

## Observation of unstable binding compounds in aqueous solution on CSI-TOF-MS

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### Introduction

Palytoxin isolated from marine organisms, is a non-peptidic potent toxin ( $LD_{50}=450\text{ng kg}^{-1}$  in mice, Fig.1). It has been reported that the toxin changes the permeability of sodium ions on the cell membrane. It is, however, unclear how the toxin acts in the cell. Conformational study of the toxin would be important for elucidating the toxic action in organisms. Inuzuka and co-workers reported that palytoxin can exist as a dimer in aqueous solution by small-angle X-ray scattering (SAXS) experiment.<sup>1</sup> On the other hand, this dimer conformation in aqueous solution has not been observed by other techniques yet.

Mass spectrometry (MS) is one of useful tools for structural study of the compounds, because MS is high sensitive detection method, therefore, it has an advantage for investigation of natural products.

In general electrospray ionization (ESI) conditions, ion source is heated from 100 to 250 degrees Celsius. In these conditions, most of the complexes are unstable and not observed. In contrast, CryoSpray ionization (CSI) method which is performed in cryogenic conditions is known as a softer ionization technique and it has successfully detected such complexes.<sup>2</sup> We report the structure of palytoxin in aqueous solution by CSI-TOF-MS.

### Materials and Methods

A Bruker Daltonik GmbH micrOTOFQ ESI-TOF (time of flight) MS (Bremen, Germany) was used in this study. Palytoxin was purchased from Wako Pure Chemical Industry. Distilled water of 1mL was added to 100 ug of palytoxin. The solution was applied to CSI-TOF MS (Flow rate; 3uL/min) Aqueous solution of palytoxin was applied to CSI-TOF-MS on positive mode.

The conditions for CSI were as follows: nebulizing gas temperature, -30 degrees Celsius; dry gas temperature, 0 degrees Celsius; End plate voltage, -4.0kV; capillary voltage, -4.5kV; flow rate of dry gas, 4L/min.

ESI experiment was carried out to compare its spectrum with that of CSI. The conditions were: dry gas temperature was 190 degrees Celsius; flow rate of dry gas, 4L/min.

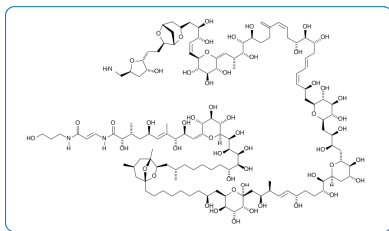


Fig. 1 Structure of palytoxin

### Results

Doubly and triply charged strong peaks of palytoxin monomers,  $[M+H+2Na]^{3+}$  and  $[M+H+Na]^{2+}$  were observed as base peaks in both ESI and CSI mode (Fig. 2A and 2B).

Triply charged dimer ions,  $[2M+Na+2H]^{3+}$  (observed at  $m/z$  1793.9858; calculated for  $C_{258}H_{448}N_6O_{108}Na$ ,  $m/z$  1793.9877) were observed with 1.1 ppm accuracy (Fig. 2B and Fig. 3A). This ion peak was not observed in ESI mode (Fig. 2A), probably due to high temperature of ion source, since only difference between CSI and ESI was temperature at the ion source.

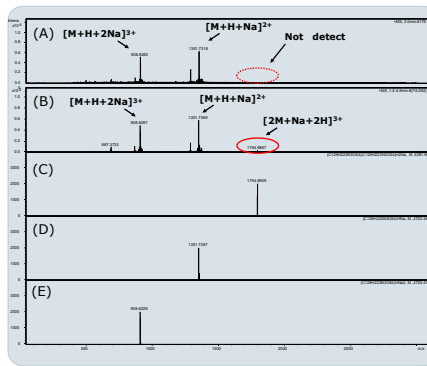


Fig. 2 The spectra of ESI (A); CSI (B); the theoretical spectrum for  $[2M+Na+2H]^{3+}$  (C);  $[M+H+Na]^{2+}$  (D);  $[M+H+2Na]^{3+}$  (E).

### Discussion

Although aqueous solution of palytoxin was subjected to ESI-TOF-MS, the dimer was not observed. It was thought that the dimer became unstable at high temperature. The solution was, therefore, injected to CSI-TOF-MS. CSI-TOF-MS revealed the triply charged ion of  $[2M+Na+2H]^{3+}$ . This result showed that palytoxin can be a dimer in aqueous solution. This result is in agreement with that of SAXS.<sup>1</sup> Interestingly, the dimer ion contained one of sodium ion and only proton-adducted ions were not detected. It has been reported that palytoxin can promote the permeation of sodium ion at cell membrane. This result suggested that the structure of palytoxin dimer might be related with the permeation.

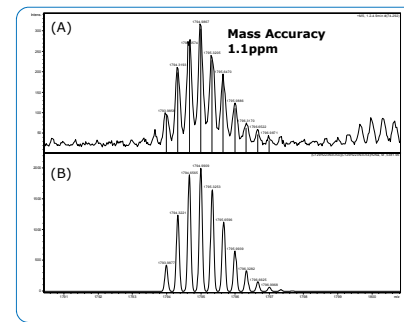


Fig. 3 The above figure (A) is zoom up spectra for dimer on CSI mode. Accuracy was within 5ppm. (B) is the calculated spectrum.

### Summary

Palytoxin was observed as a dimer in CSI method, and the accuracy was 1.1 ppm. On the other hand, this dimer structure is not observed in ESI mode. It was suggested that this dimer structure might be involved with the toxic action. It was showed that CSI-TOF-MS is a powerful tool for observation of unstable complexes.

#### References:

- (1) T. Inuzuka, T. Fujisawa, H. Arimoto, and D. Uemura. 2007. Molecular shape of palytoxin in aqueous solution. *Org. Biomol. Chem.* 5, 897-899.
- (2) Yamaguchi, K., 2007. In: Gross, M. L., Caprioli, R. M. (Eds.), *The Encyclopedia of Mass Spectrometry*, vol. 6. Elsevier, USA, pp. 502-512.

### Conclusions

- Palytoxin was observed as a dimer in CSI method.
- the result of this study agreed with that of Inuzuka.
- Palytoxin exists as a dimer in aqueous solution.
- CSI-TOF-MS is powerful tool for observation of unstable complexes.