

Profiles of ILY, VLY and Sm-hPAF Interaction with Human CD59

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Abstract. *Background:* The molecular features of a new member of the bacterially -derived cytolysin family were examined. In particular, the interactive mechanisms of intermedilysin (ILY), vaginolysin (VLY), and *Streptococcus mitis*-derived human platelet aggregation factor (Sm-hPAF) with human CD59 (hCD59) were analyzed. *Materials and Methods:* Molecular models of VLY and Sm-hPAF were constructed based on X-ray data of ILY (protein data bank ID=1S3R), and their interactive profiles with hCD59 were examined using molecular simulation. *Results:* Non-binding (NB) energy between ILY and hCD59 was three orders of magnitude higher than the energy between VLY and hCD59. NB energy between Sm-hPAF and hCD59 was similar to that between VLY and hCD59. *Conclusion:* A hydrogen bond (ILY Arg432-hCD59 Glu76) was observed between ILY and hCD59, and a stronger interaction was formed by flexible adjustment between them.

Some bacterially-derived cytolytic toxins form clusters on the target cell membranes and lyse cells by pore formation. These toxins bind to cell membrane cholesterol as a receptor and are called cholesterol-dependent cytolysins (CDCs). CDCs are produced by various bacterial strains [e.g. *Clostridium perfringens*, perfringolysin O (PFO); *Streptococcus pneumoniae*, pneumolysin (PLY); *Streptococcus pyogenes*, streptolysin O (SLO); *Listeria monocytogenes*, listeriolysin O (LLO)], and form the CDC family (1-5).

Streptococcus intermedius-derived intermedilysin (ILY) exhibits human-specific cell lysis but not cholesterol-

dependent cell-lytic activity (6, 7). It is thought that ILY binds selectively to human CD59 (hCD59) as a receptor protein (8). Recently, *Gardnerella vaginalis*-derived vaginolysin (VLY) and *Streptococcus mitis*-derived human platelet aggregation factor (Sm-hPAF) were reported as new types of cell-lytic toxins (9, 10). VLY is thought to be the offending bacteria in preterm birth. VLY has homology of 55% or more to ILY and exhibits human-specific cytolytic activity. Sm-hPAF has four ILY-like domains (domains 1-4) and an external domain (domain 0), and its cholesterol dependency is significantly weaker than that of traditional CDCs. Sm-hPAF is a multifunctional factor and has platelet-aggregatory and cell-lytic activity. These toxins exhibit similar behavior in cell lytic analysis using human erythrocytes and seem to interact with hCD59 as well as ILY. In the present study, we examined the profiles of interaction between these cytolysins (namely ILY, VLY, Sm-hPAF) and hCD59. We previously analyzed the 11mer region of these cytolysins and report on the different features of the 11mer region of ILY (type A), CDC (type C), VLY (type D), and Sm-hPAF (type D) (11). In the present study, the behavior of the 11mer neighborhood region in the interaction between cytolysins and hCD59 was analyzed.

Materials and Methods

Molecular modeling of cytolysins. Molecular models of VLY and Sm-hPAF were constructed using X-ray data of ILY (protein data bank ID=1S3R) as template, as previously described (12). The molecular structures of ILY, VLY, and Sm-hPAF were overlapped at the same position in three-dimensional coordinates, and the interaction with hCD59 was analyzed.

Interactive analysis with hCD59. Non-binding energies (index of molecular interaction) and electrostatic potential fields between hCD59 (protein data bank ID=2J8B) and modeled cytolysins were examined using the insightII-discover (Accelrys Inc., San Diego, CA, USA). The region features of the 11mer [dipole moment, solvation free energy (dGW)] of cytolysins were analyzed using MOPAC (Fujitsu Inc., Tokyo, Japan) as previously described (13, 15).

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Table I. *Nonbinding-energy between intermedilysin (ILY), vaginolysin (VLY), Streptococcus mitis-derived human platelet aggregation factor (Sm-hPAF) and human CD59.*

	ILY	VLY	Sm-hPAF
Vdw (kcal/mol)	234199	433.3	351.9
Elect (kcal/mol)	-33.8	-227.8	-238.7
Total (kcal/mol)	234165	205.5	113.2

Vdw: Van der Waals energy, Elect: electric energy, Total: total energy=Vdw energy + Elect energy.

Results

Non-binding energy between ILY, VLY, Sm-hPAF and hCD59. The van der Waals (Vdw) energy (index of attracting force) between ILY and hCD59 was 234,199 kcal/mol, significantly larger than that between VLY (433.3 kcal/mol), Sm-hPAF (351.9 kcal/mol) and hCD59 (Table I). The electrical energy between ILY and hCD59 was -33.8 kcal/mol, also larger than that between VLY (-227.8 kcal/mol), Sm-hPAF (-238.7 kcal/mol) and hCD59. Attractive force was generated between VLY, Sm-hPAF and hCD59, although it was weaker than that between ILY and hCD59.

Profiles of interaction between cytolysins and hCD59. The Arg451, Tyr436, Tyr434, Tyr460, Arg432 amino acid residues of the ILY molecule are located at the point of interaction with the hCD59 molecule (Figure 1A). The hydrogen atom of ILY Arg432 formed a hydrogen bond with the oxygen atom of hCD59 Glu76. Tyr460 of ILY domain 4 existed at a good position of regulating the interaction with hCD59. The expansion of the positive electrostatic potential (ESP) field at ILY domain 4 and negative ESP field at a lower area of hCD59 was observed. The ILY 11mer region was located outside the ESP field and did not interact directly with hCD59.

In interactive analysis between VLY and hCD59, two hydrogen bonds were observed. The Lys447 (nitrogen atom) and Arg419 (hydrogen atom) of VLY domain 4 formed a hydrogen bond with Tyr62 (hydrogen atom) and Glu76 (oxygen atom) of hCD59, respectively (Figure 1B). A positive ESP field at VLY domain 4 and negative ESP field at a lower area of hCD59 were observed, as well as the interaction between ILY and hCD59. In Sm-hPAF domain 4, Lys403 (nitrogen atom) and Arg375 (hydrogen atom) formed a hydrogen bond with hCD59 Tyr62 (hydrogen atom) and Glu76 (oxygen atom), respectively (Figure 1C). Positive and negative ESP fields were observed at Sm-hPAF domain 4 and lower area of hCD59. 11mer regions of VLY and Sm-hPAF were located outside the positive ESP field and moved freely.

Table II. *Dipole moment intensity and solvation-free energies of 11mer regions.*

	Dipole moment (debye)	dGW (kJ/mol)
ILY	16.153	-871.7
VLY	52.023	-1286.2
Sm-hPAF	52.259	-1292.8

ILY: Intermedilysin; VLY: vaginolysin; Sm-hPAF: *Streptococcus mitis*-derived human platelet aggregation factor.

11mer region profile in interaction with hCD59. The direction of the dipole moment of the ILY 11mer region was to the upper left (Figure 2) and was directed toward the positive electrostatic potential field at ILY domain 4 (Figure 1). This ILY moment intensity was 16.153 debye (Table II). Dipole moment directions of VLY and Sm-hPAF were to the lower left (Figure 2), and were directed to the outer side of domain 4 (Figure 1). The dipole moment intensities of VLY and Sm-hPAF were 52.023 and 52.259 debye, respectively. Solvation free energy (stereo-hydrophobicity dGW: lower value means higher hydrophobicity) of the ILY 11mer region was -871.7 kJ/mol, higher than those of VLY (-1286.2 kJ/mol) and Sm-hPAF (-1292.8 kJ/mol) (Table II).

Discussion

The Asp22, Phe23, Phe47 residues of hCD59 are located at unrelated positions to the direct interaction with ILY; however, amino acid mutation of these hCD59 residues was found to reduce the reactivity to ILY (16), and they seem to regulate the interaction between ILY and hCD59. Indeed, electrostatic repulsion by a positive ESP field was observed between ILY and the upper region of hCD59 (Figure 1A). One hydrogen bond (ILY Arg432-hCD59 Glu76) was observed between ILY and hCD59, and these molecules interacted flexibly. On the other hand, two hydrogen bonds (VLY Arg419-hCD59 Glu76, VLY Lys447-hCD59 Tyr62) were observed between VLY and hCD59 in molecular dynamics (MD) analysis (Figure 1B), and these molecules interacted rigidly. From these findings, we consider that the elaborate fitting of VLY with hCD59 was consequently disturbed compared with ILY, and the non-binding energy was thought to be three order smaller than the interaction between ILY and hCD59. In interactive analysis of Sm-hPAF with hCD59, two hydrogen bonds (Sm-hPAF Arg375-hCD59 Glu76, Sm-hPAF Lys403-hCD59 Tyr62) were observed, and a similar non-binding energy profile and ESP field profile was obtained (Figure 1C).

CDC interacted with cholesterol in the 11mer region, and the cholesterol dependency of CDC was suppressed by mutation of the 11mer region. CDC 11mer substitution to

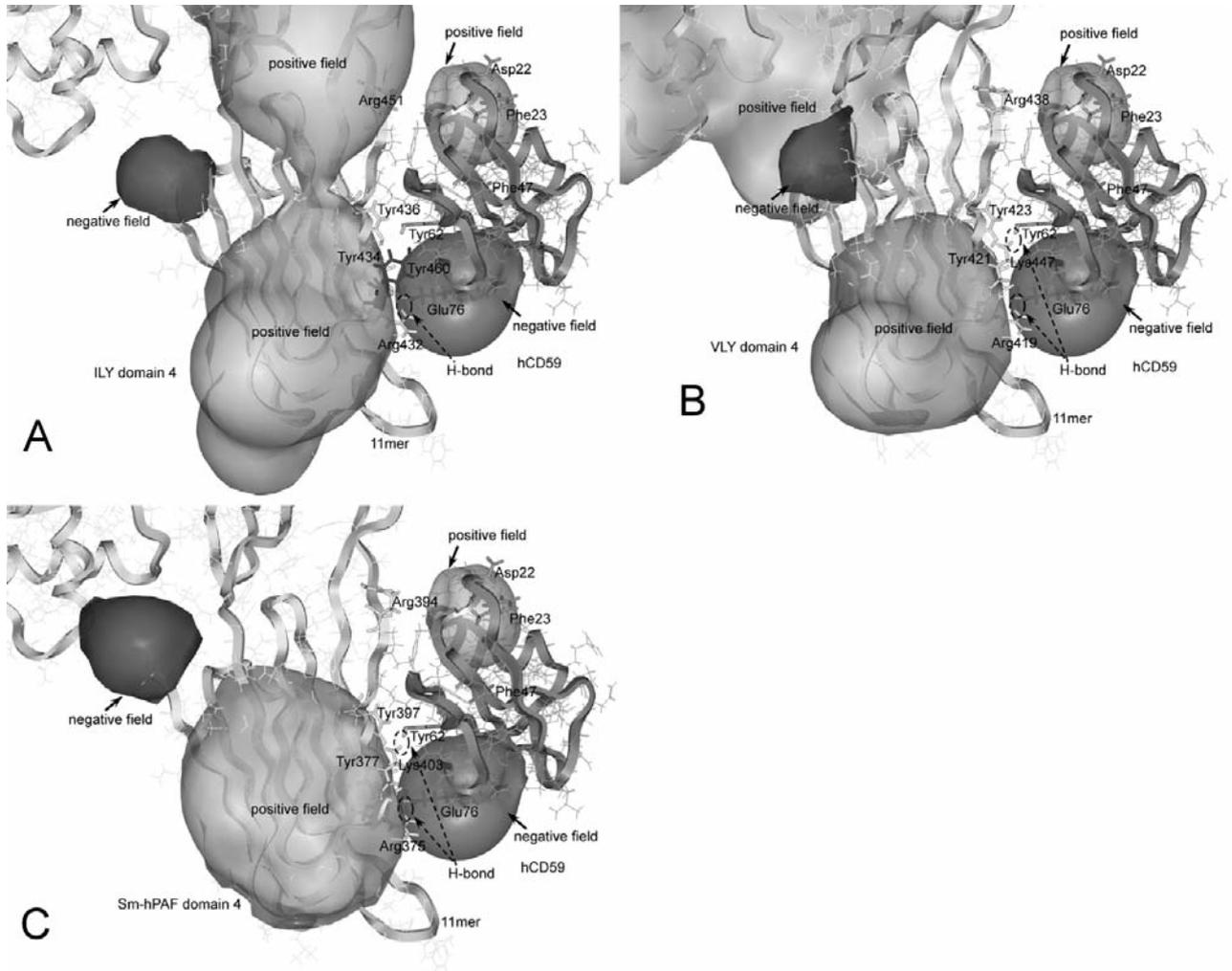


Figure 1. Profiles of interaction between cytolysins and hCD59. Electrostatic potential field of intermedilysin (ILY) (A), vaginolysin (VLY) (B), and *Streptococcus mitis*-derived human platelet aggregation factor (Sm-hPAF) (C) domain 4 and hCD59 are shown as light gray (positive charged field) and dark gray clouds (negative charged field).

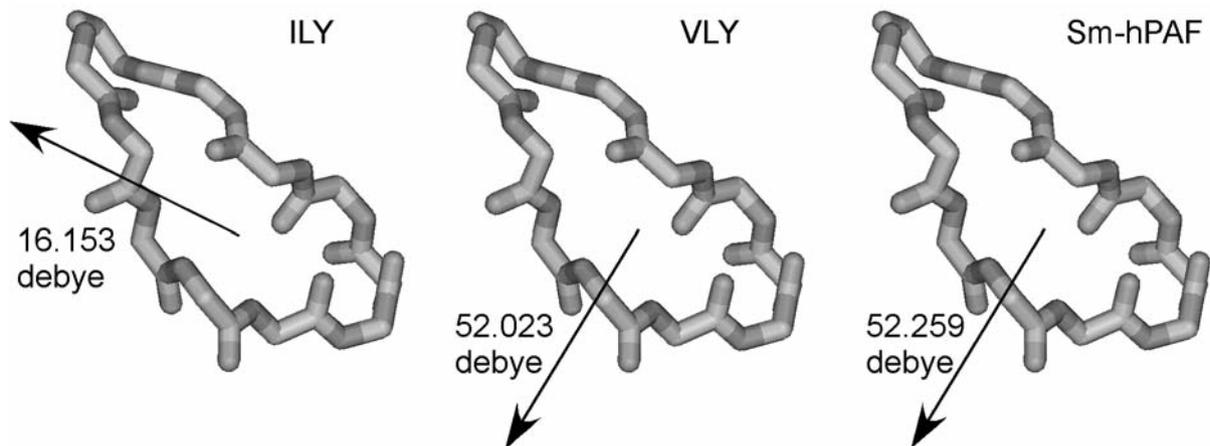


Figure 2. Dipole moment profiles of 11mer regions of cytolysins. Dipole moment direction (arrow) and intensity of intermedilysin (ILY) (16.153 debye), vaginolysin (VLY) (52.023 debye), and *Streptococcus mitis*-derived human platelet aggregation factor (Sm-hPAF) (52.259 debye) are shown.

ILY type-11mer resulted in cholesterol-independent cytolytic activity. In molecular dynamics simulation, 11mer substitution from the CDC type to ILY type caused a significant change in reactivity with cholesterol. The 11mer region of ILY moved freely during interactive analysis with hCD59 and did not interact directly with the receptor molecule (*i.e.* hCD59).

In interactive analysis between CDC and cholesterol, the 11mer region directly interacted with cholesterol, and the 3-hydroxy group of cholesterol was observed to be a key structure in their interaction (11). Structural features of cholesterol-dependent/independent cytolysin are involved in molecular pathogenesis and infectious disease therapy. We are now performing more detailed interactive analysis of these cytolysins with hCD59. Moreover, it is interesting how cytolysins with new cholesterol-independent activity, such as Sm-hPAF and VLY, have appeared.

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