# Structural studies of the CBM complex in NFκB activation

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## Abstract

NFκB activation pathway is important in inflammatory response, cell activation, proliferation, and cell survival. The CBM complex composed of caspase recruitment domain family (CARDs), B-cell chronic Lymphocytic leukemia lymphoma 10 (BCL10), and mucosa-associated lymphoid tissue lymphoma translocation gene 1 (MALT1) is one of the key complexes to perform signal transduction. It has been shown that some diseases are correlated with the CBM complex mutants which could lead NFκB to constitutive activation or inhibition. Although some single domain structures of the CBM complex have been solved, the mechanism of protein-protein interaction in the CBM complex remains largely unknown. In order to understand how the molecular interactions in the CBM complex, we made different length of constructs and mutants of the individual molecules in the CBM complex to obtain proteins for structural studies and biochemical assay. Here we report the crystal structure of the MALT1 death domain at a resolution of 2.2 Å. In addition, the results from the assays of multiangle light scattering and the isothermal titration calorimetry experiments provide the significant structural and biochemical information of the binding affinity and the stoichiometry in the CBM complex. These results help us to elucidate how the CBM complex interacts with each other and regulates downstream signaling pathways.

***Keywords – CARMA1, BCL10, MALT1, X-ray crystallography***

## Introduction

The nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB) is a protein complex that is considered participates in the regulation of cell activation, survival, proliferation, inflammation and immune responses [1]. During last 30 years, NFκB has been identified as a crucial transcription factor and has lots of research to illuminate the NFκB pathway [2]. In T cell and B cell activation NFκB pathway, ternary complex of CARDs family, BCL10 and MALT1 known as the CBM complex, is a significant component to trigger NFκB activation [3]. In the CBM complex, previous results show that CARMA family use CARD domain interact with BCL10 CARD domain and BCL10 use C-terminal region interact with N-terminal region of MALT1 [4]. However, there are no reports to date on the biochemical or detailed structural characterization of any N terminal of MALT1. Although the structures of MALT1 DD and IG domain already published on the protein data bank (PDB ID 2g7r and 3k0w), there are few articles investigating on these crucial region which can interact with BCL10. It has been shown that some diseases are correlated with the CBM complex mutants which could lead NFκB to constitutive activation or inhibition [5]. In our research, we report the crystal structure of MALT1 DD at a resolution of 2.2 Å which has higher resolution than before. With multiangle light scattering (MALS) and isothermal titration calorimetry experiments, these biochemical information provide a new insight of designing new drugs for inflammatory or immune response.

## Experiments

### Protein expression and purification

All constructs were expressed by *E.coli* system. For *E.coli* system, the plasmids were transformed into Rosetta (DE3) cells, and cultured in the LB medium. Protein expression was induced by Isopropyl β-D-1-thiogalactopyranoside and cells were harvested and lysed in different lysis buffer. The supernatants were clarified by centrifugation and loaded onto Ni-NTA resin binding. After binding, Ni-NTA resin was washed by wash buffer and eluted by elution buffer for several times. The eluted samples were purified by Size exclusion chromatography (SEC) or ion-exchange chromatography. After purification, we combined SDS-PAGE to check the purity of the protein.

### X-ray crystallography

By using hanging drop or sitting drop method in different crystallization screening kits, some conditions are proper for further optimization. After optimization, the crystals brought to the NSRRC in Taiwan, Hsinchu beamline 13B and 15A for collecting the diffraction data. The data are scaled by the software HKL2000 and do the molecular replacement by Phenix phaser-MR. Then, the data are refined by Phenix refinement.

### Multiangle light scattering

The samples are purified by FPLC and check by SDS-PAGE. MALS was done by ÄKTA pure (GE Healthcare) with ENrich™ SEC 650 column (Bio-Rad) for separating protein with different size and Viscotek TDA 305 (Malvern) as the MALS major measure machine. The data are calculated by OmniSEC software.

## Results

We expressed and purified the CARMA1, BCL10 and MALT1 separately in *E.coli* expression system. After trying different crystallization conditions, the crystals of MALT1 DD can be obtained (Fig. 1). The crystals brought to the NSRRC for collecting the diffraction pattern and scaled by the software HKL2000. The structure is solved by using the molecular replacement

method (PDB ID 2g7r) by Phenix phaser-MR. After solving the phase and refineing the data, we obtained the MALT1 DD structure from A28 to P124 at a resolution of 2.2 Å (Fig. 1). Compare to previous structure, our structure has higher resolution than before (PDB ID 2g7r) and has two loops (P49 to R52 and G65 to S70) which do not observe the electron density map in the previous structure (Fig. 2). In MALT1 DD structure, the helix 6 in the c-terminal has a long extension helix. The long extension helix in helix 6 is quite different from other DD. In the cryo-EM structure of BCL10-MALT1 at the resolution of 4.9 Å , the helix 6 of MALT1 shows a kink in the structure, not the extension one (PDB ID 6gk2) [6]. Therefore, the real function of MALT1 helix 6 in DD still need further experiment to elucidate. Recently, we received the new crystals in MALT1 DD-IG. Although the crystals have not collecting the diffraction pattern yet, we hope these crystals can provide more information of MALT1.

In order to determine the characteristic of BCL10 and MALT1, MALS and ITC are used for measuring the molecular weight, binding affinity and the stoichiometry of BCL10-MALT1 complex. In MALS results, BCL10 and DD and DD-IG1 domain of MALT1 shows monomer in the solution (Fig. 3). However, DD-IG1-IG2 domain of MALT1 shows dimer. Interestingly, these MALT1 fragment interact with BCL10 in a 1:1 ratio. These results indicate that IG2 domain of MALT1 has the dimerization function. Although IG2 domain was similar with IG1 domain with rmsd of 1.6Å and 27% sequence identity, they did not participate in the same biological function. In both ITC and MALS data shows BCL10 forms complex with MALT1 in the 1:1 ratio (ITC data not shown). Therefore, the IG2 domain may play a crucial role for MALT1 from dimer into monomer to form complex with BCL10.

## Discussion

In previous studies, MALT1 may interact with BCL10 through IG domain (140-330) of MALT1 and the BCL10 deletion (107-119) decrease the NFκB activation [7]. In 2018, cryo-EM structure shows BCL10 use CARD (1-115) to interact with MALT1 DD (29-124) and the interaction residues are R87 and Q97 in BCL10 to interact with E75 and R55 in MALT1 [6]. In our research, BCL10 c-terminal deletion also affects the complex formation. Otherwise, both DD and IG1 domain of MALT1 can form complex with BCL10 after SEC. However, the MALS cannot correctly calculate the molecular weight of BCL10-MALT1 IG domain complex. Therefore, we suppose that BCL10 and MALT1 complex may interact with multiple interaction regions. If the crystal structure of BCL10-MALT1 complex solved, it may clearly elucidate the interaction region between BCL10 and MALT1.

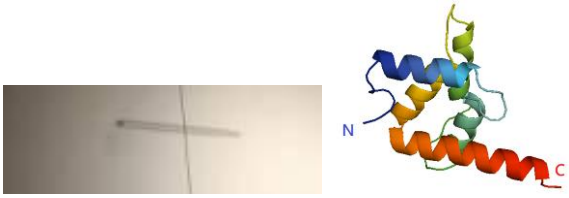


Fig. 1. The crystals and the structure of MALT1 DD.

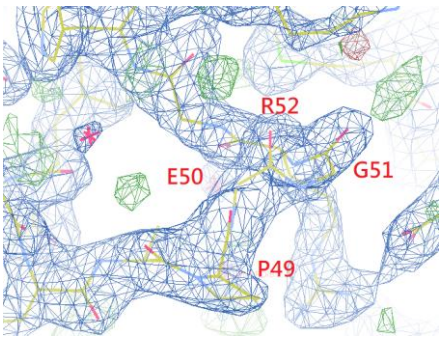


Fig. 2. The electron density map of MALT1 P49 to R52.

| Protein | Theoretical | SEC result | MALS result | Composite Ratio |
|---|---|---|---|---|
| BSA (standard) | 66.463 kDa | | 66.312 kDa | |
| BCL10 | 28 kDa | 35 kDa | 29 kDa | Monomer |
| MALT1-DD | 15 kDa | 14 kDa | 15 kDa | Monomer |
| MALT1-DD-IG | 27 kDa | 33 kDa | 28 kDa | Monomer |
| MALT1-DD-IG1-IG2 | 40 kDa | 78 kDa | 76 kDa | Dimer |
| BCL10 complex with MALT1-DD | BCL10: 28 kDa MALT1: 15 kDa | 44 kDa | 41 kDa | BCL10:MALT1=1:1 |
| BCL10 complex with MALT1-DD_IG1 | BCL10: 28 kDa MALT1: 27 kDa | 44 kDa | 52 kDa | BCL10:MALT1=1:1 |
| BCL10 complex with MALT1-DD_IG1-IG2 | BCL10: 28 kDa MALT1: 40 kDa | 80 kDa | 69 kDa | BCL10:MALT1=1:1 |

Fig. 3. The MALS results of MALT1, BCL10 and MALT1-BCL10 complex.

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