BL-5A, BL-17A/2018G143

Crystal structures of unmodified and AGE-modified human CRMP2

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1 Introduction

CRMP2 has been reported to function in regulation of synaptic transmission, microtubule (MT) dynamics and vesicle trafficking in cells and the developing brain. As a MT associating protein, CRMP2 reportedly promotes MT polymerization, stabilization and bundling. Enhanced carbonyl stress underlies a subset of schizophrenia and CRMP2 has been identified as a major target of AGE modification under enhanced carbonyl stress in iPS cells derived from schizophrenia patients. Moreover, AGE-modified sites of CRMP2 have been precisely determined through advanced mass spectrometric approaches. Here, we report crystal structures of both unmodified and AGE-modified human CRMP2 [1].

2 Experiment

The C532 construct (Human CRMP2 residues 1-532 with a His tag) was bacterially expressed and purified, and AGE-modified CRMP2 was prepared by incubation with glyoxal. Crystals of unmodified and AGE-modified CRMP2 were grown using hanging drop vapor diffusion method. X-ray diffraction datasets were collected at Photon Factory beamline BL-5A and BL-17A. The structures were solved by molecular replacement using crystal structure of CRMP2 residues 13-516 (PDB code 5MKV) as a search model.

3 Results and Discussion

We determined the structures of unmodified and AGE-modified CRMP2 at 2.26 and 2.00 Å, respectively. After several cycles of interactive refinements, we established final structure models with respective R_{free}/R_{work} values of 17.1%/19.3% and 18.3%/20.4% (PDB codes: 6JV9 and 6JVB).

Crystal structures of unmodified and AGE-modified CRMP2 display tetrameric asymmetric packing (Fig. 1a). And remarkably, the AGE sites are wildly distributed on both the outer surface around the functional domains and the inner self-assembly sites, D-hook (dynamic binding surface for dimerization) and T-site (tacking surface for tetramerization) (Fig. 1b). Moreover, there are obvious structural differences in the N-terminal β2-β3 region including AGE target lysines in T-sites between the two structures (Fig. 1c). Taken together, our results provided structure insights on the molecular mechanism of dysfunction of CRMP2 under enhanced carbonyl stress.

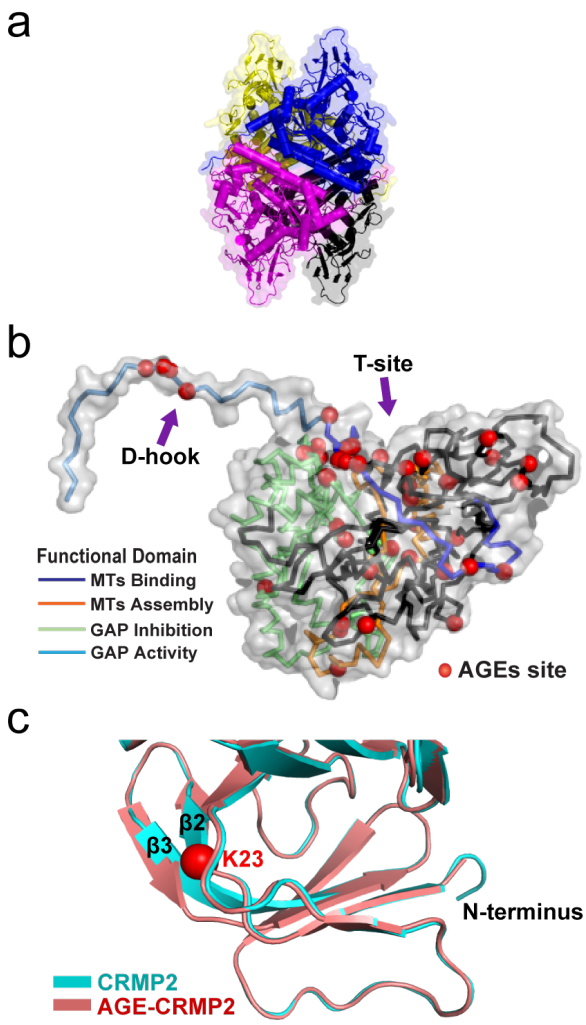

Fig. 1: Crystal asymmetric packing (a), AGE sites distribution (b) and structural differences between unmodified and AGE-modified CRMP2 (c).

References

[1] M. Toyoshima and X. Jiang *et al.*, *Life Sci Alliance* e201900478, 5, 2 (2019).

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