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Inter-domain interactions of TDP-43 as decoded by NMR

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ABSTRACT

TDP-43 inclusions have been found in ~97% ALS as well as an increasing spectrum of other neurodegenerative diseases including Alzheimer's. TDP-43 contains an ubiquitin-like fold, two RRMs and a prion-like domain, but whether they interact with each other remains unknown due to being intrinsically aggregation-prone. Nevertheless, this knowledge is pivotal to understanding physiological functions and pathological roles of TDP-43. Here as facilitated by our previous discovery which allowed NMR characterization of TDP-43 and its five dissected fragments, we successfully decoded that TDP-43 does have dynamic inter-domain interactions, which are coordinated by the intrinsically-disordered prion-like domain. Thus, TDP-43 appears to undergo conformational exchanges between “closed” and “open” states which are needed for its functions. Our study thus offers a mechanism by which cellular processes might control TDP-43 physiology and proteinopathy by mediating its inter-domain interactions.

1. Introduction

TDP43 inclusions have been found in ~97% amyotrophic lateral sclerosis (ALS) and ~45% frontotemporal dementia (FTD) patients [1–4], as well as an increasing spectrum of other neurodegenerative disorders, which include ALS/parkinsonism–dementia complex of Guam, dementia with Lewy bodies (DLB), Pick's disease, argyrophilic grain disease and corticobasal degeneration [2]. Very recently, TDP-43 has been identified as a key player in Alzheimer's disease [5].

The 414-residue TDP43 is an intrinsically aggregation-prone protein [3], which contains the N-terminal region, two RNA recognition motifs (RRM1 and RRM2), and C-terminal prion-like domain (Fig. 1A). Previously, structure determinations by crystallography and NMR revealed that both RRM1 and RRM2 adopt the canonical RRM-fold, which are capable of binding a large variety of nucleic acids [6,7]. On the other hand, due to severe aggregation, only very recently, as facilitated by our previous discovery that protein aggregation can be significantly minimized by reducing salt concentrations [8,9], we successfully decrypted that the TDP-43 N-terminus encodes a novel ubiquitin-like fold coexisting with its unfolded form in equilibrium [10], while the prion-like domain is intrinsically disordered but undergoes a disorder-to-order self-assembly into β-rich but dynamic oligomers [11].

Now a key question arises as whether TDP-43 domains are interacting with each other. This piece of knowledge is absolutely essential for utilizing the structural knowledge obtained from the isolated domains to comprehend the properties of the full-length TDP-43. For instance, the existence of the inter-domain interactions may alter the aggregation mechanism and biochemical features of TDP-43, such as its interaction with nucleic acids. Moreover, the knowledge is also extremely critical for understanding the physiology and proteinopathy of TDP-43 in cells, which might be under tight control of cellular processes through modulating its inter-domain interactions. Indeed, Ser-phosphorylation was shown to play a key role in TDP-43 pathogenesis but the underlying molecular mechanism still remains elusive [12–14].

Previously, functional characterization indeed implies that the N- and C-termini of TDP-43 might interact with each other [4]. Unfortunately so far no structural evidence has been available on this issue, mostly due to the challenge to characterize the aggregation-prone TDP-43 by high-resolution biophysical methods such as NMR spectroscopy. Here, again as facilitated by our previous discovery [8,9], we attempted to investigate TDP-43 inter-domain interactions by NMR characterization of TDP-43 and its five differentially-dissected fragments (Fig. 1A). Our study decodes that TDP-43 does have dynamic inter-domain interactions coordinated by the intrinsically-disordered prion-like domain, thus providing a

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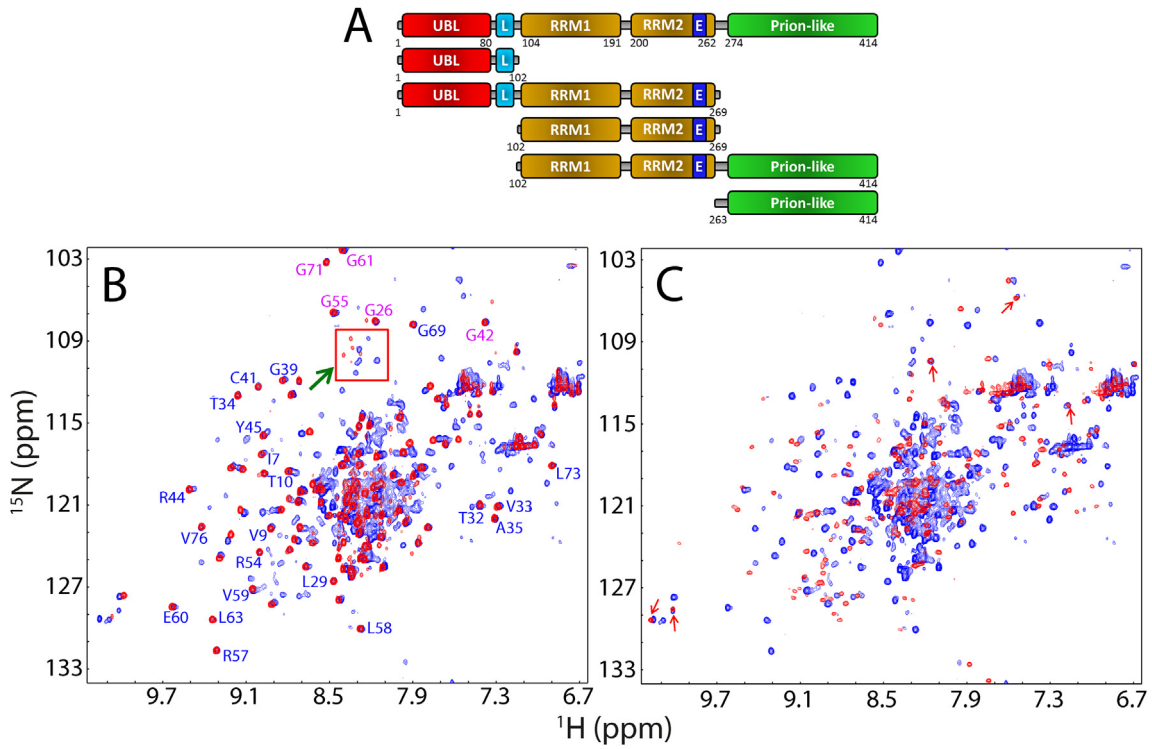

Fig. 1. (A) Domain organization of TDP-43 and five differentially-dissected fragments studied here. (B) Superimposition of NMR $^{1}H-^{15}N$ HSQC spectra of TDP-43 (1–269) in 1 mM phosphate buffer at pH 5.0 (blue) and TDP-43 (1–102) in Milli-Q water at pH 4.0 (red). Some N-Domain residues with their HSQC peaks superimposable in two fragments are labeled. The red box is used to indicate HSQC peaks of five Gly residues from the unfolded form. (C) Superimposition of HSQC spectra of TDP-43 (1–269) (blue) and TDP-43 (102–269) (red) in 1 mM phosphate buffer at pH 5.0. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

key to understanding the structure–function relationship of TDP-43.

2. Materials and methods

2.1. Protein samples

The DNA fragments encoding the full-length TDP-43 and its five differentially-dissected fragments (Fig. 1A) were amplified by PCR reactions with designed primers from the full-length TDP-43 gene and subsequently cloned into a modified vector pET28a with 6 His residues at C-terminus, which we previously used [10,11]. The expression vectors were transformed into and overexpressed in *Escherichia coli* BL21 (DE3) cells (Novagen). The recombinant proteins except for TDP-43 (102–269) were all found in inclusion body. As a result, TDP-43 (102–269) was purified by a Ni^{2+}-affinity column (Novagen) under both native conditions and denaturing condition, while for other proteins, their pellets were first dissolved in a phosphate buffer (pH 8.5) containing 8 M urea and subsequently purified by a Ni^{2+}-affinity column under denaturing conditions in the presence of 8 M urea. The fractions containing the recombinant proteins were acidified by adding 10% acetic acid and subsequently purified by reverse-phase (RP) HPLC on a C4 column eluted by water-acetonitrile solvent system. The HPLC elutions containing pure recombinant proteins were lyophilized.

The generation of the isotope-labeled proteins for NMR studies followed a similar procedure except that the bacteria were grown in M9 medium with the addition of $(^{15}NH_4)_2SO_4$ for $_{15}N$ labeling and $(^{15}NH_4)_2SO_4/[^{13}C]$-glucose for double labeling [10,11]. The purity of the recombinant proteins was checked by SDS–PAGE gels and their molecular weights were verified by a Voyager STR matrix-assisted laser desorption ionization time-of-flight-mass spectrometer (Applied Biosystems). The concentration of protein samples was determined by the UV spectroscopic method in the presence of 8 M urea. Briefly, under the denaturing condition, the extinct coefficient at 280 nm of a protein can be calculated by adding up the contribution of Trp, Tyr and Cys residues [15].

2.2. NMR experiments

All NMR experiments were acquired on an 800 MHz Bruker Avance spectrometer equipped with pulse field gradient units as described previously [10,11]. To achieve the sequential assignment of the prion-like domain, a pair of triple-resonance experiments HNCACB, CBCA(CO)NH were collected for the sequential assignment on a $^{15}N-/^{13}C$-double labeled sample of 500 μM, while ^{15}N-edited HSQC-TOCSY and HSQC-NOESY were collected on a ^{15}N-labeled sample at a protein concentration of 500 μM [11].

To assess the inter-domain interactions of TDP-43 by comparing the conformational properties of the full-length TDP-43 and its five differentially-dissected fragments (Fig. 1A), their NMR experiments were acquired at a protein concentration of 40 μM in 1 mM phosphate buffer at pH 5.0 because the full-length TDP-43, TDP-43 (1–269) and TDP-43 (102–414) became aggregated quickly at higher concentrations.

To map out the interacting residues in TDP-43 N-domain and RRMs, ^{15}N-labeled N-Domain TDP-43 (1–102), TDP-43 (102–269) composed of two RRMs, and TDP-43 (1–269) containing both N-Domain and two RRMs at a protein concentration of 40 μM in 1 mM phosphate buffer (pH 5.0) were respectively titrated by adding the unlabelled prion-like domain TDP-43 (263–414) at different molar ratios. For TDP-43 (1–102) and TDP-43 (1–269), the addition of the unlabelled prion-like domain even at a molar ratio of 0.5 triggered severe precipitation of the samples and thus no further NMR

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