Studies of the Monodipole-macrodipole Interactions within α-Helices Using the Point-charge Systems for Alanine

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Our previous quantum mechanical calculations using polyalanine model systems showed that the monodipole-macrodipole interactions selectively stabilize α-helices and make it possible for α-helices to be formed in hydrophobic environment where the solvent effect is not available. The monodipole-macrodipole interactions in α-helices were studied molecular mechanically using various point-charge systems available. The results show that all the point-charge systems used in the calculations produce the monodipole-macrodipole interactions up to about 60% compared to the results of the quantum mechanical calculations. The results of molecular mechanical calculations are explained and discussed compared to the results of the quantum mechanical calculations.

Key Words : α-Helices, Alanine, Force field, Folding

Introduction

Molecular mechanics (MM) has been widely used in studying protein folding.[1] MM uses force field (FF) parameters to calculate energies and many FF parameters have been developed so far.[2-10] Because the accuracies of MM calculations largely depend on their MM energies, the importance of FF parameters cannot be overemphasized. To test the accuracies of FF parameters the energies of MM calculations on model molecules are usually compared to the results of quantum mechanical (QM) calculations on the same systems.[9-10] The problem of these procedures, however, is that the energies from QM calculations cannot be separated into a certain energy from each individual bonding or individual nonbonding interaction because the QM energies include all the interactions combined altogether. This make it unable to check the accuracy of each FF parameter of MM: charge system, bond, angle, torsion, hydrogen bond, van der Walls, etc. Therefore the accuracies of FF parameters are tested as a whole, not individually, and the accuracy of each FF parameter is left unknown. This kind of approach works in systems where the contribution from one individual interaction can be compensated by the substitution from another interaction. For example, the energy of H-bond can be calculated by only the electrostatic interactions between the charges given to atoms involved as in AMBER[3,4] or by a special formula like Lenard Jones 10-12 as in DREIDING.[11]

In this study we focus on the point-charge systems being used in MM calculations. The accuracy of a charge system in a FF is very important in determining the quality of the whole FF because it can be checked easily that a little change of charges creates substantial difference in energy.[7] However, there has been no reliable way to evaluate the point-charge systems by the reason as explained above.

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Recently we proposed that monodipole-macrodipole interactions within α-helices stabilize the α-helical conformation more effectively in hydrophobic environments, providing a means for α-helices to be formed in hydrophobic environment with very little solvation effects.[12] The differential energies between two α-helices of $(Ala)_7\_N$ and $(Ala)_4\_N$ result from the dipole-dipole interactions between the two groups, the first alanine at the N-terminus of $(Ala)_7\_N$ and the last three alanines at the C-terminus of $(Ala)_7\_N$. The differential energies include only the pure electrostatic energies between the two alanine groups because all the other bonding and nonbonding interactions are canceled out during the process of energy differentiation. Therefore, the monodipole-macrodipole interaction model system can be used to exclusively evaluate the point-charge systems without paying any attention to the other force-field parameters of all the bonding and nondbonding interactions.

Recently many MM studies with force fields have been carried out to study the folding mechanism of α-helics.[13-17] Here we studied six point-charge systems among all point-charge systems currently available using the monodipole-macrodipole interactions within α-helices: two AMBER[3,4] force fields, CFF91,[5,6] CVFF,[2] CHARMM,[8] and the quantum mechanically developed one by Bellido *et al.*[7]

Calculation Methods

In order to study the monodipole-macrodipole interaction in α-helices using point-charge systems the same model system and procedures were used as described in our previous report.[12] This time only the classical electrostatic energies were calculated and all the other bonding and nonbonding interactions were turned off during the MM calculations.

Table 1 shows the point-charge systems used for the current MM calculations. Each point-charge system was used for all alanines in the model systems of polyalanines.

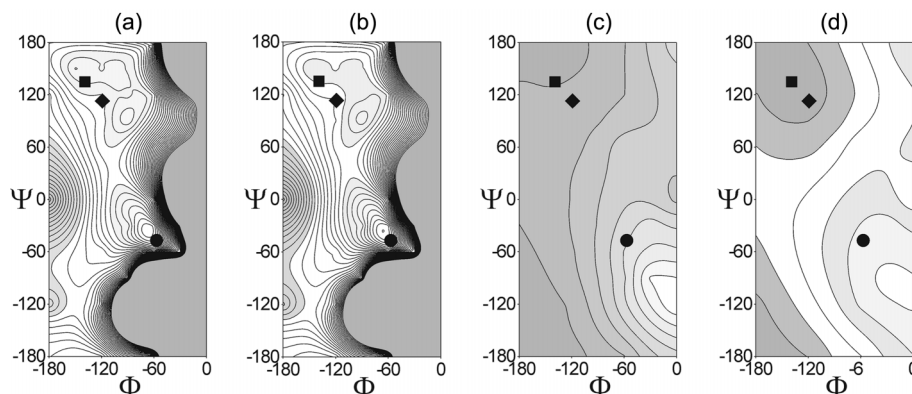**Table 1.** The point-charge systems for the polyalanines used in the MM electrostatic energy calculations

Atom	Label	CVFF[a]	AMBER1[b]	AMBER2[c]	CFF91[d]	BELLIDO[e]	CHARMM[f]
N	N	-0.5000	-0.4630	-0.5200	-0.6503	-0.4100	-0.3500
C	CA	0.1200	0.0350	0.2150	0.1578	0.0730	0.1000
H	HN	0.2800	0.2520	0.2480	0.4395	0.2540	0.2500
H	HA	0.1000	0.0480	0.0000	0.0530	-0.0450	0.0000
C	C	0.3800	0.6160	0.5260	0.3964	0.7190	0.5500
O	O	-0.3800	-0.5040	-0.5000	-0.3964	-0.5910	-0.5500
C	CB	-0.3000	-0.0980	0.0310	-0.1590	-0.4500	0.0000
H	HB	0.1000	0.0380	0.0000	0.0530	0.1500	0.0000

[a]From ref. 2. [b]From ref. 3. [c]From ref. 4. [d]From ref. 5&6. [e]From ref. 7. [f]From ref. 8.
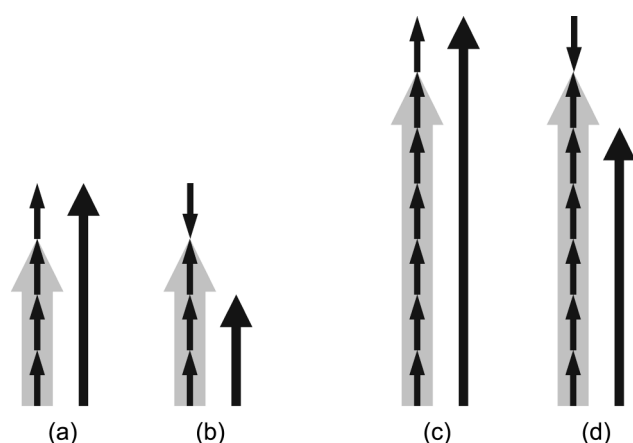
Figure 1. Schematic diagram for the monodipole-macrodipole interactions (from ref. 12). The small black arrow indicates the dipole of each alanine monomer (monodipole), the big gray arrow indicates the total dipole moment of the α-helix (macrodipole) in which the one alanine at the N-terminus is excluded while the long black arrow indicates the resulting total dipole moment of the α-helix. As indicated by the orientation of the dipole moment vector, the N-termini are positive (at the top) and while the C-termini are negative (at the bottom). (a) (Ala)$_4$_Nα; a 4-alanine peptide in which all alanines are in the α-helical conformations. (b) (Ala)$_4$_Nβ; the same as (Ala)$_4$_Na except the alanine at the N-terminus is in β-sheet conformation. (c) (Ala)$_7$_Nα. (d) (Ala)$_7$_Npβ or (Ala)$_7$_Naβ.

For the CONH$_3$ and NHCH$_3$ groups added at the respective N- and C-termini of the polyalanines used, the same point charges for NH and CO of alanine in Table 1 were used for NH of NHCH$_3$ and CO of CONH$_3$ and equal charges are assigned for the four atoms of the remaining CH$_3$ to make neutral the whole NHCH$_3$ and CONH$_3$ groups.
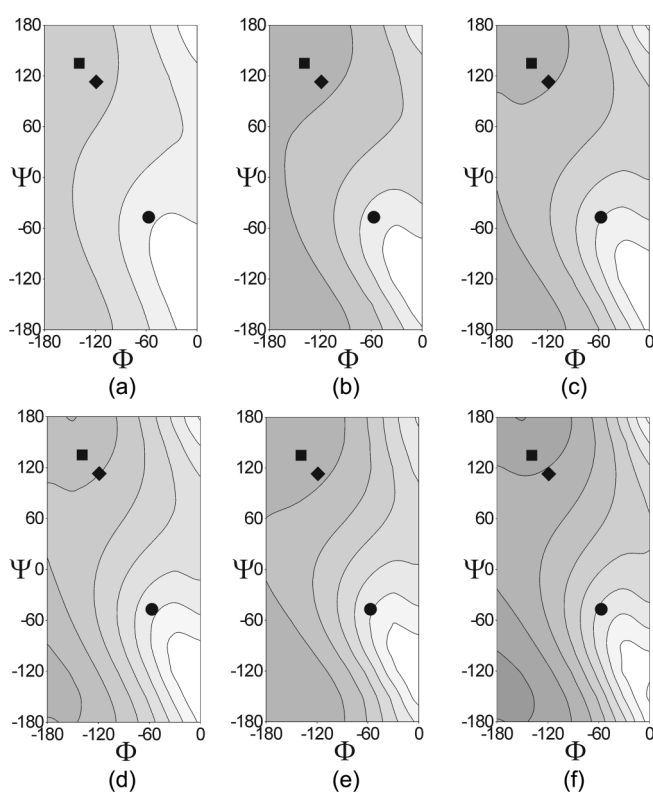
The additional effect of three alanines added at the C-terminus of the (Ala)$_4$_N to make (Ala)$_7$_N can be calculated in three steps as explained in our previous report.[12]

i) The MM electrostatic energies for (Ala)$_4$_N are calculated by changing the torsional angles of the last alanine at the N-terminus keeping all the other alanines fixed in the α-helical conformation.

ii) The same procedure for MM electrostatic energies for (Ala)$_7$_N.

iii) Calculate the differential energies between the MM electrostatic energies for (Ala)$_4$_N and (Ala)$_7$_N.

The MM calculations were carried out for torsional angles of $\phi = -180°$ to $0°$ and $\psi = -180°$ to $180°$ with increments of $30°$, leading to 91 points. We also considered three additional conformations corresponding to α-helix (α: $\phi = -57°$ and $\psi = -47°$), parallel β-sheet (pβ: $\phi = -119°$ and $\psi = 113°$), and antiparallel β-sheet (aβ: $\phi = -139°$ and $\psi = 135°$).

Figure 2. Conformational surfaces for the amino terminus of (Ala)$_4$_N and (Ala)$_7$_N from gas phase HF/6-31G** calculations (from ref. 12). The α-helix ($\phi = -57$, $\psi = -47$; indicated by ●), the parallel β-sheet ($\phi = -119$, $\psi = 113$; indicated by ◆), and the antiparallel β-sheet ($\phi = -139$, $\psi = 135$; indicated by ■) conformations are shown on each map. The bright region indicates stable conformations and the dark region indicates unstable conformations. The contour spacing is 1.0 kcal/mol. (a) The total energy of gas phase (Ala)$_4$_N. (b) The total energy for gas phase (Ala)$_7$_N. (c) The energy difference between gas phase (Ala)$_4$_N and (Ala)$_7$_N. (d) The total dipole moment of gas phase (Ala)$_7$_N. The contour spacing is 2.0 Debye.

Figure 3. Same as for Figure 2c except the classical electrostatic energies using the point-charge systems in Table 1: (a) CVFF, (b) AMBER1, (c) AMBER2, (d) CFF91, (e) BELLIDO, (f) CHARMM. The contour spacing is 0.5 kcal/mol.

Results

Figure 3 shows the results of MM calculations for the differential electrostatic energies between $(Ala)_4\_N$ and $(Ala)_7\_N$ for various ϕ and ψ torsional angles of the alanine at the N-terminus with the structure of the remaining residues fixed in an α-helical conformation. All maps are in a shape similar to the QM results of a polyalanine case in Figure 2c, but the relative energy difference between the minimum and maximum on each map are different from each other. These results indicate that all point-charge system produce the monodipole-macrodipole interactions though they failed to reproduce the QM results.

All Maps in Figure 3 seem to have minima and maxima at the same conformations of ϕ and ψ angles. However, the

energy gradients between them are different each other, resulting different relative differential energies between α versus pβ and between α versus aβ. Table 2 shows that from QM calculations the energy difference between α versus pβ in Figure 2c is 3.96 kcal/mol and 3.90 kcal/mol between α versus aβ.[12] However, all MM results show that the differential energy differences are between 1.08 and 2.28 kcal/mol for α versus pβ, and between 1.00 and 2.05 kcal/mol for the α versus aβ. The results indicate that the point-charge system used in MM calculations reproduce the monodipole-macrodipole interaction from about 30% to 60% of the QM results depending on the kinds of the point-charge systems.

The point-charge systems of CAHRMM and BELLIDO show the best monodipole-macrodipole interactions (Table 2): for CHARMM the differential energies are 2.28 kcal between α versus pβ and 2.05 kcal/mol between α versus aβ, for BELLIDO 2.21 kcal/ between α versus pβ and 2.04 kcal/mol between α versus aβ. The worst case is CVFF which shows 1.08 kcal for the differential between α versus pβ and 1.00 kcal/mol between α versus aβ.

Each map in Figure 4 shows the dipole moments of $(Ala)_7\_N$ for each point-charge system used for the MM calculations. All maps show qualitatively the same pattern with the same locations of minima and maxima. Considering the differential energies and differential dipole moments in Table 2 together, the system producing best differential dipole moment creates the best differential energies and vice versa, showing quite a good correlation between them. This indicates that the monodipole-macrodipole interactions deeply relate to the differential dipole moments of the model systems of polyalanines because they are from the electrostatic interactions between the dipole moments within α-helices.

Discussion

QM calculations effectively including the solvation energy show that the α-helical conformation is selectively stabilized by the solvent effects relative to the β-sheet conformation,[18] which make it possible for α-helices to be formed in hydrophilic environment, like in water. However, this conflicts with the fact that most α-helices found in proteins are unstable in water by themselves implying that most α-helices are not formed in water.

Table 2. Comparison of the differential electrostatic energies and differential dipole moments from the MM electrostatic energy calculations for the amino terminus alanine having an α-helix or β-sheet conformation

	CVFF	AMBER1	AMBER2	CFF91	BELLIDO	CHARMM	QM[e]
ddE_(α-pβ)[a]	-1.00	-1.28	-1.50	-1.88	-2.04	-2.05	-3.60
ddE_(α-aβ)[b]	-1.08	-1.37	-1.60	-2.09	-2.21	-2.28	-3.96
dD_(α-pβ)[c]	5.47	6.08	6.53	7.23	8.02	7.63	8.10
dD_(α-aβ)[d]	5.50	6.04	6.62	7.52	8.03	8.17	8.80

[a]ddE_(α-pβ) = dE_(α) - dE_(pβ), where dE_(x) is the differential energy between $(Ala)_7\_Nx$ and $(Ala)_4\_Nx$. [b]ddE_(α-ab) = dE_(α) - dE_(aβ), negative ddE indicates that α-helical conformation has stronger dipole-dipole interaction than the β-sheet conformations. [c]dD_(α-pβ) = D_(α) - D_(pβ), where D_(x) is the total dipole moment of $(Ala)_7\_Nx$. [d]dD_(α-aβ) = D_(α) - D_(aβ). [e]From ref. 12. *The detailed atomic point-charge information for the charge systems used in the calculations is in Table 1.
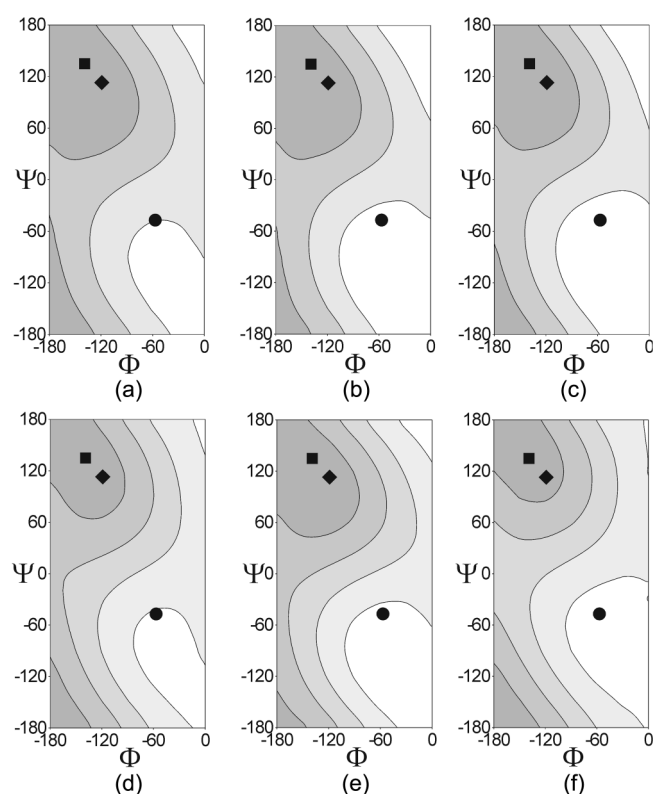
Figure 4. Same as for Figure 2d except the total dipole moment of (Ala)$_7$_N using the point-charge systems in Table 1: (a) CVFF, (b) AMBER1, (c) AMBER2, (d) CFF91, (e) BELLIDO, (f) CHARMM. The contour spacing is 2.0 Debye.

Many studies have been carried out for the interactions between the dipole moment of α-helices and the functional groups added at the end of α-helices. α-helices have dipole moment along their axes by the appropriate alignment of NH and CO functional groups of each residue consisting α-helices.[19,20] Our recent QM calculations showed that monodipole-macrodipole interactions within α-helices stabilize α-helices.[12] This implies that α-helices can be formed even in hydrophobic environment like inside of proteins. The results also showed that the monodipole-macrodipole interaction increases with the length of the α-helix, explaining why the process of α-helix formation is cooperative.

Now we studied this monodipole-macrodipole interaction within α-helices using six point-charge systems using the same model systems as used in the previous QM calculations.[12] The similarities among the maps in Figure 3 and the map of Figure 2c indicate that the point-charge systems all produce the monodipole-macrodipole interactions within α-helices because of the appropriate alignment of NH and CO functional groups in α-helices. However, as the charges assigned to atoms change according to the point-charge system used, the contribution of the monodipole-macrodipole interactions are quantitatively different from system to system though they look similar qualitatively.

The maps for the dipole moments of (Ala)$_7$_N in Figure 4

are quite alike each other and also look similar to Figure 2d from QM calculations. However, Table 2 shows that there are some discrepancies among the point-charge systems for both the total and differential dipole moments. Careful examination of the differential energies and the differential dipole moments in Table 2 makes it clear that there exists a well correlation between them, confirming our previous results that the electrostatic energies from the interactions between monodipole moments within α-helices (as illustrated in Figure 1) dominate the differential energies.[12]

The monodipole-macrodipole interactions within α-helices does not affect the folding time of α-helices substantially because the rate-determining step for the formation of α-helices is the formation of the first turn in α-helices when the dipole-dipole interactions are still very small. However, the effects of the dipole-dipole interaction become greater with the length of α-helices and may play some important role in the elongation processes of α-helices. If the monodipole-macrodipole interactions in α-helices are included correctly, α-helices become more stable, which results less frequent occurrence of folding and unfolding of helix segments shown at both ends of an α-helix during the folding and unfolding process of the whole α-helix.[21]

Our previous results showed that in water the neutralization effects of water molecules reduce the contribution of the dipole-dipole interactions in α-helices.[12] However, in hydrophobic environment like inside of proteins where the solvent effect is little, the contribution may become large enough to alter the final structure of proteins. In the early stage of protein folding the environment inside of a protein is quite different to that of the final structure and is known to be more like the gas phase.[22-24] Because the nucleation of α-helices is very fast and occurs in the early stage of protein folding before the hydrophobic collapse,[25] the monodipole-macrodipole interactions within α-helices may play some crucial roles affecting the final shapes of proteins.

Margulis *et al.* showed that different force fields produced different results in their molecular dynamics simulations about the folding and unfolding processes of α-helices.[26] This indicates that force fields have to be improved to produce consistent results irrespective to the kinds of force fields used in MM calculations. Force fields are usually developed based on small molecules by optimizing their parameters according to the QM results on the same molecules. However, this leaves the possibility that the parameters optimized using interactions occurring within short distances may not work for the systems where long distance interactions are important. Therefore, the new generation force fields should also consider the long distance interactions, mostly the electrostatic interactions, in addition to the short distance interactions. In this respect, our results will provide a good way in checking and improving force fields, especially the point-charge systems.

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