Molecular Profiles of Cholesterol-dependent Cytolysin Family-derived 11mer Regions

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Abstract. *Background: Cholesterol-dependent cytolysins (CDCs) are secreted from various types of bacteria and are involved in various diseases (e.g. abscess formation). Traditional CDCs has a conserved 11mer region, which is a key structure in membrane recognition. Materials and Methods: Based on the X-ray data of traditional CDC perfringolysin O (PFO), molecular models of intermedilysin (ILY), pyolysin (PLO), vaginolysin (VLY), and Streptococcus mitis-derived human platelet aggregation factor (Sm-hPAF) were constructed. The 11mer regions of these models were extracted, and their molecular features were analyzed. Results: The dipole moments of these 11mer regions were classified into four types, and their stereo-hydrophobicity (dGW) was different. It was thought that these results influenced the species specificity and membrane recognition of each cytolysin. Conclusion: Traditional CDCs, ILY, PLO, and VLY consisted of four domains (domains 1 to 4). Domain 0 existed on the N-terminal side in Sm-hPAF in addition to these four domains. The 11mer sequence of Sm-hPAF is the same as that of VLY, but Sm-hPAF has slightly different characteristics (e.g. species specificity, membrane recognition, cholesterol dependency) compared to VLY. Dynamic structure analysis of domain 0 might clarify these differences.*

Some bacteria-derived cytolytic toxins, such as perfringolysin O (PFO), pneumolysin (PLY), streptolysin O (SLO), listeriolysin O (LLO), alveolysin (ALV), and suilysin (SLY) form clusters on target cell membranes and lyse cells by pore formation (1, 2). Their cytolytic activity is cholesterol-dependent, and they are called cholesterol-dependent cytolysins (CDCs). CDCs have been found in various bacterial strains (*e.g. Clostridium perfringens*: PFO, *Streptococcus pneumoniae*: PLY, *Streptococcus pyogenes*: SLO: *Listeria monocytogenes*: LLO, *Bacillus alvei*: ALV, *Streptococcus suis*: SLY). CDC family members have a conserved undecapeptide (11mer) region, the amino acid sequence of which is ECTGLAWEWWR. These traditional CDC 11mer regions play a key role in cell membrane recognition (3-5).

Intermedilysin (ILY) was found to be secreted from *Streptococcus intermedius*, and exhibited human cell-specific cytolytic activity (6). It was confirmed using a mutation experiment of the 11mer region, that the ILY 11mer region has species-specific cytolytic activity (7). *Arcanobacterium pyogenes* synthesized a cholesterol-dependent cytolysin named pyolysin (PLO), which had cholesterol-dependent cytolytic activity, but no human cell specificity (8, 9). Vaginolysin (VLY) was produced by *Gardnerella vaginalis* and is thought to be the offending bacterium in preterm birth (10, 11). VLY has 55% or more homology to ILY and exhibits human-specific cytolytic activity. Moreover, *Streptococcus mitis*-derived human platelet aggregation factor (Sm-hPAF) was found to be a multifunctional factor having not only cytolytic activity but also platelet aggregation activity (12). These cytolysins have a unique 11mer region, which might play a key role in controlling species specificity and cholesterol dependency. In the present study, we constructed molecular models of these cytolysins based on X-ray data of PFO (protein data bank ID=1PFO), and analyzed their 11mer structural features.

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Key Words: Cytolysin, cholesterol, membrane, 11mer region.

Materials and Methods

Structural construction of CDCs. Molecular modeling of target CDCs were performed using the homology modeling technique with the homology module (Accelrys Inc., San Diego, CA, USA), as previously described (13). The X-ray data set of PFO (1PFO)

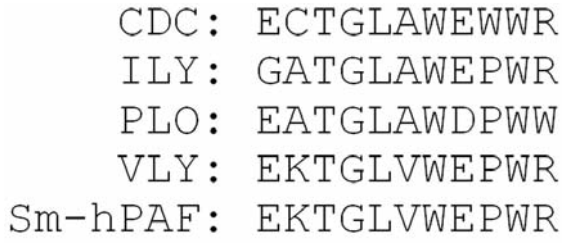

Figure 1. *Amino acid sequences of 11mer regions of examined cytolysins. CDC: Conserved sequence of cholesterol dependent cytolysin; ILY: intermedilysin; PLO: pyolysin; VLY: vaginolysin; Sm-hPAF: Streptococcus mitis-derived human platelet aggregation factor.*

was used as the modeling template. The energy minimization of modeled CDCs was performed using a consistence valence forcefield. The z-matrix data for the 11mer regions of modeled CDCs were extracted from each model, and molecular orbital (MO) analysis was performed with PM3 Hamiltonian using MOPAC (Fujitsu Ltd., Japan) (7). Solvation free energies (dGW) were determined from MO parameters (14).

Results

Molecular features of 11mer regions. Traditional CDCs have a conserved 11mer sequence (ECTGLAWEWWR), and amino acid substitutions have been observed in other types of cytolysins as well (*e.g.* ILY, PLO, VLY, and Sm-hPAF) (Figure 1). Solvation free energy (dGW: lower dGW means higher hydrophobicity), which is an index of structure-dependent hydrophobicity, of these 11mer regions was analyzed (Table I). The dGW values of ILY and PLO 11mer regions were –440.6 and –457.3 kJ/mol, respectively. The range of traditional CDC 11mers (*e.g.* PFO, PLY, LLO, SLO, ALV, SLY) is –554.7 to –448.1 kJ/mol. The dGW of the VLY 11mer region was –490.0 kJ/mol. Sm-hPAF had the most hydrophobic 11mer region (-655.8 kJ/mol) among the tested CDCs.

The dipole moment direction type, which is an index of reactivity, of 11mer regions in the CDCs is shown in Figure 2. The dipole moment of ILY (1.731 Debye) was significantly smaller than that of traditional CDCs (Table I), and the direction faced the intramolecular site, crowded with other amino acid residues (type A in Figure 2). The moment of PLO (2.427 Debye) was smaller than that of traditional CDCs, as well as that of the ILY moment, but it faced the outer side (type B). In traditional CDCs, dipole moment intensity is in the range of 8.028 to 9.022 Debye, and faces the outer side of the molecule (type C). VLY (3.647 Debye) and Sm-hPAF (8.540 Debye) showed the fourth type of dipole moment direction, facing downward and to the left, on the far side of the surface of the space (type D).

Table I. *Structural parameters of 11mer regions. Dipole moment and (HOMO)-(LUMO) parameters were obtained from the data in vacuum condition.*

	dGW: Solvation free energy (kJ/mol)	Dipole moment (Debye)	Moment type	HOMO energy (eV)	LUMO energy (eV)	HOMO–LUMO energy gap (eV)*
ILY	–440.6	1.731	A	–8.081	0.076	8.157
PLO	–457.3	2.427	B	–8.076	0.100	8.176
PFO	–554.7	9.022	C	–8.323	–0.298	8.025
PLY	–448.4	8.028	C	–8.426	–0.181	8.245
LLO	–498.3	8.367	C	–8.316	–0.166	8.150
SLO	–502.3	8.133	C	–8.426	–0.184	8.242
ALV	–448.1	8.297	C	–8.425	–0.181	8.244
SLY	–502.6	8.215	C	–8.416	–0.178	8.238
VLY	–490.0	3.647	D	–7.992	–1.351	6.641
Sm-hPAF	–655.8	8.540	D	–3.559	–1.202	2.358

Intermedilysin (ILY), pyolysin (PLO), perfringolysin O (PFO), pneumolysin (PLY), listeriolysin O (LLO), streptolysin O (SLO), alveolysin (ALV), suilysin (SLY), vaginolysin (VLY), *Streptococcus mitis*-derived human platelet aggregation factor (Sm-hPAF). *The absolute value of HOMO–LUMO energy gap.

Analysis of 11mer region interaction with cholesterol molecule. In interactive analysis of 11mer region-mutated SLO and ILY, the position of the 3-hydroxyl group (3-OH) in the cholesterol molecule influenced non-binding energy (index of the interaction between materials), between cytolysin and cholesterol (7). In the present study, the interaction between the 11mer region and cholesterol was analyzed in various cytolysins, and the results are shown in Table II. The position of the 3-OH group in cholesterol was different in three binding patterns (case 1, 2, 3). When the 3-OH of cholesterol was located near the Glu^{492} of ILY (case 1), the non-binding energy (NBE) between cholesterol and ILY was significantly lower (–2.603 kcal/mol) than that of SLO (3.175 kcal/mol). The dependency on cholesterol is different in ILY and SLO; therefore, it seemed that case 1 was appropriate as the interaction index model. When the ILY 11mer region was mutated to the SLO type [ILY(ECW)], NBE had a positive value (6.462 kcal/mol), as did that for SLO. A positive NBE value means an incorporative relationship. In contrast, the 11mer-mutated SLO [SLO(GAP)], which has an ILY type 11mer, had a negative NBE value (–2.593 kcal/mol), as did that for ILY. In case 1 NBE analysis, the NBE of PLO (–1.616 kcal/mol), VLY (–3.345 kcal/mol), and Sm-hPAF (–1.972 kcal/mol) also had negative values. Although the effect of cholesterol on hemolysis was different between ILY and SLO, there was no difference in the NBE positions of ILY and SLO in case 2 and 3 (Figure 3, Table II); therefore, these positions should not be considered.

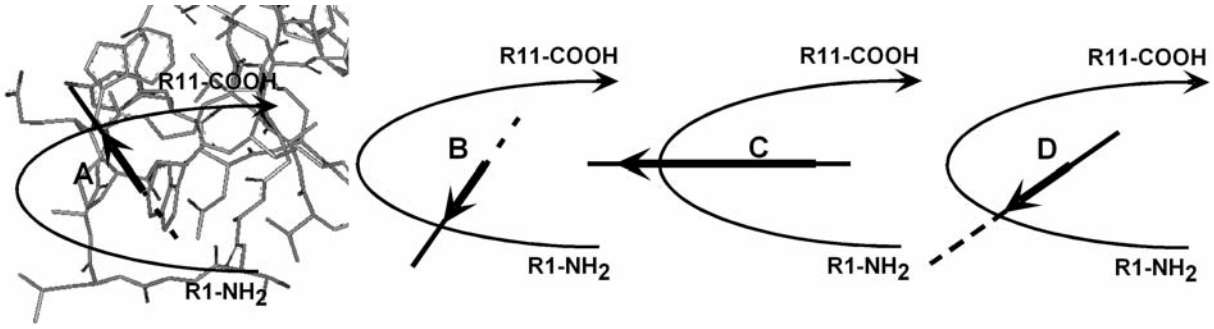

Figure 2. *Dipole moment direction of 11mer regions. Dipole moment direction of ILY (type A), PLO (type B), CDC (type C), VLY and Sm-hPAF (type D).*

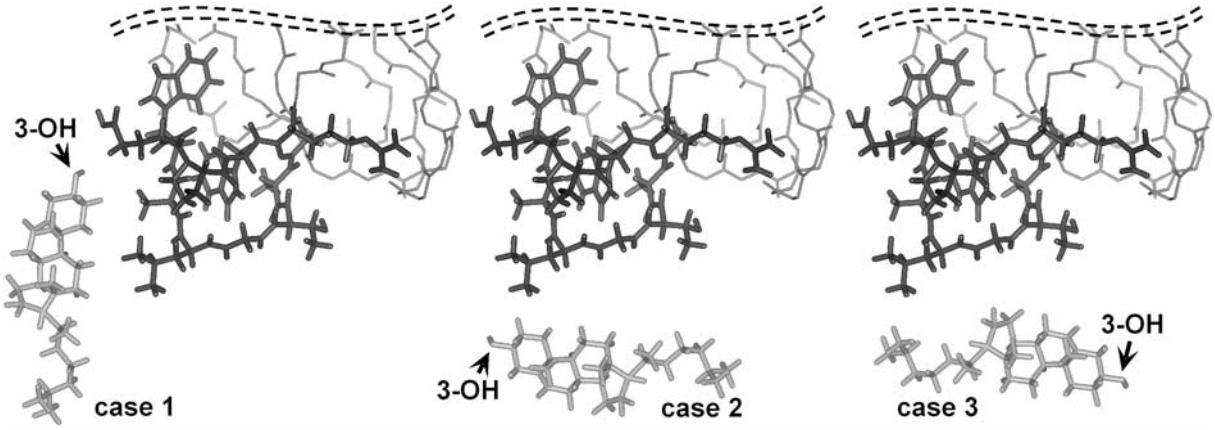

Figure 3. *Profile of interaction between cytolysin 11mer region and a cholesterol molecule. The 3-OH position in cholesterol is different in each simulation condition (case 1-3).*

Discussion

In the present study, we assessed the characteristics of the 11mer region of bacteria-derived cytolysins, using the molecular modeling and molecular orbital (MO) analysis. The dipole directions of ILY and PLO differed respectively from those of traditional CDCs. It was interesting that VLY and Sm-hPAF had the fourth type of dipole moment direction (Figure 2). Although the moment direction was the same in VLY and Sm-hPAF, the strength of that in VLY was smaller (3.647 Debye) than that of Sm-hPAF (8.540 Debye). The degree of stereo-hydrophobicity (dGW) of Sm-hPAF (–655.8 kJ/mol) was smaller than that of VLY (–490.0 kJ/mol). These findings suggest that the dynamic structure of the 11mer region was influenced by the structure of the whole cytolysin molecule. The amino acid sequence of the 11mer region was the same in VLY and Sm-hPAF, but differences in the three-dimensional 11mer structure might arise because structural differences of other parts affect the 11mer structure. The (HOMO)-(LUMO) energy gap (index of chemical reactivity; the smaller the absolute value of the

Table II. *Intermolecular parameters between cytolysin 11mers and cholesterol. Cholesterol and 11mer region of each cytolysin are located as shown by case 1, 2, and 3 in Figure 3, and non-binding energies were determined using InsightII-Discover.*

	Non-binding energy (kcal/mol)			Cholesterol effect on hemolysis
	Case 1	Case 2	Case 3	
ILY	–2.603	–3.321	–4.813	–
ILY(ECW)[1]	6.462	–3.470	–1.339	+++
SLO	3.175	–3.392	–4.835	+++++
SLO(GAP)[1]	–2.593	–3.325	–5.81E-33	++++
PLO	–1.616	–1.842	–2.470	ND
VLY	–3.345	–3.899	–5.143	ND
Sm-hPAF	–1.972	–1.861	–2.380	+++, –[2]

Intermedilysin (ILY), streptolysin O (SLO), pyolysin (PLO), vaginolysin (VLY), *Streptococcus mitis*-derived human platelet aggregation factor (Sm-hPAF). ILY(ECW) is an ILY mutant, which has the traditional cholesterol dependent cytolysin type 11mer. SLO(GAP) is a SLO mutant, which has the ILY type 11mer. ND: Not determined. [1]Results summarized from reference 7; [2]cholesterol effect on human erythrocytes: +++, rabbit erythrocytes: –.

HOMO-LUMO energy gap, the higher the reactivity) was estimated from the values of HOMO and LUMO obtained by MO analysis. The absolute energy gap value in the 11mer region of VLY (6.641) and Sm-hPAF (2.358) was smaller than in other cytolysins [8.025 (PFO) to 8.245 (PLY)]. The 11mer regions of VLY and Sm-hPAF had higher reactivity than in other cytolysins.

The 3-acetylated cholesterol analogue (3-OH group substituted with an acetyl group) did not inhibit the hemolysis of SLO (data not shown), and the 3-OH group has an important role in the cytolytic activity of cholesterol-dependent cytolysin. The membrane recognition of cytolysin is necessary for the cell membrane destruction process, and the lipid raft domain takes part in the membrane recognition of cytolysins. In the lipid raft, it was assumed that the hydroxyl groups of cholesterol molecules would be positioned along the water-accessible outer side. This consideration does not contradict the result of NBE simulation in which the interaction energy between cholesterol and cytolysin (*e.g.* ILY, SLO) differs only under case 1 conditions. ILY is a typical CDC, but in a broader sense, ILY might be cholesterol-dependent because it needs the lipid raft to interact with the cell membrane. From the NBE values, VLY and Sm-hPAF seemed to have cholesterol-independent features, as did ILY, or it was thought that they showed extremely low cholesterol dependency compared with traditional CDCs (*e.g.* SLO). Cholesterol affected the interaction between Sm-hPAF and human erythrocytes, but did not influence the interaction with rabbit erythrocytes (12). As for Sm-hPAF, its behavior in cholesterol was different from that of other cytolysins. Only the Sm-hPAF has domain 0 on the *N*-terminal side, and the dynamic structure of domain 0 might have various influences on membrane and cholesterol recognition. These domain 0 effects are currently under investigation. Moreover, Sm-hPAF is a multifunctional factor, and confirmation of its role not only in infectious disease, but also in cancer is at an advanced stage.

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