect in weakening the stacking, especially for the charged system. As the dielectric constant increases to 4.0, the parallel stacking energy is comparable for the charged and the neutral systems, whereas the T-shaped stacking energy is also similar for different systems (except R^+_T). As the dielectric constant increases further to 80.0, the interactions of the charged systems generally become weaker than the neutral ones (except R^+_T).

ASSOCIATED CONTENT

Supporting Information

MP2/6-31+G(d,p) (x, y) PES for different parallel stacking models; different energy terms as a function of ω ; electrostatic and dispersion energies as a function of the (x, y) coordinates; and the complete author list of ref 50 and 51. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Notes

The authors declare no competing financial interest.

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