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Structural basis of the phosphorylation dependent complex formation of neurodegenerative disease protein Ataxin-1 and RBM17

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ABSTRACT

Spinocerebellar Ataxia Type1 (SCA1) is a dominantly inherited neurodegenerative disease and belongs to polyglutamine expansion disorders. The polyglutamine expansion in Ataxin-1 (ATXN1) is responsible for SCA1 pathology. ATXN1 forms at least two distinct complexes with Capicua (CIC) or RNA-binding motif protein 17 (RBM17). The wild-type ATXN1 dominantly forms a complex with CIC and the polyglutamine expanded form of ATXN1 favors to form a complex with RBM17. The phosphorylation of Ser776 in ATXN1 is critical for SCA1 pathology and serves as a binding platform for RBM17. However, the molecular basis of the phospho-specific binging of ATXN1 to RBM17 is not delineated. Here, we present the modeled structure of RBM17 bound to the phosphorylated ATXN1 peptide. The structure reveals the phosphorylation specific interaction between ATXN1 and RBM17 through a salt-bridge network. Furthermore, the modeled structure and the interactions between RBM17 and ATXN1 were validated through mutagenesis study followed by Surface Plasmon Resonance binding experiments. This work delineates the molecular basis of the interaction between RBM17 and the phosphorylated form of ATXN1, which is critical for SCA1 pathology. Furthermore, the structure of RBM17 and pATXN1 peptide might be utilized to target RBM17–ATXN1 interaction to modulate SCA1 pathogenesis.

1. Introduction

Polyglutamine diseases are dominantly inherited neurodegenerative disorders caused by an expansion of the CAG (encoding glutamine)-trinucleotide repeat region in the disease-related genes. Nine polyglutamine diseases are so far known including Huntington's disease, dentatorubropallidoluysian atrophy, spinobulbar muscular atrophy, and six spinocerebellar ataxias types (SCA) [1]. The length of polyglutamine expansion is shown to be correlated with the age of disease onset and the disease severity [2]. Even though the polyglutamine expanded proteins are ubiquitously expressed in all cell types, pathogenesis occurs only in specific brain areas. While the mechanisms of pathogenesis are poorly understood, intranuclear and cytoplasmic aggregates containing the polyglutamine expanded protein are found in affected neurons in all polyglutamine diseases as a common hallmark of the diseases.

SCA1 is one of the nine polyglutamine expansion neurodegenerative diseases. A major pathological feature of SCA1 is selective loss of Purkinje cells in the cerebellar cortex leading to progressive loss of motor coordination, speech impairment and problems with swallowing and breathing, eventually causing death [3,4]. The polyglutamine expansion in Ataxin-1 (ATXN1) is responsible for SCA1 [5,6]. ATXN1 contains AXH domain at the middle, and a polyglutamine repeat at the N-terminal region of ATXN1. ATXN1 has several binding partners. In nucleus, ATXN1 forms at least two complexes: one containing transcriptional repressor CIC and the other containing RBM17. Wild-type (non-expanded form) ATXN1 prefers to form a complex with CIC, while polyglutamine expanded ATXN1 favors to form a complex with RBM17 [7]. However, both ATXN1–RBM17 and ATXN1–CIC complexes are implicated in SCA1 disease and the balance between these two complexes might be critical for SCA1 pathogenesis [8].

CIC is a transcription factor containing HMG-box domain and predominantly expressed during granule cell development in the cerebellum, hippocampus and olfactory bulb. CIC is involved in ErbB signaling and central nerve system (CNS) development [9,10]. The evolutionarily conserved N-terminal region of CIC interacts with the AXH domain of ATXN1, and the reduction of CIC protein level

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partially rescues SCA1-like phenotype in a mouse model [11]. We recently determined the crystal structure of ATXN1 AXH domain bound to CIC and revealed that ATXN1 undergoes reconfiguration from homodimer to hetero-tetramer upon CIC binding [12].

RBM17, also known as SPF 45, is a part of the spliceosome complex regulating mRNA splicing and abundant in Purkinje cell nuclei [7,13]. RBM17 is shown to mediate the toxicity of polyglutamine expanded ATXN1 in *Drosophila* model of SCA1 [7]. It was shown that Retinal degeneration, ommatidial disorganization and fusion, and interommatidial bristle loss were worsened when polyglutamine expanded ATXN1 and RBM17 was co-expressed than wild-type ATXN1 and RBM17, which is consistent with that RBM17 preferentially interacts with polyglutamine expanded ATXN1 and that the interaction between ATXN1 and RBM17 is implicated with SCA1 pathology [7]. The phosphorylation of Ser776 of ATXN1 by MSK1 pathway serves a binding platform for RBM17. Furthermore, the Ser776 phosphorylation is critical for SCA1 pathology as well as for the stability of ATXN1 [7,14–16]. However, the biological consequence of the phosphorylation of ATXN1 leading to RBM17 complex formation is not clearly understood. Despite of the importance of RBM17–ATXN1 interactions in SCA1 pathology, molecular basis of the phosphorylation dependency of RBM17–ATXN1 interaction has not been delineated. Here, we present the structure of RBM17 bound to ATXN1 peptide by molecular modeling studies. The structure reveals the phospho-specific interaction between RBM17 and ATXN1. Furthermore, we validated the structure and the interaction by mutagenesis study with SPR binding experiments. This work provides molecular understanding on the phospho-specific interaction of RBM17 toward ATXN1 implicated in SCA1 pathology.

2. Materials and methods

2.1. Molecular modeling

To predict the complex structure of RBM17 and the phosphorylated ATXN1 (pATXN1) 6mer peptide, molecular modeling studies were conducted using the X-ray crystal structure of RBM17 complexed with ULM5 peptide (PDB code: 2PEH) [17]. The protein structure was cleaned up, and the 3D structure of the peptide was generated by Concord and energy minimized using MMFF94s force field and MMFF94 charges in Tripos SYBYL-X2.0. The flexible docking was carried out using GOLD v5.0.1 with the binding site defined as 10 Å around the co-crystallized ULM5 peptide. The ligand was docked using the similarity, region, and H-bond constraints, and evaluated by the GoldScore scoring function. The computational calculations were undertaken on an Intel® Xeon™ Quad-core workstation with Linux Cent OS release 5.5.

2.2. Protein expression and purification

All proteins were expressed using *Escherichia coli* BL21 (DE3) cell line at 18 °C with 1 mM isopropyl β-D-1-thiogalactopyranoside, and purified by Ni–NTA (Qiagen) affinity chromatography followed by HiTrapQ (GE healthcare) anion-exchange and Superdex75 (GE healthcare) size exclusion chromatography. All mutants were generated using Quick Change Site-directed mutagenesis kit (Stratagen).

2.3. Surface plasmon resonance (SPR)

All interactions between RBM17 and ATXN1 were measured by Surface Plasmon Resonance technique using Biacore 3000 or T200 (GE Healthcare). A running buffer composed of 150 mM NaCl, 10 mM HEPES PH7.4, 3 mM EDTA and 0.005% (v/v) Tween20 was used for all experiments and a buffer containing 1 M NaCl or 2 M NaCl, 10 mM HEPES pH 7.5, 3 mM EDTA, and 0.005 % (v/v) Tween 20 was used as a regeneration buffer. The data were analyzed using Bia Evaluation software (GE Healthcare).

3. Results

3.1. Prediction of the ATXN1 and RBM17 complex structure

The polyglutamine-expanded ATXN1 preferentially interacts with RBM17, and the phosphorylation of Ser776 in ATXN1 is shown to be critical for the binding to this partner protein [7,15]. In order to predict the complex structure of the phosphorylated ATXN1 (pATXN1) and RBM17, molecular modeling studies were performed. It was reported that RBM17 (a.k.a SPF45) can bind to both ATXN1 and ULM5, and their binding motifs have high sequence similarity (Fig. 1A) [17]. Therefore, the X-ray crystal structure of RBM17 (301–401 a.a.) complexed with ULM5 peptide (PDB code: 2PEH) was used for the modeling of pATXN1–RBM17 complex structure. We focused on the region where ULM5 peptide demonstrated the tight interactions with RBM17 (see the region within the double-ended arrow in Fig. 1A), and the co-crystallized ULM5 6mer peptide was modified to pATXN1 6mer (RRWpSAP). Then, flexible docking of pATXN1 peptide onto RBM17 was carried out, and the resulting complex structure was further refined by the energy minimization.

The complex structure shows that pATXN1 6mer peptide binds tightly to RBM17 through the strong hydrophobic interactions and intensive salt-bridge/H-bonding network (Fig. 1B and E). Also, their overall structures show very good shape complementarities. Especially, $Trp775^{pATXN1}$ nicely occupies the deep hydrophobic pocket (Fig. 1C and D), which is formed by the helices αA and αB along with the hydrophobic side chains on the β-sheets of RBM17 (Fig. 1B) [17]. It makes the strong hydrophobic interactions with the surrounding residues, i.e., Phe377, Leu372, Val382, and Met312 of RBM17. Also, the NH moiety of the $Trp775^{pATXN1}$ indole ring forms the H-bond with $Glu325^{RBM17}$ (Fig. 1E). The residues located at the tip of the β-hairpin formed by the strands β3' and β4 of RBM17 [17], i.e., Arg375, Tyr376, and Phe377, appear to contribute to the binding with pATXN1. $Phe377^{RBM17}$ makes an orthogonal π–π stacking interaction with $Trp775^{pATXN1}$ and stacks with the alkyl group of $Arg774^{pATXN1}$. This threefold stacking among $Trp775^{pATXN1}$, $Phe377^{RBM17}$, and $Arg774^{pATXN1}$ stabilizes the complex structure of RBM17 and pATXN1. The backbone amide and carbonyl groups of $Tyr376^{RBM17}$ and $Gly379^{RBM17}$ make a hydrogen bonding with the backbone of the pATXN1 peptide. Interestingly, the guanidyl group of $Arg375^{RBM17}$ makes a strong salt-bridge interaction with the phosphorylated $Ser776^{pATXN1}$, and $Arg375^{RBM17}$ is stabilized by another salt-bridge with the carboxylate group of $Glu329^{RBM17}$. This tight interaction network among phospho-$Ser776^{pATXN1}$, $Arg375^{RBM17}$ and $GLu329^{RBM17}$ reflects that the interaction of ATXN1 and RBM17 requires the phosphorylation of this $Ser776^{pATXN1}$ residue [15]. Compared with the interactions of ULM5 peptide bound in RBM17, pATXN1 peptide makes additional strong H-bonding network with RBM17 using $Arg773^{pATXN1}$ residue. It is notable that the corresponding residue in ULM5 is Ser336 as shown in Fig. 1A. The guanidyl group of $Arg773^{pATXN1}$ interacts with the backbone carbonyl of $Glu325^{RBM17}$ and the carboxylate of $Glu329^{RBM17}$. This $Glu329^{RBM17}$ residue on the helix αA makes the salt-bridge interaction with $Arg375^{RBM17}$ in β3′ and stabilizes RBM17. At the end of the helix αA, the carboxylate groups of $Asp319^{RBM17}$ and $Glu325^{RBM17}$ form the salt–bridge interactions with $Arg774^{pATXN1}$. Altogether, molecular modeling results demonstrate that pATXN1 tightly binds to RBM17 with the strong electrostatic and hydrophobic interactions as well as good shape complementarities.

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