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An in silico study of the effect of SOD1 electrostatic loop dynamics on amyloid-like filament formation

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Abstract

Superoxide dismutase [Cu–Zn], or SOD1, is a homo-dimeric protein that functions as an antioxidant by scavenging for superoxides. A wide range of SOD1 variants are linked to inherited, or familial, amyotrophic lateral sclerosis, a progressive and fatal neurodegenerative disease. Aberrant SOD1 oligomerization has been strongly implicated in disease causation, even for sporadic ALS, or SALS, which accounts for ~90 % of ALS cases. Small heat shock proteins (sHSP) have been shown to protect against amyloid fibril formation in vitro, and the sHSP α B-crystallin suppresses in vitro aggregation of SOD1. We are seeking to elucidate the structural features of both SOD1 amyloid formation and α B-crystallin amyloid suppression. Specifically, we have used a flexible docking protocol to refine our model of a SOD1 non-obligate tetramer, postulated to function as a transient desolvating complex. Homology modeling and molecular dynamics (MD) are used to supply the missing structural elements of a previously characterized SOD1 amyloid filament, thereby providing a structural analysis for the observed gain of interaction. This completed filament is then further modified using MD to provide a structural model for protofibril capping of SOD1 filaments by α B-crystallin.

Keywords

Superoxide dismutase; Amyotrophic lateral sclerosis; Electrostatic loop; α B-crystallin

Introduction

The conversion of peptides and proteins from their soluble forms into insoluble fibrillar aggregates is responsible for a variety of pathological conditions, including Alzheimer's disease (AD), Parkinson's disease (PD), and amyotrophic lateral sclerosis (ALS). Not surprisingly, therapeutic development has been driven by the paradigm that formation of these highly organized fibrillar aggregates leads to neurodegeneration and death (Hardy and Higgins 1992)—yet the relevance of plaques, or extracellular fibrils, to AD pathogenesis remains unclear, as substantial neuronal dysfunction occurs prior to the appearance of

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Electronic supplementary material

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fibrillary deposits (Klein et al. 2001). In fact, there is a growing body of evidence to suggest that these fibrils may simply be end-stage products, with the primary toxic element being a pre-fibrillar oligomer (Caughey and Lansbury 2003). The failure of therapeutic strategies based on fibril elimination (Kirkitadze et al. 2002; Haass and Selkoe 2007) is now leading to a shift in focus away from deposition and towards aggregation and oligomerization as mechanisms underpinning pathogenesis (Redler et al. 2014).

ALS is linked to misfolding and aggregation of superoxide dismutase (SOD1), with over 90 % of sporadic cases and also the remaining familial cases associated with a wide array of inherited mutations. Although it is known that oligomeric assemblies of both wild-type (wt) and mutant SOD1 are precursors to larger and detergent-insoluble aggregates, the structural events triggering oligomerization remain to be determined. It has also been found that mutant misfolded SOD1 can convert wtSOD1 in a prion-like fashion (Grad et al. 2011), and that misfolded wtSOD1 can be propagated by release and uptake of protein aggregates (Grad et al. 2014). The conformational change hypothesis of globular protein aggregation (Kelly 1998) is based on the concept that partial unfolding constitutes an initiating event. These partially unfolded conformational states, while thermodynamically distinct from the native state, can be structurally similar to it and accessed by thermal fluctuations (Chiti and Dobson 2009). A recent NMR analysis of SOD1 folding and misfolding pathways identified thermal fluctuations within the electrostatic loop, or loop VII, of SOD1 that mediate the formation of aberrant oligomers (Sekhar et al. 2015). Structural and dynamic change affecting this loop has previously been identified as a shared property of 13 familial ALS-related SOD1 variants (Molnar et al. 2009).

Small heat shock proteins (sHsp) are ATP-independent molecular chaperones that are widely expressed in numerous species. They are induced in various cell types when subjected to stress stimuli, where they bind to misfolded proteins to prevent irreversible aggregation. Aided by ATP-dependent chaperones, this high molecular mass complex is then refolded to a competent state. All sHsps contain variable N- and C- termini, in addition to a conserved α -crystallin domain critical for chaperone activity and oligomerization. Two vertebrate sHsps, α A-crystallin and α B-crystallin, are major cytoplasmic components of the human eye lens (Ecroyd and Carver 2009), and α B-crystallin is also expressed in several other cell types, including neurons (Horwitz 2003). The chaperone activity of the sHSP dimer of the α -crystallins is thought to necessitate either subunit exchange or subunit dissociation that exposes various hydrophobic interface sites (Gu et al. 2002; Claxton et al. 2008). This in turn results in the exposure of substrate-binding sites that facilitate the sequestration of the target complex, thus preventing aggregation. In addition to maintaining homeostasis by protecting against protein misfolding, some sHsps have also been shown to protect against amyloid fibril formation (Ecroyd and Carver 2009). Most notably, α B-crystallin has been identified as a suppressor of spinocerebellar ataxia 3 (SCA3) toxicity (Bilen and Bonini 2007), most likely through the formation of a transient α B-crystallin/ataxin-3 complex (Robertson et al. 2010). Recently, α B-crystallin has also been shown to suppress SOD1 aggregation in vitro, most likely by binding to aggregated forms of SOD1 and preventing fibril elongation (Yerbury et al. 2013).

By focusing on solvent-exposed intramolecular backbone hydrogen bonds as physico-chemical descriptors for protein packing (Fernández and Scheraga 2003; Fernández and Ridgway 2003), we have previously developed a mechanism of action for small heat shock proteins (sHsp) (Healy and King 2012; Healy 2012). This model in turn has been utilized to provide a structural basis for describing the modulation of SCA3 toxicity by α B-crystallin (Healy et al. 2014). Most recently, we have presented a role for a transient, non-obligate oligomer in the formation of SOD1 fibrillar aggregates (Healy 2015). This paper seeks to build on this analysis by probing two SOD1 pathogenic variants, G37R and H46R, in order to investigate further the possibilities of this oligomeric model, and also to provide a structural basis for understanding the suppression of SOD1 aggregation by α B-crystallin.

Methods

The crystal structures for wild type (wt), the G37R mutant, and the metal-deficient H46R mutant of human superoxide dismutase are available from the RCSB (www.rcsb.org) as PDB entries 2C9V (Strange et al. 2006), 1AZV (Hart et al. 1998), and 1OZT (Elam et al. 2003), respectively. Although the β -barrel core of the H46R mutant is preserved relative to wt SOD1, both the zinc and electrostatic loops exhibit significant disorder. The missing residues in 1OZT were “grafted” from the appropriate regions in 2C9V and the secondary structure refined using the loop-refinement capability of the MODELER protocol, as implemented in the Discovery Studio program suite. After adding hydrogens, all proteins were subjected to a short energy minimization using the CHARMM force field (Brooks et al. 1983).

The complete H46R amyloid-like filament was next minimized by steepest descent and conjugate gradient, using the Generalized Born with simple Switching (GBSW) implicit solvent model. A fixed-atom restraint was then applied to those residues in the crystal structure that were well characterized, and the complex was heated to 300 K over 100 ps. The structure shown in Fig. 1b was obtained after equilibration at 300 K for 250 ps. The SHAKE algorithm was employed to keep bonds involving hydrogen atoms at their equilibrium length, allowing the use of a 2-fs time step.

Co-ordinates for the core domain of human α B-crystallin crystallized with a palindromic peptide, used to mimic the sHsp C-terminal region, are available from the RCSB (www.rcsb.org) as PDB entry 4M5S (Hochberg et al. 2014). A model of the H46R dimer bound to α B-crystallin was generated by aligning the linear portion of the electrostatic loop for the H46R mutant dimer with the C-terminal peptide found in 4M5S, and superimposing the two proteins using of the MODELER protocol, as implemented in the Discovery Studio program suite. This has the effect of replacing the cleft between the β_5 and β_6 strands of the SOD1 β -barrel core with the β_4 – β_8 hydrophobic groove of the α B-crystallin core as the point of contact for the extended SOD1 electrostatic loop responsible for the “gain-of-interaction” (GOI) found in the amyloid-like filament. This complex was minimized by steepest descent and conjugate gradient, using the Generalized Born with simple Switching (GBSW) implicit solvent model. A harmonic restraint was then applied to all residues except those previously identified as disordered in 1OZT. The complex was heated to 300 K over 100 ps. After equilibration at 300 K for 250 ps, a 1-ns molecular dynamics (MD) production

was run at 300 K. The SHAKE algorithm was employed to keep bonds involving hydrogen atoms at their equilibrium length, allowing the use of a 2-fs time step.

The SwarmDock algorithm combines local docking and particle swarm optimization to find low-energy orientations for ligand and receptor proteins (Torchala et al. 2013). Normal modes are used to model transitions between bound and unbound conformations, and low-energy conformations are returned as a ranked list of clustered docking poses (Moal and Bates 2010). We have previously used the rigid docking protocol ZDOCK (Chen et al. 2003) to identify a transient, non-obligate tetramer of wt SOD1 that is postulated to play a role in the formation of aberrant SOD1 protein aggregates. Using the SwarmDock algorithm, we have undertaken a full-blind flexible protein–protein dock of the G37R SOD1 dimer to identify the extent of any conformational change associated with such a protein–protein interaction (PPI). The pose shown in Fig. 1a was returned by the SwarmDock server (<http://bmm.crick.ac.uk/~SwarmDock/>) as the lowest energy structure for the highest-ranked cluster, post-filtering for non-funnel-like energy structures.

Results and discussion

Based on the pattern of solvent-exposed hydrogen bonds identified for SOD1, we have previously proposed a role for SOD1 oligomerization based on the need to protect, or desolvate, these areas of vulnerability shared by wtSOD1 and several pathogenic mutants including G37R (Healy 2015). The rigid protein–protein docking solution of wt SOD1 that gave the greatest solvent excluded surface area upon dimer–dimer contact was found to be a pose where the electrostatic loop of one dimer–dimer contact is “patched” by residue Trp₃₂ of the other. In turn, the D₂ symmetry of the tetramer ensured that a corresponding Trp₃₂ patch is also found further along the interface. The optimum pose from the flexible protein–protein dock of G37R SOD1, Fig. 1a, exhibits a similar symmetry with the Trp₃₂ (shown as space-filled residue in Fig. 1a) of one dimer contact patching the electrostatic loop (shown as a line ribbon in Fig. 1a) of the other. While the root mean squared difference (RMSD), for the main chain atoms, between the rigid solution for wtSOD1 and flexible solution for G37R is relatively modest, at 2.1 and 1.4 Å for the “receptor” and “ligand” dimers respectively, the conformational change upon oligomerization does yield a substantial increase, from 1370 Å² for wtSOD1 to 2720 Å² for G37R, in solvent excluded surface area (SASA) upon dimer–dimer contact. The increase in SASA for the G37R mutant reflects a more effective oligomeric assembly. Given the main-chain RMSD values, it is clear that much of this increased SASA is attributable to greater packing efficiency of the side chains at the dimer–dimer interface of the G37R oligomer. However, a recent study has demonstrated a close association between subunit flexibility and the assembly of protein complexes (Marsh and Teichmann 2014). In turn, computational studies have indicated that 64 % of the G37R SOD1 residues are more flexible than the wild type (Khare and Dokholyan 2006), where the highest flexibility computed are for those helix-forming residues within the electrostatic loop. This correlates well with the dramatic subunit asymmetry observed in the G37R crystal structure, as well as with the higher crystallographic B-factors measured for those loop residues (Hart et al. 1998). Computationally, the more flexible G37R backbone allows for the sampling of a greater number of low-frequency normal modes, allowing greater conformational deformation upon subunit binding. This in turn would reflect the increased

conformational fluctuations associated with PPIs for flexible proteins in solution. More recently, it has been observed that a destabilizing mutation in the enzyme dihydrofolate reductase (DHFR) leads to a functional tetramerization of the otherwise monomeric enzyme (Bershtein et al. 2012). Thus PPIs have been shown to compensate for the protein destabilization that often accompanies mutation.

As we have previously demonstrated (Healy et al. 2009, 2014; Healy 2011), exposure of a region such as the short helix present in the electrostatic loop, where the hydrogen bonds are not well “wrapped” by non-polar carbonaceous groups, to solvent can lead to a loss of secondary structure. In this case, loss of secondary structure, and thus conformational change within the electrostatic loop, would be due to the disruption of Asn₁₃₁–Ser₁₃₄, Glu₁₃₂–Thr₁₃₅, and Glu₁₃₃–Lys₁₃₆ intra-helical hydrogen bonds, all of which we have shown to be vulnerable to solvent exposure (Healy 2015). Therefore, the secondary structure of the electrostatic loop, and thus the structural integrity of SOD1, is facilitated by formation of a transient, non-obligate SOD1 oligomer such as that shown in Fig. 1a.

Such a distortion as that described above would no longer require patching by the Trp residue, as the loss of the intra-helical hydrogen bonds, due to solvent exposure, negates the need for desolvation. X-ray structures with disordered loops have been reported for several SOD1 variants, including the H46R mutant (Antonyuk et al. 2005). An apo H46R crystal structure has also been obtained consisting of amyloid-like filaments in which adjacent dimers interact through a GOI contact between the unstructured electrostatic loop of one dimer and the cleft defined by edges of the β_5 and β_6 strands of an adjacent dimer (Elam et al. 2003). Our model of this filament, where the disordered loops have been refined as described, is shown in Fig. 1b. The orientation of the constituent β -strands perpendicular to the filament axis facilitates a closer packing of the β -barrels. As can be seen from Fig. 2a, and as noted previously (Elam et al. 2003), the GOI interfaces are symmetric around a dyad formed by the Leu₄₂ and Leu₁₂₆ residues of the participating subunits. The contacts at the loop–cleft interface are shown in Fig. 2b. In this model structure, a total of nine hydrogen bonds are responsible for the GOI, formed between the four residues making up the linear portion of the unstructured electrostatic loop and three residues on the β_5 - and one residue on the β_6 -strands.

The sHsp α B-crystallin co-localizes with SOD1 inclusions in humans (Kato et al. 1997), and increases in abundance in the spinal cord as ALS progresses in mouse models (Wang et al. 2008). More recently, the formation of a high molecular mass complex between SOD1 and α B-crystallin was shown to inhibit SOD1 aggregation in vitro by acting primarily on fibril elongation (Yerbury et al. 2013), an observation that would explain the presence of α B-crystallin in SOD1-positive inclusions. For the filament in Fig. 1b, such inhibition would necessitate a disruption of the GOI between the electrostatic loop and the β_5 – β_6 cleft. Since the α B-crystallin core domain has been previously shown capable of preventing amyloid fibrillation (Hochberg et al. 2014), we have constructed a SOD1- α B-crystallin model complex, Fig. 3a, where the cleft between the β_5 and β_6 strands of the SOD1 β -barrel core is replaced in the GOI with the β_4 – β_8 hydrophobic groove of the α B-crystallin core. Exposure of this groove has been shown to be sufficient in preventing amyloidogenic aggregation, even in the absence of the sHsp N- and C- termini (Hochberg et al. 2014; Mainz et al. 2015).

Model construction involved essentially replacing a peptide that co-crystallized with the α B-crystallin with the linear portion of the electrostatic loop of one of the dimers in Fig. 1b, and using MD to refine the interface.

A Ramachandran diagram for two residues of the short helix is used to analyze the 1-ns MD trajectory, as shown in Fig. 3b. The clustering of values for Glu₁₃₂, culminating in a final value for ϕ of -66.9° and a value for ψ of -51.3° (black square, Fig. 3b) highlights the relatively early formation of the N-terminal portion of the helix. The greater dispersion observed for Thr₁₃₅, culminating in a final value for ϕ of -45.5° and a value for ψ of -65.8° (black triangle, Fig. 3b) highlights the greater difficulty in reforming the C-terminal portion of the helix. Though this may be due in part to the harmonic restraint applied to the β -barrel core, nevertheless a well-defined element of secondary structure within the electrostatic loop can be seen in Fig. 3a. The extent of reformation of the short helix in the electrostatic loop is substantial, with a contraction of the length of the C $_{\alpha}$ (Asn₁₃₁)-C $_{\alpha}$ (Thr₁₃₇) helical axis from 14.23 Å at the beginning of the MD production run to a value of 11.07 Å for the structure shown in Fig. 3a. The contacts at the loop-groove interface for this modeled complex are shown in Fig. 3c. The single hydrogen bond is in marked contrast to the strength of interaction predicted for the amyloid filament, and while asparagine is formally hydrophilic, the orientation of the methylene group in the Asn side chain, coupled with the C-H bonds of the two adjacent glycines, does constitute a weakly hydrophobic interface to the β_4 - β_8 hydrophobic groove of the α B-crystallin. The combination is consistent with the observation that the intervention of α B-crystallin in amyloidogenesis involves a weak, transient interaction with the amyloid building blocks (Mainz et al. 2015).

While the existence of a transient non-obligate SOD1 tetramer must be considered speculative, the fact that two very different protein-protein docking methodologies, a previous fast-Fourier transform rigid protocol and the current full-blind flexible protocol, converged to near-identical solutions for both wtSOD1 and a pathogenic variant, does lend support to the thesis. Further support is provided by the fact that allowing for conformational change results in a substantial gain of SAS area upon oligomerization. More intriguing is the symmetry of the predicted tetramer relative to that of the dimer. It has been previously noted that in the evolution of protein complexes, dihedral and cyclic symmetries are geometrically related (Levy et al. 2008). Specifically, a complex with D_n symmetry can be formed from 2 n-mers with C_n symmetry (or n dimers with C₂ symmetry). This is approximately the relationship we see between the dimer characterized in the asymmetric symmetry unit (ASU) of both wtSOD1 and the G37R mutant, and our predicted tetramer. Such a relationship also raises the possibility that the ASU for a SOD1 variant crystallized in a space group higher than P2₁ (2C9V) or P4₁ symmetry (1AZV) might consist of a structure with more than two subunits. The G85R SOD1 mutant, available as pdb entry 2ZKX, crystallizes in the I2₁2₁2₁ space group, and the ASU is comprised of a tetramer nearly identical structure to shown in Fig. 1a.

In addition to the α -crystallin domain (ACD) shown in Fig. 3, sHsps are also composed of a flanking N-terminal domain and a smaller C-terminal polar extension. The latter contains a conserved IXI motif. While the N-terminal region more than likely contains one or more peptide binding regions, and the variable C-terminal loop functions as a patching extension

critical to higher-order oligomerization, recent work implicates the ACD of α B-crystallin in the prevention of amyloid fibrillation (29). More recently, it has been shown that α B-crystallin uses different interfaces to capture amorphous and amyloid clients (Mainz et al. 2015; Kulig and Ecroyd 2012), with the β_4 – β_8 groove of the ACD mediating the interaction with A β_{1-40} to inhibit fibril elongation. Aggregation assays indicate that this termination of fibrillary elongation occurs through the formation of complexes involving weak, transient interactions (Kulig and Ecroyd 2012). Interestingly, the idea that the non-polar elements of otherwise formally hydrophilic residues, in this case the methylene group in the Asn side chain, can be responsible for weak hydrophobic interactions has previously been highlighted in our study of the sHsp *Mycobacterium tuberculosis* α -crystallin (*Mtb* Acr) (Healy and King 2012). In that study, it was noted that for wheat HSP16.9 and *Mtb* Acr, even when the hydrophobic character of the residue is not conserved, the orientation of the side chains of the polar residues, Lys₆₆ in wheat HSP16.9 and Tyr₁₁₇ in *Mtb* Acr, is such that only the non-polar segments of the side chains are directed towards the interior of the β_4 – β_8 groove, thus maintaining the groove's hydrophobic character.

Thus we postulate the possibility that a non-obligate tetramer of SOD1 can be transiently formed both to compensate for the destabilizing effect of mutations, and to protect the erstwhile solvent-exposed hydrogen bonds of the electrostatic loop. Structural distortion, such as that which accompanies metal depletion, could interfere with this oligomerization, thus exposing the loop to the destabilizing effects of water. The subsequent loss of secondary structure would facilitate a new gain of interaction between the electrostatic loop of one dimer and the cleft between the β_5 and β_6 strands of the other. The packing and orientation of the of the SOD1 β -barrel strands that results is typical of that found in amyloid filaments. The β_4 – β_8 hydrophobic groove of the α B-crystallin core can substitute for the SOD1 β_5 – β_6 cleft, although doing so results in an interface stabilized by a single hydrogen bond and a weak hydrophobic interaction. The resulting reconstitution by α B-crystallin of the secondary structure of the SOD1 electrostatic loop provides a structural model for protofibril capping, and a possible mechanism for the observed in vitro suppression of SOD1 aggregation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Fig. 1.

a The lowest energy structure for the highest-ranked cluster for the flexible protein–protein dock of the G37R SOD1 dimer, where the electrostatic loop is shown as a *line-ribbon*, and the Trp32 “patching” residue is shown as *space-filled residue*. **b** Our model of the H46R filament, where the disordered loops have been refined as described, with the H46R mutation shown as *ball* and *stick*

Fig. 2.

a The symmetric "gain-of-interaction" (GOI) in the amyloid-like filament, formed by contact between the unstructured electrostatic loop of one dimer and the cleft defined by edges of the β_5 and β_6 strands of an adjacent dimer, showing the Leu₄₂ and Leu₁₂₆ dyad of the participating subunits. **b** The hydrogen-bonding contacts at the electrostatic loop/ β_5 - β_6 cleft interface

Fig. 3.

a The SOD1/αB-crystallin complex modeled as described in the text. **b** Trajectory analysis, of the 1 ns MD production run for the SOD1/αB-crystallin complex, shown as a Ramachandran diagram for two residues of the short helix: Glu₁₃₂ (*square, orange*) and Thr₁₃₅ (*triangle, teal*); the final angles for Glu₁₃₂ ($\phi = -66.9^\circ$, $\psi = -51.3^\circ$) and Thr₁₃₅ ($\phi = -45.5^\circ$, $\psi = -65.8^\circ$) shown as a *black square* and *black triangle*, respectively. **c** The contacts at the electrostatic loop/β₄-β₈ groove interface of the SOD1/αB-crystallin complex