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ALS-causing profilin-1-mutant forms a non-native helical structure in membrane environments☆,☆☆

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ABSTRACT

Despite having physiological functions completely different from superoxide dismutase 1 (SOD1), profilin 1 (PFN1) also carries mutations causing amyotrophic lateral sclerosis (ALS) with a striking similarity to that triggered by SOD1 mutants. Very recently, the C71G-PFN1 has been demonstrated to cause ALS by a gain of toxicity and the acceleration of motor neuron degeneration preceded the accumulation of its aggregates. Here by atomic-resolution NMR determination of conformations and dynamics of WT-PFN1 and C71G-PFN1 in aqueous buffers and in membrane mimetics DMPC/DHPC bicelle and DPC micelle, we deciphered that: 1) the thermodynamic destabilization by C71G transforms PFN1 into coexistence with the unfolded state, which is lacking of any stable tertiary/secondary structures as well as restricted ps-ns backbone motions, thus fundamentally indistinguishable from ALS-causing SOD1 mutants. 2) Most strikingly, while WT-PFN1 only weakly interacts with DMPC/DHPC bicelle without altering the native structure, C71G-PFN1 acquires abnormal capacity in strongly interacting with DMPC/DHPC bicelle and DPC micelle, energetically driven by transforming the highly disordered unfolded state into a non-native helical structure, similar to what has been previously observed on ALS-causing SOD1 mutants. Our results imply that one potential mechanism for C71G-PFN1 to initiate ALS might be the abnormal interaction with membranes as recently established for SOD1 mutants.

1. Introduction

Amyotrophic lateral sclerosis (ALS), the most common motor neuron disease, clinically characterized by a relentless progressive loss of motor neurons in the brain and spinal cord, was first described in 1869 but its mechanism still remains a great mystery [1]. In 1993, human superoxide dismutase 1 (SOD1) was identified to be the first gene associated with familial ALS and to date > 20 ALS-causative genes have been identified [1,2]. In 2012, mutations in the profilin 1 (PFN1) gene were found to cause ALS [1,3,4]. Human PFN1 is an 140-residue small protein (Fig. S1A) that regulates actin polymerisation with mechanisms not entirely understood as well as other cellular functions including actin dynamics, membrane trafficking, neuronal synaptic structure and activity, small GTPase signaling through binding to an array of proteins carrying poly-L-proline motifs [5–7]. Additionally, PFN1 also binds to lipids such as phosphatidylinositol-4,5-bisphosphate (PIP2) [5,6], as well as reversibly interacts with membranes [8].

PFN1 adopts a well-structured globular fold, in which a seven-stranded antiparallel β-sheet is sandwiched by N- and C-terminal α-helices on one face of the sheet; and three small helical regions on the opposite face [9–11]. Recently, ALS-causing mutations have been demonstrated to thermodynamically destabilize PFN1 [11], as well as to cause structural perturbations to the native state to different degrees [12,13] with the extent of these phenomena correlating with their tendency to aggregate *in vitro* and in mammalian cells. Interestingly, the C71G mutation has been characterized to structurally trigger a significant change of the native state [12], and to thermodynamically destabilize its stability [11]. Protein aggregation is universally characterized by all neurodegenerative diseases [14,15], and for PFN1-associated ALS, aggregation of ALS-causing mutants has been previously proposed to trigger ALS by gain-of-toxic-function or/and loss-of-normal function mechanisms [16]. Very recently, the C71G-PFN1 mutant has been characterized to cause ALS phenotypes in mice by a gain of toxicity, and the acceleration of motor neuron degeneration preceded the accumulation of its aggregates [17]. As such, it is of both fundamental and therapeutic interest to explore the underlying mechanism for the gain of toxicity of C71G-PFN1.

Noticeably, although PFN1 and SOD1 share no functional similarity,

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☆☆ The structure coordinate of C71G-PFN1 in DPC micelle has been deposited in PDB with ID of 5WXW and the associated NMR data were deposited in BMRB with ID of 36042.
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it was also found that the mutant PFN1 and mutant SOD1 models show the most striking similarity in the relentless progression of motor neuron degeneration in neuropathology [17]. This observation is reminiscent of the recent report that it is the abnormal insertion of the SOD1 mutants into ER membranes in motor neurons, which triggers ER-stress, an initial event in ALS pathogenesis [18]. Indeed, ALS-causing SOD1 mutants have been previously revealed to be also capable of tightly associating with the outer mitochondrial membrane as an integral membrane protein, with the association even resistant to high ionic strength and high pH [19]. Furthermore, our NMR studies deciphered a common biophysical mechanism by which ALS-causing truncation [20] or depletion of zinc ion [21] transforms the cytosolic SOD1 into membrane-interacting forms. Briefly, these ALS-causing factors act to delay/impair the maturation of the unfolded nascent state into the well-folded SOD1 native structure which adopts an eight-stranded Greek-key β-barrel. As a consequence, the amphiphilic or/and hydrophobic regions universally existing in proteins including SOD1 are unlocked and exposed for abnormally interacting with membranes, which is driven by hydrogen bond energetics gained from the establishment of a large number of hydrogen bonds upon forming helical conformations in membrane environments [22,23].

Here, we attempted to understand the biophysical mechanisms by which the ALS-causing mutation triggers aggregation of PFN1 and leads to a gain of toxicity by determining the high-resolution conformations and dynamics of both wild-type (WT) and C71G mutant of PFN1 in aqueous buffers and membrane environments with NMR spectroscopy. The results decode that C71G-PFN1 coexists with the unfolded state, which is highly disordered and lacking of any stable secondary structures with unrestricted backbone motions on ps-ns time scale. Most importantly, while the wild-type (WT) PFN1 only reversibly interacts with membranes required for its physiological functions; C71G-PFN1 acquires abnormal capacity in strongly interacting with membranes by forming a non-native helical conformation as determined by NMR. Therefore, our results implies that like SOD1 mutants [18], the acquired capacity of C71G-PFN1 in strongly interacting with membranes might at least represents one potential mechanism for triggering the onset of ALS. Furthermore, the coupled abilities in membrane interaction and aggregation might significantly enhance its toxicity responsible for ALS pathogenesis.

2. Results

2.1. WT-PFN1 only reversibly interacts with membrane

To allow unambiguous comparison of NMR parameters of WT- and C71G-PFN1, we first achieved NMR sequential assignment of WT-PFN1 by analyzing a pair of triple-resonance NMR spectra CBCA(CO)NH, HN(CO)CACB; and Fig. S1B presents its (ΔCα-ΔCβ) chemical shifts, which represent a sensitive indicator of secondary structures for both folded and unfolded proteins [20,21,24–26]. Despite a slight difference in buffer pH values between our present sample (pH 6.8) and a previous one for determining NMR structure of WT-PFN1 (pH 6.4) [9], the (ΔCα-ΔCβ) chemical shifts are almost identical (Fig. S1B). Furthermore, only a small set of HSQC peaks showed significant shifts upon lowering pH value from 6.8 to 6.0 (Fig. S1C). Furthermore, even at pH 4.0, WT-PFN has HSQC spectrum with only well-dispersed peaks, many of which are superimposable to those at pH 6.8.

To further assess the interaction of WT-PFN1 with membrane, we titrated WT-PFN1 with large bicelles composed of dimyristoylphosphatidylcholine (DMPC) and dihexanoylphophatidylcholine (DHPC) at a q value of 4, which resemble native bilayer membranes [27], as we previously conducted on identifying the membrane-interacting subdomain within the TDP-43 prion-like domain [26]. Gradual addition of DMPC/DHPC bicelle triggered the stepwise shifts of a small set of HSQC peaks (Fig. 1A). Interestingly, when the residues with shifted HSQC peaks were mapped back to the crystal structure of WT-PFN1 [11], all residues are located on the N- and C-terminal helices constituting one face of the structure, except for Gly95, Lys105 and Lys108. Gly95 is located in a missing loop in the crystal structure (PDB ID of 4X1L), while Lys105 and Lys108 are within another loop but have close contacts with the residues of the N- and C-terminal helices (Fig. 1B and C). However it is important to point out that some residues with shifted HSQC peaks may not be directly involved in binding to bicelles as the shift of HSQC peaks can result not only from the direct binding of the amide protons to bicelles, but also from the changes of conformations/dynamics induced by the binding event [28].

Furthermore, even at a ratio of 1:600 (PFN1:Bicelle), no disappearance was observed for HSQC peaks and also the peak shifts remained unsaturated. This observation indicates that the binding affinity of WT-PFN1 to bicelles is relatively low and reversible. Moreover, the interaction with bicelle appears to have no significant effect on the three-dimensional structure of PFN1 because no significant change was observed on its HSQC spectra. Interestingly, however, as judged from what we previously observed on the native VAPB-MSP domain and SOD1 that their HSQC peaks showed no shifts upon being exposed to membrane environments [20,21,29,30], the current result implies that WT-PFN1 indeed has weak capacity in reversibly interacting with membranes, which is completely consistent with the previous result that WT-PFN1 needs to be reversibly localized to the plasma membrane, which is required for its physiological functions [8].

2.2. C71G-PFN1 co-exists with the unfolded state

Exactly as previously reported [11], we also found that the majority of the recombinant C71G-PFN1 protein was in inclusion body. On the other hand, however, in 2005 we found that unlike the well-folded proteins following the “Salting-in” rule that protein solubility increases upon adding salt ions over the range of low salt concentrations (usually < 300–500 mM), “insoluble” proteins could only be solubilized in aqueous solution with the minimized salt concentrations [20,21,25,26,29–32]. This allowed us to determine NMR structures of aggregation-prone TDP-43 N-domain [25] and C-terminal domain [26], as well as ALS-causing SOD1 mutants [20,21]. Here we have tested a variety of buffer conditions and found that despite becoming aggregated in high-salt buffers, the C71G-PFN1 protein could be dissolved in 1 mM phosphate buffer at different pH values, thus allowing our further characterization of the conformations of C71G-PFN1.

At pH 6.8, the C71G-PFN1 protein appeared to form tiny aggregates even at a protein concentration of 5 μM, thus giving unacceptable CD spectrum with the machine HT > 700 voltage (Fig. 2A) and no detectable NMR heteronuclear single quantum coherence (HSQC) signals. However, at pH 6.0, we were able to collect a high-quality HSQC spectrum of C71G-PFN1, in which two sets of HSQC peaks were detected: one has weak intensity but large ^{1}H- and ^{15}N-spectral dispersions with many peaks even superimposable to those of WT-PFN1 at pH 6.0; while another has strong intensity but narrow spectral dispersions typical of a highly disordered state (Figs. 2B and 3A). However, it is impossible to reach a conclusion that the unfolded population is much higher than the folded one because in general even for the same protein at the same molar concentration, the unfolded form will give rise to HSQC peaks with the intensity many time higher than those of the folded form due to the significant differences in relaxation times for the folded and unfolded forms. The existence of the exchanges between the folded and unfolded states on the μs-ms time scale will further complex the relationship between HSQC peak intensities and populations, which is dependent of exchange rates, populations and chemical shift differences. Depending on the exchange time scales, different NMR dynamics methods are needed to quantify the exchange parameters, as we previously utilized HSQC-NOESY experiment to characterize the millisecond conformational exchanges of the TDP-43 N-domain [25], as well as CPMG-based relaxation dispersion experiments to investigate the microsecond conformational exchanges of EphA4 receptor in