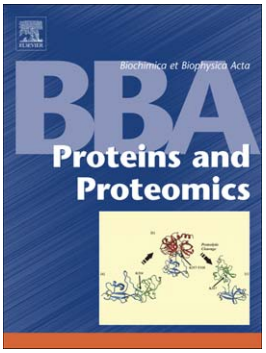


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# The unfolding mechanism of monomeric mutant SOD1 by simulated force spectroscopy

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**Abstract**

Mechanical unfolding of mutated apo, disulfide-reduced, monomeric superoxide dismutase 1 protein (SOD1) has been simulated via force spectroscopy techniques, using both an all-atom (AA), explicit solvent model and a coarse-grained heavy-atom Gō (HA-Gō) model. The HA-Gō model was implemented at two different pulling speeds for comparison. The most-common sequence of unfolding in the AA model agrees well with the most-common unfolding sequence of the HA-Gō model, when the same normalized pulling rate was used. Clustering of partially-native structures as the protein unfolds shows that the AA and HA-Gō models both exhibit a dominant pathway for early unfolding, which eventually bifurcates repeatedly to multiple branches after the protein is about half-unfolded. The force-extension curve exhibits multiple force drops, which are concomitant with jumps in the local interaction potential energy between specific $\beta$-strands in the protein. These sudden jumps in the potential energy coincide with the dissociation of specific pairs of $\beta$-strands, and thus intermediate unfolding events. The most common sequence of $\beta$ strand dissociation in the unfolding pathway of the AA model is $\beta$-strands 5, 4, 8, 7, 1, 2, then finally $\beta$-strands 3 and 6. The observation that $\beta$-strand 5 is among the first to unfold here, but the last to unfold in simulations of loop-truncated SOD1, could imply the existence of an evolutionary compensation mechanisms, which would stabilize $\beta$-strands flanking long loops against their entropic penalty by strengthening intramolecular interactions.

*Keywords:* Protein misfolding, Molecular dynamics simulation, Superoxide dismutase 1, Amyotrophic Lateral Sclerosis, Single molecule force spectroscopy, Coarse-grained protein model.

## 1. Introduction

Over 160 mutations throughout the homodimeric antioxidant protein Cu, Zn superoxide dismutase (SOD1) have been found to be associated with a familial form of amyotrophic lateral sclerosis (fALS), affecting about 1/5 of those with autosomal dominant inheritance [1, 2]. Many of these mutations have been observed to show weakened dimer and/or folding stability [3, 4, 5, 6, 7, 8], and accelerate fibril elongation (but not always fibril nucleation) [7, 8]. In fALS cases, and in at least some of the more prevalent cases of sporadic ALS, patients display intraneuronal immunoreactivity to SOD1 misfolding-specific antibodies [9, 10, 11]. This suggests a potential fundamental role for the misfolding and propagation of SOD1 in the pathogenesis of at least some cases of ALS.

Many proteins implicated in misfolding-related degenerative disease form neurotoxic aggregates by intermolecular association of partially unfolded structures of monomer [12, 13].In the context of SOD1 misfolding related ALS, coarse-grained simulations of the unfolding pathway of WT and mutant SOD1 monomer exhibit multiple partially-unfolded intermediates that can determine fibril morphology [14]. The gain of transient oligomer interactions due to the partial disorder of the protein in the electrostatic loop region has been investigated computationally [15]. Thus, a molecular dissection of unfolding intermediate structures in more accurate, all-atom simulation models can provide insight into the conformational pathway involved in the seeding of multimeric oligomers that are involved in the infectious prion-like propagation [16, 17] of this currently incurable disease.

SOD1 has a $\beta$ barrel structure with two long loops dressing the core. Both of these loops have residues enabling metals to bind to the protein. A Cu ion imparts

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