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In Silico Study of the Ion Channel Formed by Tolaasin I Produced by *Pseudomonas tolaasii*

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A toxin produced by *Pseudomonas tolaasii*, tolaasin, causes brown blotch disease in mushrooms. Tolaasin forms pores on the cellular membrane and destroys cell structure. Inhibiting the ability of tolaasin to form ion channels may be an effective method to protect against attack by tolaasin. However, it is first necessary to elucidate the three-dimensional structure of the ion channels formed by tolaasin. In this study, the structure of the tolaasin ion channel was determined *in silico* based on data obtained from nuclear magnetic resonance experiments.

Keywords: Tolaasin ion channel, *Pseudomonas tolaasii*

Pseudomonas tolaasii causes brown blotch disease in *Agaricus bisporus* and *Pleurotus ostreatus* [1]. A toxin produced by *P. tolaasii* was identified to be a lipodepsipeptide that forms an ion channel [2]. This toxin was named tolaasin and seven analogs have been identified [1]. Of all the analogs, tolaasin I is the most abundant one. Its primary structure was determined by Nutkins *et al*. [8], and its three-dimensional structure was determined using nuclear magnetic resonance (NMR) spectroscopy by Jourdan *et al*. [5]. Tolaasin forms an ion channel that allows ions to penetrate the membrane [4], but it is not known how tolaasin forms these ion channels. To control brown blotch disease caused by *P. tolaasii*, cultivation houses are typically fumigated and the water is sterilized. However, this treatment cannot completely prevent blotch disease; thus, alternative methods are needed. Because tolaasin causes disease, compounds inhibiting tolaasin have been examined. Several compounds were previously shown to inhibit tolaasin [7] and were primarily glucosylated carboxylic acids. The tolaasin inhibitors bind to tolaasin, which was shown by NMR spectroscopy [7]. It has been known that tolaasin forms pores on cellular membrane and destroys cell structure [3]. Inhibiting the ability of tolaasin to form ion channels may be an effective method to protect against attack by tolaasin. However, it is first necessary to elucidate the three-dimensional structure of ion channels formed by tolaasin. Therefore, in this study, the tolaasin ion channel was built *in silico* using data obtained from NMR experiments.

The three-dimensional (3D) structure of tolaasin I monomer was determined using NMR spectroscopy [5, 7]. Tolaasin I consists of the following amino acid sequence: β-hydroxyoctanoyl-D-But[1]-D-Pro-D-Ser-D-Leu-D-Val-D-Ser-D-Leu-D-Val-L-Val-D-Gln-L-Leu-D-Val-D-But-D-*allo*Thr-L-Ile-L-Hse-D-Dab-L-Lys[18], where D-But, L-Hse, and D-Dab denote *Z*-dehydroaminobutyric acid, L-homoserine, and D-2,4-diaminobutyric acid, respectively [4]. This protein contains a lactone ring between D-alloThr[14] and L-Lys[18], and a left-handed α-helix between D-Pro[2] and D-alloThr[14]. The β-hydroxyoctanoyl chain is parallel to the helix.

Since it was first shown that the tolaasin-induced ion channel formation was inhibited by zinc ion, the ability of the zinc ion to penetrate the lactone ring was assessed. The diameter of the backbone of the lactone ring is 5Å × 7Å, but there is no hole in its inner surface owing to the van der Waals clouds. As a result, the ion channel formed by tolaasin I was believed to consist of a trimer or tetramer of tolaasin I. There are a few methods to determine the 3D structure of peptides or proteins. Of these, molecular modeling has both advantages, such as no requirement for real biological samples, and disadvantages, such as low reliability. In this study, we tried to determine the 3D structure of the ion channel formed by tolaasin I using molecular modeling.

The structures of the tolaasin I monomer and the ion channel induced by tolaasin I were built using Sybyl (Tripos, St. Louis, MO, USA). Molecular modeling calculations

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and graphical representation were performed using Sybyl 7.3 on an Intel Core 2 Quad Q6600 (2.4 GHz) computer running a Linux operating system (CentOs 5.0 WS).

To determine the 3D structure using molecular modeling, a template with high sequence similarity was needed. Peptides or proteins with a similarity higher than at least 30% were not found in the protein databank (PDB). Therefore, other ion channels that had a similar appearance were searched for, to be used as a template. There are many ion channels deposited in the PDB. At least a trimer of tolaasin is believed to be needed to form an ion channel. The length of the tolaasin I monomer determined using NMR spectroscopy was 23Å. The average thickness of the cellular membrane is 30Å, so a monolayer of tolaasin I was not large enough to form an ion channel, and a double layer of tolaasin I would be needed to form an ion channel. Since most ion channels that are formed from a trimer are much longer than tolaasin I, they do not need to form a double layer. In addition, we were unable to accurately align tolaasin I to these proteins as a template. Tetramers deposited in the PDB were then searched. There are several nonredundant tetramer ion channels including 3IFX, 3E83, 3CO2, 2H95, 2OVC, 1P7B, and 1BHP in PDB. All tetramer ion channels except human KCNQ1 were not aligned with tolaasin I using the Sybyl program [6]. KCNQ1 is a human voltage-gated potassium channel that plays an important role in the repolarization phase of the cardiac action potential. Its gene is located in chromosome 11 and corresponds to 676 amino acids. KCNQ1 forms heteromultimers with other potassium channel proteins such as KCNE1 and KCNE3. Its 3D structure is not deposited in PDB, but it was obtained from the supplementary material of a paper published by Kang *et al*. [6]. A KCNE1 tetrameric ion channel was observed in the X-ray crystallographic structure, which was complexed with the monomer chain B. Each monomer of the tetramer contained 238 residues between Cys^{122} and Gln^{359}. In the case of KCNE1, only the 3rd α-helix, known as the transmembrane domain between Leu^{45} and Leu^{71}, was included. This α-helix was 34Å in length. In the study by Kang *et al*. [6] data on both the open ion channel mode and closed mode were provided, but the former was selected as a template for homology modeling of the ion channel formed by tolaasin I because the channel was expected to be in the open mode.

Since the template contained 238 residues and the target only contained 18 residues, the residues to be used for alignment needed to be selected. Of the 238 residues of the template, the residues surrounding Val^{355} were most similar to the residues surrounding Val^{12} of tolaasin I. Therefore, the backbones of monomer A of the template and the target were aligned using the fit monomer tool provided by the Sybyl program. Similar experiments were conducted for the monomers B, C, and D. After alignment, the template was deleted. Fig. 1A shows that four tolaasin I molecules

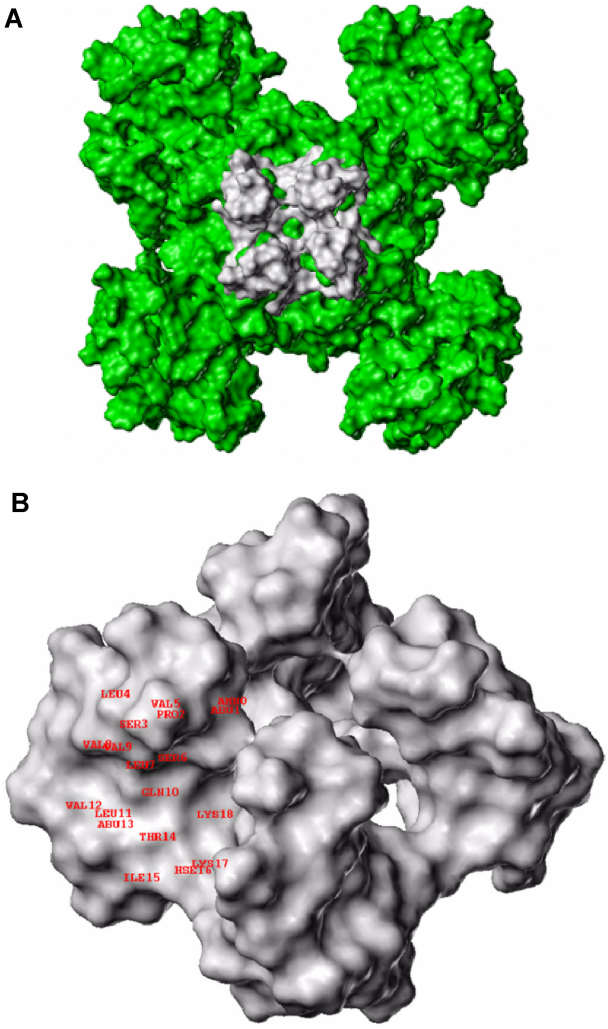

Fig. 1. Three-dimensional molecular models of tolaasin I and the induced ion channel.
A. Four tolaasin I molecules overlapped on the tetramer KCNQ1, a human voltage-gated potassium channel. **B**. Tolaasin-I-induced an ion channel.

were overlapped on KCNQ1. The tolaasin I molecules and KCNQ1 form calyces and flowers, respectively. The NMR experiments provided information on the binding between tolaasin I and cations such as zinc, sodium, and calcium [unpublished results]. Because the addition of cations changed the chemical shifts and intensities of the ^{1}H peaks related to the protons of L-Hse^{16} and D-Dab^{17}, we know where these two residues should be placed in the ion channel; the two residues should face the inner surface of the channel. Fig. 1B shows the structure of the ion channel formed by tolaasin I. As mentioned above, four tolaasin I molecules could form an ion channel. Ser, Gln, Thr, and Lys were placed in the inner surface, and Leu, Val, and Ile were placed on the outer surface. L-Hse^{16} and D-Dab^{17} residues were positioned in the inner surface. The hole through the ion channel had a diameter of 3Å.

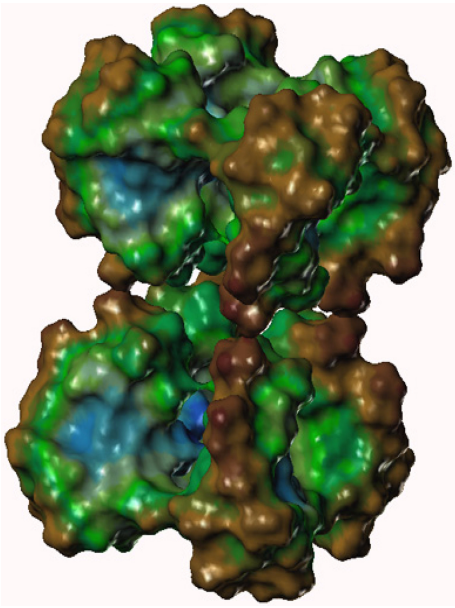

Fig. 2. Octamer tolaasin-I-induced ion channel.

Since the length of tolaasin I is 23Å, which was determined using NMR spectroscopy [7], it is not long enough to penetrate through the cellular membrane. Therefore, a double-layered tetramer is needed. To form a double-layered tetramer, a plane was prepared in the N-terminals of the tetramer shown in Fig. 1B. The tetramer was reflected on the plane and another tetramer was created. As a result, an ion channel was formed by tolaasin I, consisting of eight tolaasin I peptides (Fig. 2). As expected, based on the appearance of the other ion channel, the inner surface of the ion channel formed by tolaasin I was hydrophilic and its outer surface was hydrophobic. In Fig. 2, the hydrophilic surface is denoted in blue color and the hydrophobic surface is represented by a brown color. Whereas the inner surface contains D-But[1], D-Ser[6], D-Leu[7], D-Gln[10], D-*allo*Thr[14], L-Hse[16], D-Dab[17], and L-Lys[18] residues, the outer surface

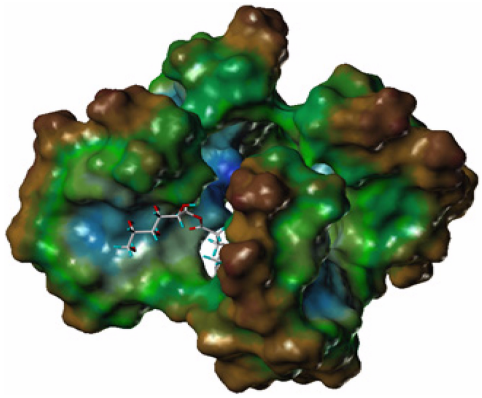

Fig. 3. A tolaasin I inhibitor, sorbitololeic acid, docked into the octamer tolaasin-I-induced ion channel.

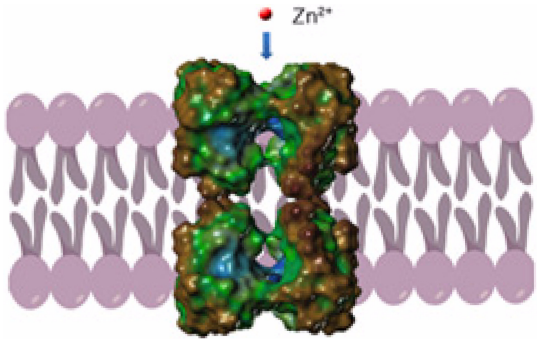

Fig. 4. Schematic representation showing the octamer tolaasin-I-induced ion channel embedded in the membrane and a zinc ion.

contained D-Pro[2], D-Ser[3], D-Leu[4], D-Val [5], D-Val[8], L-Val[9], L-Leu[11], D-Val[12], D-But[13], and L-Ile[15] residues and a β-hydroxyoctanoyl chain.

Some glucosylated carboxylic acids were shown to inhibit the tolaasin I monomer [7]. These tolaasin I inhibitors (TIF) may bind to the tolaasin-I-induced ion channel. One of these inhibitors, sorbitololeic acid, docked into the octamer (Fig. 3). The docking study was carried out on a Linux system (CentOs 5.0 WS) using FlexX (Tripos) on Sybyl 7.3. All residues of the ion channel formed by tolaasin I were considered to be a potential docking site. The selection radius for docking was 6.5Å. The docking process was iterated 30 times for the ligand. As shown in Fig. 3, sorbitololeic acid bound to the center of the hole and blocked the entrance, which prevented the zinc ion from approaching the ion channel or changing its appearance.

Technically, the Sybyl program cannot be used to dock a zinc ion into the ion channel. The diameter of the zinc ion is 1.4Å and the size of the inner hole of the ion channel formed by tolaasin I is approximately 3Å, which is enough for the zinc ion to penetrate the hole. This phenomenon is schematically shown in Fig. 4.

In conclusion, the structure of the ion channel formed by tolaasin I, determined *in silico* based on data obtained from nuclear magnetic resonance experiments, may be double-layered tetramers.

Acknowledgments

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