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SALS-linked WT-SOD1 adopts a highly similar helical conformation as FALS-causing L126Z-SOD1 in a membrane environment

Liangzhong Lim, Jianxing Song *

Department of Biological Sciences, Faculty of Science, National University of Singapore, Republic of Singapore

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ABSTRACT

So far >180 mutations have been identified within the 153-residue human SOD1 to cause familial amyotrophic lateral sclerosis (FALS), while wild-type (WT) SOD1 was intriguingly implicated in sporadic ALS (SALS). SOD1 mutations lead to ALS by a dominant gain of cytotoxicity but its mechanism still remains elusive. Previously functional studies have revealed that SOD1 mutants became unexpectedly associated with organelle membranes. Indeed we decoded that the ALS-causing truncation mutant L126Z-SOD1 with an elevated toxicity completely loses the ability to fold into the native β-barrel structure but acquire a novel capacity to interact with membranes by forming helices over hydrophobic/amphiphilic segments. Very recently, the abnormal insertion of SOD1 mutants into ER membrane has been functionally characterized to trigger ER stress, an initial event of a cascade of cell-specific damages in ALS pathogenesis. Here we attempted to understand the mechanism for gain of cytotoxicity of the WT SOD1. We obtained atomic-resolution evidence that the nascent WT SOD1 without metalation and disulfide bridge is also highly disordered as L126Z. Most importantly, it owns the same capacity in interacting with membranes by forming very similar helices over the first 125 residues identical to L126Z-SOD1, plus an additional hydrophobic helix over Leu144-Ala152. Our study thus implies that the WT and mutant SOD1 indeed converge on a common mechanism for gain of cytotoxicity by abnormally interacting with membranes. Moreover, any genetic/environmental factors which can delay or impair its maturation might act to transform SOD1 into cytotoxic forms with the acquired capacity to abnormally interact with membranes.

1. Introduction

Amyotrophic lateral sclerosis (ALS), the most common motor neuron disease clinically characteristic of the degeneration of motor neurons in the brain and spinal cord, was first described in 1869 but its mechanism still remains a great mystery [1]. While a list of genes has been linked to familial ALS (FALS), the cause of sporadic ALS (SALS) accounting for ~90% of ALS cases still remains poorly understood. Nevertheless, sporadic and familial ALS affect the same neurons with the clinical similarities, and consequently it is believed that both forms of the disease converge on common pathways or/and involve common factors.

In 1993, human superoxide dismutases 1 (SOD1) comprising ~1% of total protein in neurons was identified to be the first gene associated with FALS [1–3]. The mature homodimeric SOD1 acts to catalyze the dismutation of superoxide radicals to molecular oxygen and hydrogen peroxide, with each subunit folding into an eight-stranded, Greek-key β-barrel and holding one copper and one zinc ions, which is further stabilized by an intramolecular disulfide bridge Cys57-Cys146 [3–5] (Fig. 1A). Currently, >180 mutations have been identified within this 153-residue protein that accounting for ~20% of total FALS cases (http://alsod.iop.kcl.ac.uk/) [4]. Amazingly, evidence has recently emerged that the wild-type (WT) SOD1 is linked to SALS [6]. It has been well established that mutations in SOD1 lead to ALS by a dominant gain of cytotoxicity but its exact mechanism still remains poorly understood [1,3,5,6].

Surprisingly, previous *in vivo* studies have deciphered that ALS-causing SOD1 mutants became associated with mitochondria and ER membranes [7–9]. In particular, a misfolded mutant SOD1 has been found to be associated with the outer mitochondrial membrane as an integral membrane protein, with the association resistant to high ionic strength and high pH [8]. Indeed, recently by NMR spectroscopy we decoded the biophysical mechanism for this transformation. Briefly, the L126Z truncation removing the C-terminal 28 residues of SOD1 leads to a mutant with an elevated cytotoxicity as compared to other ALS-causing point mutants, which completely loses the ability to fold into the native β-barrel structure and becomes highly-disordered even in the presence of zinc ion. Consequently, the disordered L126Z-SOD1 acquires a novel capacity to interact with membranes energetically

* Corresponding author.
E-mail address: dbssjx@nus.edu.sg (J. Song).

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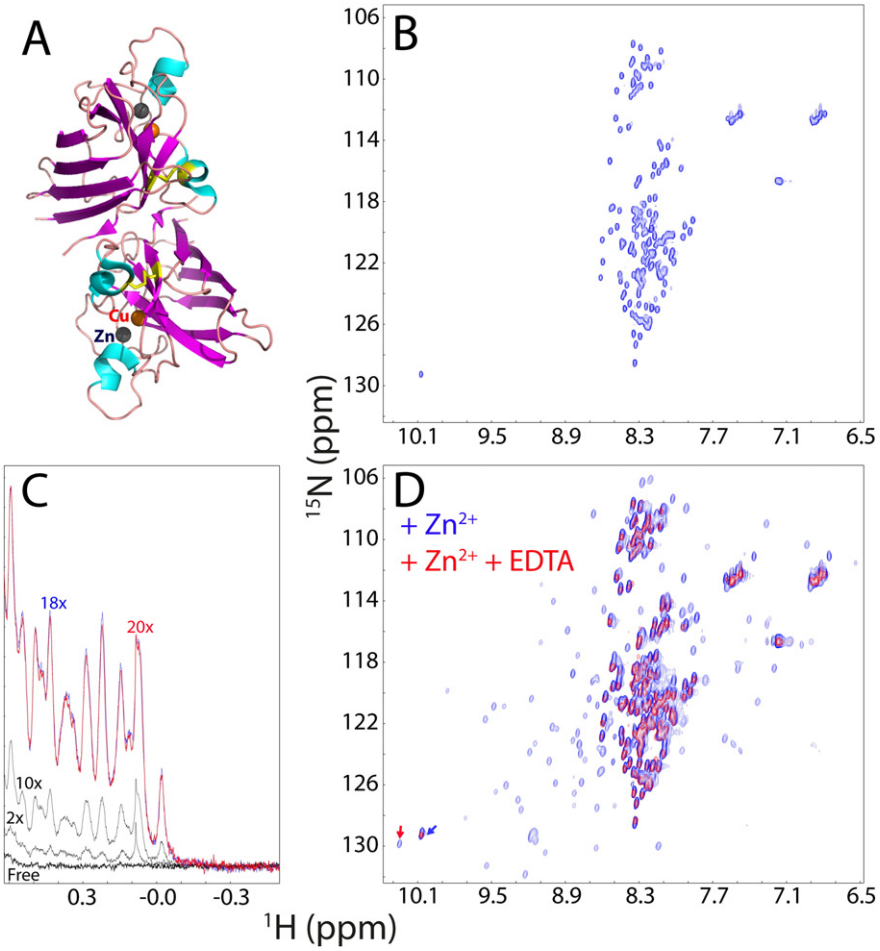

Fig. 1. Zn^{2+} plays a central role in initiating the maturation of the WT human SOD1. (A) Ribbon representation of the dimeric crystal structure of the mature and active human SOD1 (PDB code of 1PU0). Zinc and copper ions are represented by grey and brown spheres respectively. (B) Two-dimensional ^{1}H–^{15}N NMR HSQC spectrum of the WT human SOD1 of the native sequence (free of any tag) without metallation and disulfide bridge. (C) Up-field 1D NMR peaks characteristic of the folded SOD1 (−0.5–0.62 ppm) in the presence of zinc at different molar ratios. (D) Superimposition of HSQC spectra of the WT human SOD1 (free of any tag) without the disulfide bridge but in the presence of zinc at a ratio of 1:40 (SOD1:zinc) (blue) and that after addition of EDTA at an equal molar ratio of zinc (red). Blue arrow is used for indicating HSQC peak of the Trp32 ring proton resulting from the unfolded ensemble while red arrow for that from the folded SOD1.

driven by forming short helices over amphiphilic/hydrophobic regions [10,11].

Most strikingly, by combining ribosome affinity purification and high-throughput sequencing, a cascade of cell type-specific damages has been now identified in mutant SOD1-mediated ALS [12]. The abnormal insertion of SOD1 mutants into ER membranes in motor neurons has been established to trigger ER-stress even without any detectable SOD1 aggregate, which represents an initial event in ALS pathogenesis [12]. Together, these findings suggest a mechanism by which the ALS-causing SOD1 mutants gain cytotoxicity by acquiring a novel capacity in interacting with membranes. Therefore, a key question arises as whether under some pathological conditions, the WT SOD1 without any mutations might also be capable of interacting with membranes? If yes, this might rationalize the linkage of the WT SOD1 to SALS in light of the recent report [12].

Here, we address this question by NMR determination of the conformations and dynamics of the WT human SOD1 of the native sequence in aqueous solution and membrane environment. We obtained atomic-resolution evidence that the nascent WT SOD1 without metalation and disulfide bridge is also highly disordered without highly-restricted backbone motions as L126Z. Most interestingly, the nascent and disordered SOD1 also owns the capacity in interacting with membranes to transform into a helical conformation. Therefore, our study implies that the WT and mutant SOD1 indeed converge on a common mechanism for gain of cytotoxicity, and to increase the effective cellular concentration of zinc might represent an important therapy for some SALS patients caused by the WT SOD1.

2. Results

2.1. The nascent WT SOD1 is highly-disordered in the absence of Zn^{2+}

Previously, to overcome severe aggregation and formation of various non-native disulfide cross-linked species for the WT human SOD1 [5,10,13,14], super-stable pseudo wild-type sequences were used in which Cys residue (Cys 6, Cys 57, Cys 111 and Cys 146) were replaced by Ala or Ser to mimic the nascent SOD1 which has not been loaded with metal ions and is lacking of the disulfide bridge. However, as compared to the human WT SOD1, these pseudo wild-type SOD1 proteins showed significantly enhanced thermodynamic stability as previously shown by various biophysical characterizations [5], and consequently they already had a folded β-barrel even without metallation and disulfide bridge [5,13,14]. On the other hand, as facilitated by our previous discovery that protein aggregation can be significantly minimized by reducing salt concentrations