Crystallography

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# Structure-based Analysis of Anti-ciguatoxin MAb 10C9Fab Recognition Mechanism for Poly-cyclic ethers

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## Introduction

Ciguatoxins are a family of marine toxins composed of trans-fused polycyclic ethers, and the principal cause of ciguatera seafood poisoning in tropical and subtropical regions. It has not yet been clarified at the atomic level the mechanism of its action nor its interaction with a protein. Here we report the crystal structures of anti-ciguatoxin antibody 10C9Fab in the ligand-free form and in complex with the ABCD-ring (CTX3C-ABCD) or the ABCDE-ring (CTX3C-ABCDE) fragments of the antigen CTX3C. Analysis of these structures provides novel insight about the interaction between polycyclic ethers and antibodies [1].

## Methods and Results

Purified 10C9Fab (10 mM Tris-HCl, pH 8.0) was concentrated to 8 mg $ml^{-1}$ for crystallization trials. For preparation of the CTX3C-ABCD–10C9Fab complex, the appropriate amount of a 500 μM CTX3C-ABCD solution in DMSO was added to the protein solution (final 10C9Fab:CTX3C-ABCD molar ratio, 1:1.7); and for the CTX3C-ABCDE–10C9Fab complex, a 5 mM CTX3C-ABCDE solution in DMSO was added to the protein solution (final 10C9Fab:CTX3C-ABCDE molar ratio, 1:1.2). Crystals were grown by the hanging drop vapor diffusion method at 20ºC. Crystals of ligand-free 10C9Fab, the CTX3C-ABCD–10C9Fab, and CTX3C-ABCDE–10C9Fab complexes suitable for data collection were grown from 0.1 M sodium cacodylate (pH 5.2) containing 0.2 M zinc acetate and 15% PEG 8000, 0.1 M sodium acetate (pH 4.6) containing 0.2 M ammonium sulfate and 30% PEG monomethyl ether 2000, and from 0.1 M trisodium citrate dehydrate (pH 5.6) containing 20% v/v isopropanol and 20% PEG 4000, respectively. A data set from a crystal of ligand-free 10C9Fab was collected at beamline NW12 under cryogenic conditions (100 K), while data sets for the complexes with CTX3C-ABCD and CTX3C-ABCDE were collected on beamlines 6a and 5a, respectively. Diffraction data for the ligand-free fragment and the two complexes were collected up to resolutions of 2.6, 2.4, and 2.3 Å, respectively. All collected data were processed using the program *HKL2000*. The structures were determined by the molecular replacement method using the program PHASER.

10C9 Fab has an extraordinary large and deep binding pocket at the center of the variable region, where CTX3C-ABCD or CTX3C-ABCDE binds longitudinally in the pocket via hydrogen bonds and van der Waals interactions. The structural analysis indicates that the keys to recognition of a polycyclic ether compound possessing a large, rigid structure like that of CTX3C are (i) the presence of a suitable pocket that form extensive van del waals contacts with the majority of the surface of the antigens and (ii) the presence of polar residues that can interact with the antigen via hydrogen bonds network.

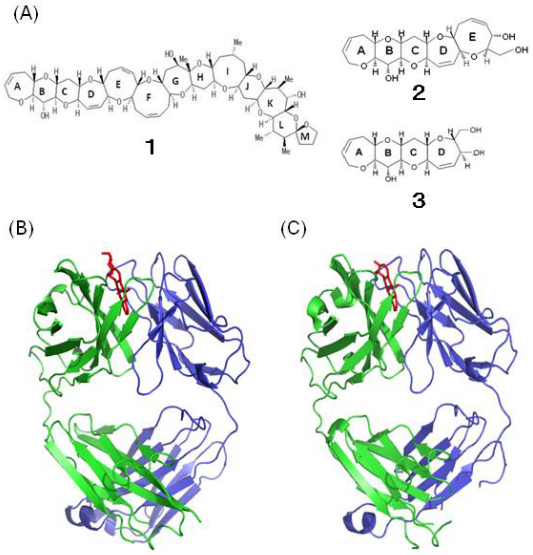


Fig. 1 (A) Molecular structures of ciguatoxin fragments: CTX3C (1), CTX3C-ABCDE (2), and CTX3C-ABCD (3). (B) Structure of the CTX3C-ABCDE–10C9Fab complex. (C) Structure of the CTX3C-ABCD–10C9Fab complex. Antigen is depicted in red.

## Reference

[1] M. Ui, Y. Tanaka, T. Tsumuraya, I. Fujii, M. Inoue, M. Hirama, and K. Tsumoto, *J. Biol. Chem*, in Press (2008).

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