Efficiency of Antimicrobial Defense: Molecular Flexibility of Natural Defensin and Artificial *Bis*-quaternary Ammonium Compound

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Abstract. *Background: Human α-defensins (such as HD5 and HD6) are typical bactericidal peptides. We examined the molecular features of HD5 and HD6 by molecular dynamics (MD) analysis. Materials and Methods: Molecular features of natural defensins and artificial bis-quaternary ammonium compounds (e.g. 1,6-polymethylenedithio)bis(1-octylpyridinium iodide: 4DTBP-m,8) were analyzed using molecular simulation techniques. Results: HD5 and HD6 had different electrostatic potential profiles, which indicated the region-dependent hydrophobicity. 4DTBP-m,8 derivatives were significantly flexible, and many conformers existed. Conclusion: HD5 and HD6 indicated antimicrobial activity by restricted conformation.*

Natural antimicrobial peptides assume an important role in the innate immune system (1), varying in structure, size, constitution (amino acid sequence) and antimicrobial activity (2, 3). These peptides have been preserved in many species, and exist in the skin, neutrophilic leukocytes, and intestines, *etc*., acting as a first-line defense against attack. Defensins are known as typical bactericidal peptides, forming a large family of antimicrobial peptides. Human defensins are cysteine-rich, cationic peptides, and their molecular masses are 3-5 kDa. Intestinal Paneth cells synthesize α-defensin 5 and 6 (HD5 and HD6), which are natural immunity factors (4-6). HD5 and HD6 consist of 32 amino acids, have six conserved cysteine residues and form three intramolecular S–S bridges. Both HD5 and HD6 are known as natural defense factors against microbial attack.

In the present study, we analyzed the molecular features (*e.g*. electrostatic potential (ESP) fields, molecular dynamics (MD), and stereo-hydrophobicity) of HD5 and HD6. Most antimicrobial peptides, including HD5 and HD6, are cationic and affect a broad range of microorganisms by disruption of negatively charged cytoplasmic membranes (7). The synthesized antimicrobial *bis*-quaternary ammonium compounds [*bis*-QACs, *e.g*. 4,4'-(1,6-hexamethylenedithio) *bis*(1-octylpyridinium iodide)(4DTBP-6,8)] are cationic surfactants (8, 9). The flexibility of defensin molecules was compared with that of *bis*-QAC, and the molecular flexibility and defense efficiency of the natural defensins and artificial molecule *bis*-QAC were considered.

Materials and Methods

Molecular analysis of defensin. The X-ray data sets of α-defensin 5 (HD5: ID=1ZMP) and 6 (HD6: ID=1ZMQ) were obtained from the RCSB protein data bank. The hydrogen atoms were added to these molecules at pH 7.0. ESP fields and MD of HD5 and HD6 were analyzed using InsightII-discover (Accelrys Inc., USA) (10, 11). The HD molecule was divided into 10 groups every 6 amino acids, and the stereo-hydrophobicity (dGW) of each group was examined using MOPAC (Fujitsu Ltd., Japan).

Analysis of bis-QACs features. The initial structure of 4DTBP-m,8 was constructed using CAChe (Fujitsu Ltd., Japan). The conformation analysis of 4DTBP-m,8 was performed using CONFLEX with MM2 forcefield. The minimal inhibitory concentration (MIC) of 4DTBP-m,8 derivatives for *Escherichia coli* K12 W3110 was measured according to a standard dilution method described previously (12).

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Key Words: Conformation, antibacterial activity, hydrophobicity, bis-quarternary compound.

Results

Molecular features of HD5 and HD6. The HD5 molecule had a negatively charged ESP field (an index of interaction), in *N*- and *C*-terminal areas, as shown in Figure 1A and 1B. HD6 had a negative ESP field at *N*- and *C*-terminals, and another area was occupied by a positive ESP field (Figure 1C). The ESP field distribution was quite different between HD5 and HD6 molecules.

MD analysis of HD5 and HD6 was performed, and the total energy (=kinetic energy + potential energy) during the simulation period (500 ps) of HD6 (average=1046.5 kcal/mol) was greater than that of HD5 (average=726.2 kcal/mol) (Figure 2A *versus* D). The kinetic energies of HD5 (average=431.2 kcal/mol) and HD6 (average=429.5 kcal/mol) were almost equal (Figure 2B and E). The total energy difference between HD5 and HD6 was derived from the difference in potential energy between HD5 (average=295.0 kcal/mol) and HD6 (average=617.0 kcal/mol) (Figure 2C *versus* F). HD5 and HD6 molecules had three S–S bonds, which kept the HD molecules rigid. Indeed, the structures of HD5 and HD6 significantly collapsed in MD analysis when three intramolecular S–S bridges had been broken (Figure 3).

HD5 and HD6 molecules were divided from the *N*-terminal into 10 regions every 6 amino acid residues, and MO analysis of each region (region 1-10) was performed (Figure 3). *N*- and *C*-terminal areas (regions 2, 9 and 10) of HD5 were more hydrophobic than other regions (*e.g.* central regions 3-6) (Figure 4A). In the HD6 molecule, the *N*-terminal regions 2 and 3 and *C*-terminal regions 9 and 10 were more hydrophobic, similar to HD5 (Figure 4D). The dipole moments (an index of reaction direction) of HD5-derived regions were different from each other (Figure 4B). HD6-derived regions had various dipole moment directions, as well as HD5 moments (Figure 4E). In region 7 of both HD5 and HD6, dipole moment intensity (an index of reaction strength) was weaker than that of other regions (Figure 4C and F).

Molecular features of bis-QACs. Antibacterial compound 4DTBP-m,8 (m=3, 4, 6, 8, 10) exhibited flexible behavior in conformation analysis, and 786-2349 conformers were detected (Table I). The MIC for *E. coli* for these derivatives is also shown. The conformer energy of these five 4DTBP-m,8 derivatives ranged from −1.302 to 15.191 kcal/mol, significantly lower than the MD total energy of HD5 and HD6 (Figure 2A and D). 4DTBP-m,8 conformers had conformation-dependent hydrophobicity. Appropriate conformers of these 4DTBP-m,8 derivatives exhibited antibacterial activity because of their adequate hydrophobicity (8, 9). Thus, synthesized *bis*-QACs had many conformations, but the expression efficiency of the antibacterial conformer was comparatively low.

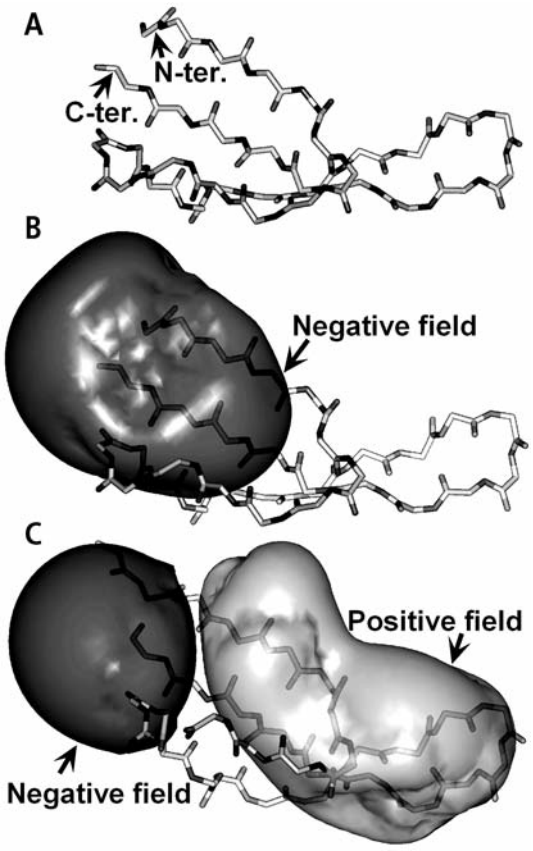

Figure 1. *Electrostatic potential (ESP) field of HD5 and HD6. Frame model of HD5 (A). N-, and C-terminal are indicated by arrows. ESP field of HD5 (B) and HD6 (C). Negative and positive fields are shown as dark-gray and light-gray clouds, respectively.*

Discussion

HD5 and HD6 molecules secreted from paneth cells had a different ESP profile (Figure 1), and it was thought that they would have different ESP-related reactivity to external factors. This is thought to be one example of the living body having two or more defense systems. In MD analysis, the potential energy (an index of response) of HD6 was larger than that of HD5 (Figure 2C and F), and it is thought that HD6 can more quickly react to an external factor than HD5 can. The rapidity of HD6 might be better than HD5 in the interception of external attack, and HD6 would thus seem to have responsibility for primary intervention against external attack. Moreover, the directionality of HD5 and HD6 against external attack is different because of the difference in their dipole moment direction profiles (Figure 4B and 4E). In both HD5 and HD6, the dipole moment intensity in region 7 was smaller than that in other regions (Figure 4C and F), and it was thought that the reactivity to external factors in region 7 was weaker than in other regions. The hydrophobicity of regions 2, 9 and 10 of HD5 was higher than

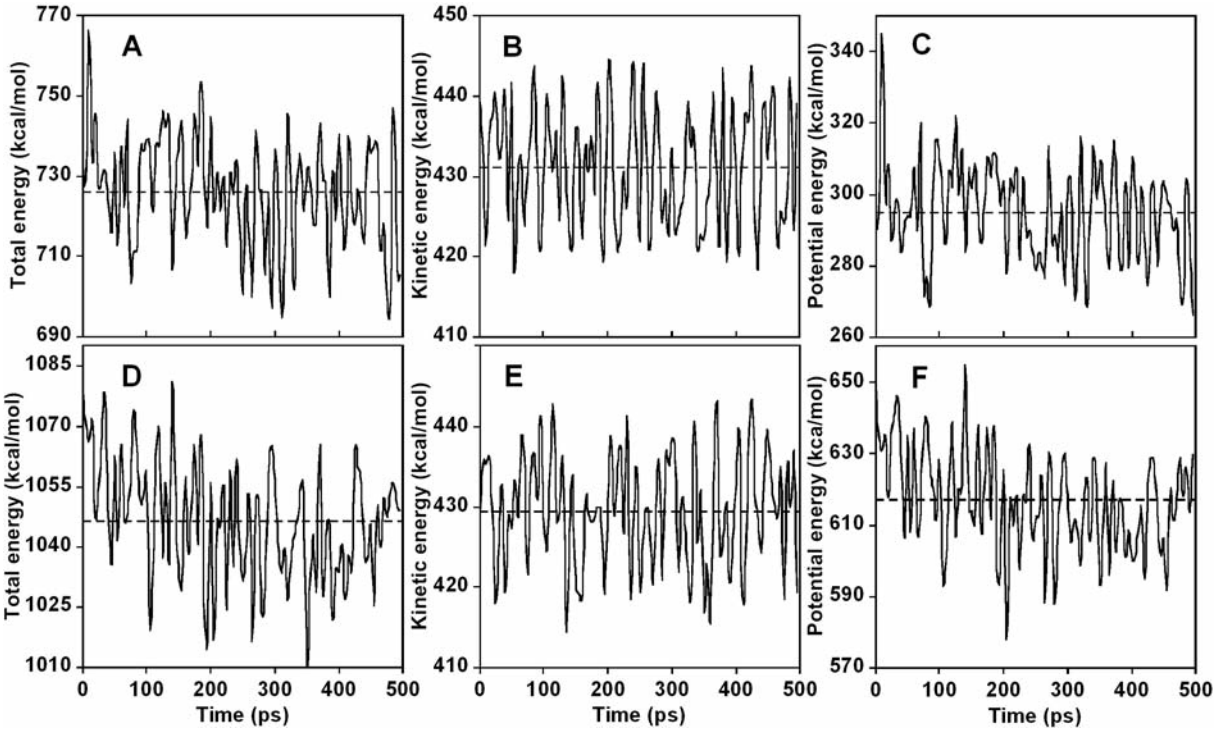

Figure 2. *Molecular dynamics (MD) energy of HD5 and HD6. Total energy of HD5 (A) and HD6 (D). Kinetic energy of HD5 (B) and HD6 (E). Potential energy of HD5 (C) and HD6 (F). Dashed lines indicate the average of each energy.*

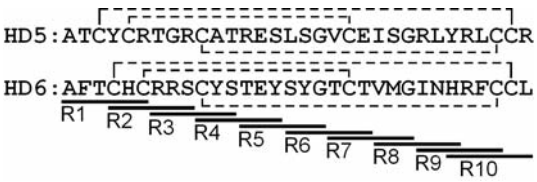

Figure 3. *Amino acid sequences of HD5 and HD6. Dashed lines indicate the S–S bridges. R1–R10 indicate the regions divided for molecular orbital (MO) analysis.*

Table I. *4DTBP-m,8 conformers. Conformation analysis of 4DTBP-m,8 derivatives were performed using CONFLEX. Effect of alkyl-bridge (m) length on minimal inhibitory concentration (MIC) in E. coli K12 W3110 was examined. These results were summarized from reference 8.*

Compound	Conformer	MIC (μM)
4DTBP-3,8	951	6.5
4DTBP-4,8	786	3.3
4DTBP-6,8	1125	1.5
4DTBP-8,8	1589	3.3
4DTBP-10,8	2349	2.1

in other regions. In the HD6 molecule, regions 2, 3 and 9, 10 were more hydrophobic than other regions. Thus, the *C*-terminal of HD5 and HD6 were hydrophobic. In the *N*-terminal region, HD6 (regions 2, 3) was comparatively more hydrophobic than HD5 (region 2). From the MD potential energy, ESP field and hydrophobicity profile of HD5 and HD6, these HD molecules should offer different defense systems (including timing, direction and strength) against external factors. Moreover, the HD molecule had three S–S bridges, which conferred molecular flexibility. Because of the existence of these S–S bridges, it was difficult for the HD molecule to have many conformations; however, HD responds to various external factors by restricting conformation, and the high biophylaxis efficiency of this natural defense system is impressive.

We had reported that the flexibility of *bis*-QACs depended on the structure of the bridge portion (8). *Bis*-QACs have many conformations, but supply few appropriate conformers which have effective antibacterial activity. The flexibility of both HD5 and HD6 molecules is inferior to those of *bis*-QACs, but these HD molecules can efficiently produce conformers that have antibacterial activity. A molecular feature of HD is thought to be one example of the good efficiency of antimicrobial substances that exist naturally. We are now examining the correlation between the molecular flexibility and the efficient activity of natural immunity-related molecules (such as HD5 and HD6).

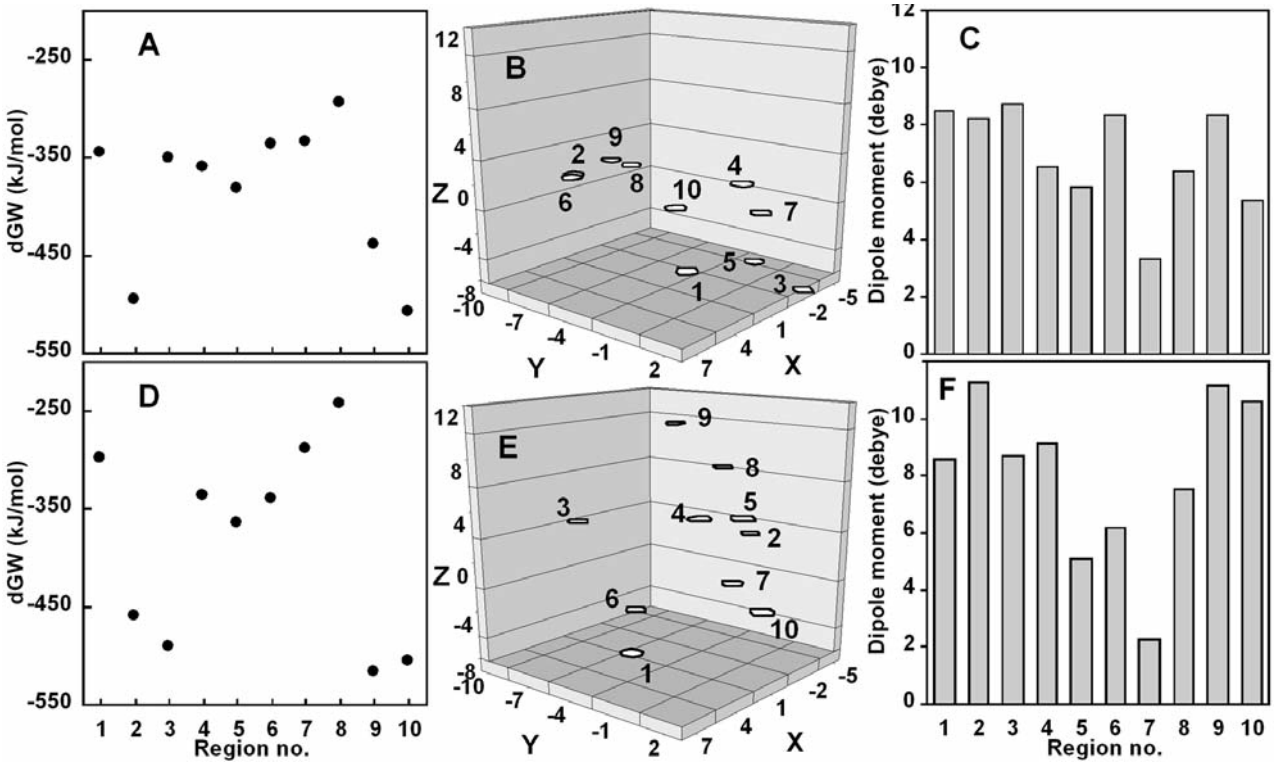

Figure 4. *Molecular profiles of HD5 and HD6. Stereo-hydrophobicity of MO analyzed regions (R1–R10 in Figure 3) of HD5 (A) and HD6 (D). Dipole moment direction of MO analyzed regions (R1–R10) of HD5 (B) and HD6 (E). Numbers in B and E indicate the analyzed regions (R1 – R10). Dipole moment intensity of analyzed regions (R1–R10) of HD5 (C) and HD6 (F).*

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