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Structural basis of protein complex formation and reconfiguration by polyglutamine disease protein Ataxin-1 and Capicua

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1 Introduction

Spinocerebellar Ataxia Type 1 (SCA1) is a dominantly-inherited neurodegenerative disease caused by polyglutamine expansion in Ataxin-1 (ATXN1) [1]. ATXN1 binds to the transcriptional repressor Capicua (CIC), and the interaction plays a critical role in SCA1 pathogenesis whereby reducing CIC levels rescues SCA1-like phenotypes in a mouse model [2, 3]. Here, we present the crystal structure of ATXN1's AXH domain bound to CIC and show that the binding pocket of the AXH domain to CIC overlaps with the homo-dimerization pocket of the AXH domain. Thus, the binding to CIC disrupts the homo-dimerization of ATXN1. Furthermore, the binding of CIC reconfigures the complex to allow another form of dimerization mediated by CIC, showing the intricacy of protein complex formation and reconfiguration by ATXN1 and CIC. Identifying the surfaces mediating the interactions between CIC and ATXN1 reveals a critical role for CIC in the reconfiguration of the AXH dimers and might provide insight into ways to target the ATXN1/CIC interactions to modulate SCA1 pathogenesis.

2 Experiment

AXH-CIC21 complex was crystallized in the presence of 3 mM CIC21 peptide (EPRSVAVFPWHSLVPFLAPSQ) in a reservoir solution containing 0.1M Calcium chloride, 24% (v/v) PEG 3350 and 4% (v/v) pentaerythritol ethoxylate by the hanging drop vapour diffusion method at 20ºC. AXH-CIC28 complex was crystallized in the presence of CIC28 peptide (MFVWTNVEPRSVAVFPWHSLVPFLAPSQ) in a mothor liquor containing 1.6M NaCl, 3% (v/v) glycerol , 16% (w/v) PEG 3350 and 1mM L-Glutathione. AXH-CIC21 crystals were soaked in a cryo solution containing 26% (w/v) PEG3350 and 10% (v/v) Glycerol. For AXH-CIC28, the mother liquor solution was used as a cryo-protectant. X-ray diffraction data were collected at the beamline 17A at Photon Factory, Tsukuba, Japan and the beamline 5C at the Pohang Accelerator Laboratory, Pohang, Korea. The data were processed using HKL2000. The AXH-CIC21 structure was determined by Se-SAD method using Phenix, and refined using CNS. The AXH-CIC28 structure was solved by molecular replacement method using the AXH apo-structure as a search model and refined using CNS program. The structure factors and coordinates of the structures were deposited at the Protein Data Bank (www.rcsb.org, PDB ID: 4J2J for AXH-CIC21, 4J2L for AXH-CIC28).

3 Results and Discussion

To understand the molecular mechanism mediating ATXN1-CIC interaction, we determined the crystal structure of ATXN1's AXH domain bound to a highly conserved N-terminal region of CIC (28-48 a.a., CIC21: EPRSVAVFPWHSLVPFLAPSQ) at 2.5 Å resolution using Single-wavelength Anomalous Dispersion (SAD) method (Table 1). The complex structure reveals that CIC21 binds to a highly hydrophobic pocket of the AXH domain (Fig. 1)

	AXH-CIC$_{21}$ (SAD)	AXH-CIC$_{28}$ (Native)
Data collection		
Space group	I222	$P4_322$
Cell dimensions		
a, *b*, *c* (Å)	77.72, 89.10, 132.66	53.50, 53.50, 276.21
$\alpha = \beta = \gamma$ (°)	90	90
Resolution (Å)	50-2.5 (2.54-2.50)	50-3.0 (3.05-3.00)
R_{sym}	0.089 (0.584)	0.106 (0.663)
$I / \sigma(I)$	52.6 (5.4)	45.2 (6.2)
Completeness (%)	100 (100)	99.9 (100)
Redundancy	14.6 (15)	12.0 (13.3)
Refinement		
Resolution (Å)	50-2.5	30-3.15
No. reflections	29,785	7,519
R_{work} / R_{free}	0.223 / 0.270	0.236 / 0.296
No. atoms		
Protein	3137	2311
Water	28	
B-factors (Å^2)		
Protein	57.00	75.56
Water	44.66	
R.m.s.d. deviations		
Bond lengths (Å)	0.007	0.013
Bond angles (°)	1.4	2.2
Ramachandran plot (%)	87.7 (12.3)	77.1 (22.9)

R_{work} : Σ $||F_{obs}|-|F_{calc}||/\Sigma|F_{obs}|$
R_{free}: Σ $||F_{obs}|-|F_{calc}||/\Sigma|F_{obs}|$ where 5% of randomly selected data were used.
R_{sym}: $\Sigma|I_{hkl}-\langle I_{hkl}\rangle|/\Sigma I_{hkl}$, where $\langle I_{hkl}\rangle$ is the mean intensity of all reflections equivalent to refection hkl.

Table 1. Data collection and refinement statistics

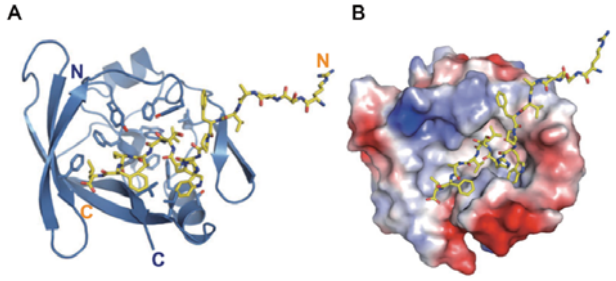

Fig. 1. Crystal structure shows that CIC21 binds to a highly hydrophobic pocket of the AXH domain. (A) Ribbon representation of the AXH domain (blue) and the bound CIC21 peptide shown in ball-and-stick (yellow). (B) Electrostatic surface representation of the AXH domain bound with CIC.

Upon further examination of the structure, we noticed that there are three molecules of the AXH-CIC21 complexes in an asymmetric unit in the crystal (Fig. 2). Among them, the structure of two AXH-CIC21 complexes suggests a possible new form of ATXN1 dimerization mediated by CIC dimer.

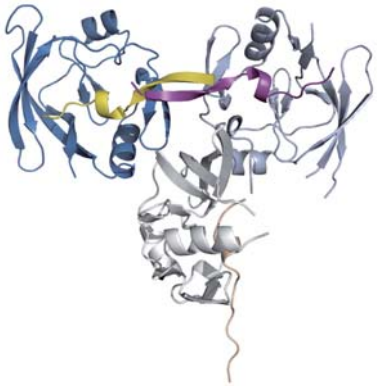

Fig. 2. Three AXH-CIC21 molecules in an asymmetric unit of the crystal.

To assess the biological relevance of this new form of AXH-CIC complex, we determined the crystal structure of the AXH domain bound to a longer CIC peptide (21-48 a.a.,CIC28:MFVWTNVEPRSVAVFPWHSLVPFLAPSQ) at 3.15 Å resolution by molecular replacement method using the AXH domain alone without CIC21 as a search model (Table 1).

There are two AXH-CIC28 molecules in an asymmetric unit of the crystals, which belong to a different space group with the AXH-CIC21 crystals (Table 1 and Fig. 3) indicating that the AXH-CIC28 molecules have a different packing environment with the AXH-CIC21. Indeed, the AXH domains make a new form of dimerization mediated by CIC28 dimer (Fig. 3). Two CIC28 molecules positioned in anti-parallel manner make an intertwining interaction between each other, and these two intertwined CIC28 molecules bridge two AXH domains.

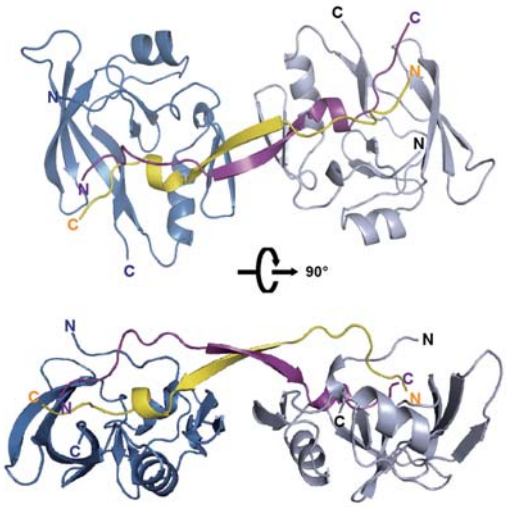

Fig. 3. Crystal structure showing CIC28-mediated heterotetramer formation with the AXH domain. Ribbon representation of the AXH-CIC28 structure showing that CIC28 reconfigures the complex formation of the AXH domain (the AXH domain is shown in blue and light blue, and the CIC28 peptide is shown in yellow and purple).

References

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