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Review

Protein folding alterations in amyotrophic lateral sclerosis ☆

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ABSTRACT

Protein misfolding leads to the formation of aggregated proteins and protein inclusions, which are associated with synaptic loss and neuronal death in neurodegenerative diseases. Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease that targets motor neurons in the brain, brainstem and spinal cord. Several proteins misfold and are associated either genetically or pathologically in ALS, including superoxide dismutase 1 (SOD1), Tar DNA binding protein-43 (TDP-43), Ubiquilin-2, p62, VCP, and dipeptide repeat proteins produced by unconventional repeat associated non-ATG translation of the repeat expansion in C9ORF72. Chaperone proteins, including heat shock proteins (Hsp's) and the protein disulphide isomerase (PDI) family, assist in protein folding and therefore can prevent protein misfolding, and have been implicated as being protective in ALS. In this review we provide an overview of the current literature regarding the molecular mechanisms of protein misfolding and aggregation in ALS, and the role of chaperones as potential targets for therapeutic intervention.

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Contents

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1. Introduction

Protein folding is an important component of cellular protein homeostasis, biological pathways vital for cellular viability that ensure constant levels of proteins are present within the cell (Sin and Nollen, 2015). When proteins fold correctly, the native three dimensional structure is formed, which is crucial for its normal biological functions (Dobson, 2003; Skolnick and Fetrow, 2000). All proteins have the generic tendency to misfold into abnormal conformations under certain circumstances, and mutations or environmental conditions can enhance protein misfolding (Herczenik and Gebbink, 2008). However, under normal conditions, cellular protein quality control (PQC) systems continuously monitor newly synthesised proteins to ensure that they are correctly folded (Amm et al., 2014). The PQC system promotes the folding of nascent polypeptide chains into their native conformation, and therefore aims to prevent inappropriate interactions between polypeptides, to inhibit protein misfolding or aggregation. However when this task is not achieved, misfolded proteins are degraded by the PQC, involving the proteasome or autophagy systems (Reynaud, 2010). Molecular chaperones located both in the cytosol (primarily heat-shock proteins (Hsps), and in the endoplasmic reticulum (ER) (principally the protein disulphide isomerase (PDI) family of proteins) prevent protein misfolding and hence are important components of the PQC (Barral et al., 2004; Buchberger et al., 2010). Chaperones recognise exposed segments of hydrophobic amino acids in the nascent peptide chain, and thus they facilitate the formation of non-covalent interactions between polypeptides, that promote generation of the correctly folded protein within the highly complex and overcrowded cellular protein milieu (Hartl et al., 2011).

Protein misfolding is a characteristic pathological feature of neurodegenerative disorders, although the proteins that misfold in each case are specific to the respective condition. Neurodegenerative diseases are marked pathologically by the accumulation of insoluble protein aggregates and/or large inclusions, in affected tissues. However, increasingly, the smaller, soluble oligomeric aggregates are being implicated as the major toxic components. Despite great advances in our understanding of these disorders, the mechanisms by which each protein misfolds, aggregates, and triggers neurodegeneration, is still unclear. This review focuses on the role of misfolded proteins in the pathogenesis of amyotrophic lateral sclerosis (ALS), and the possible role of protein chaperones as novel therapeutic targets.

2. Protein folding, misfolding and the formation of protein disulphide bonds

Protein folding is a physical process by which a polypeptide, consisting of a randomly oriented sequence of amino acids, folds into a functional native protein *via* covalent bonds (Bhattacharyya et al., 2016). Secondary structure provides the protein with regular geometry and is defined by patterns of hydrogen bonding, either α-helical or β-sheets. When these structures fold into a globular structure, hydrophobic regions are usually masked or their exposure to the protein surface is minimised. This is driven by hydrophobic interactions and stabilised by non-covalent interactions (Uversky and Dunker, 2010). After forming the secondary structures, folding involves the formation of the tertiary structures, and then quaternary structures, involving the assembly of subunits in a multi-domain protein. Post-translational modifications, such as disulphide bond formation formed by PDI in the ER, are also an important aspect to protein folding (Schulz and Schirmer, 2013) and are formed shortly before proteins find their most energetically favourable native conformation. Finally, recent studies have highlighted the importance of the quinary structure on protein folding (Cohen et al., 2015; Monteith et al., 2015). Quinary structure is the local cellular environment the protein is contained in. In physiologically relevant conditions, in which proteins are tightly packed, rather than in conventionally studied dilute buffered solutions, interactions at the protein surface with the cytosolic environment, have recently been shown to be important in maintaining the stability of proteins (Cohen et al., 2015; Monteith et al., 2015).

Disulphide bond formation occurs within the ER lumen, which provides an ideal environment for protein folding, due to its oxidising conditions (Oka and Bulleid, 2013). The ER is equipped with numerous ER-resident chaperones that catalyse disulphide bond formation, and thus assist in the native oxidative folding of proteins, as part of the PQC. (Hudson et al., 2015). Membrane and secretory proteins normally transverse the ER, where oxidative protein folding occurs (Dudek et al., 2015), due to the presence of a secretory signal peptide. In contrast, cytoplasmic proteins lack a secretory leader and hence do not normally transit the ER. Hence disulphide bond formation is rare in cytoplasmic proteins, but does occur in some instances.

Disulphide bonds are important for the structural stability of proteins and they also promote the assembly of multi-protein complexes (Frand and Kaiser, 1998; Woycechowsky and Raines, 2000). Disulphide bonds are introduced into protein substrates by a diverse set of pathways and the PDI family of proteins are key to this process (Bulleid and Ellgaard, 2011). PDI has two thioredoxin-like active sites, which both contain two cysteine residues. Disulphide bonds form between these two cysteines and the protein substrate, by oxidation. PDI is also responsible for the reduction and isomerization of these disulphide bonds. For PDI to catalyse the formation of disulphide bonds in unfolded proteins, it must be re-oxidised. ER oxidoreductin 1 (ERo1) is an oxidoreductase that utilises a redox cofactor, flavin adenine dinucleotide (FAD), and molecular oxygen, to generate disulphide bonds de novo. This reaction is catalysed by peroxiredoxin 4 and glutathione peroxidase, which produces hydrogen peroxide to oxidise PDI (Nguyen et al., 2011; Wang et al., 2014; Zito et al., 2010). Additionally, oxidised glutathione (GSSG) and vitamin K oxidoreductase can re-oxidise PDI after substrate oxidation, by directly interacting with PDI (Appenzeller-Herzog et al., 2010; Lappi and Ruddock, 2011; Ruddock, 2012). In contrast, quiescin sulfhydryl oxidase (QSQX) also facilitates disulphide bond formation by directly oxidising protein substrates (Codding et al., 2012; Raje and Thorpe, 2003). Substrate oxidation also results in the production of reduced PDI. Although the ER introduces disulphide bonds into substrate proteins with high precision, errors in this process can lead to the formation of non-native disulphide bonds in these substrates. The reduced form of PDI mediates isomerisation reactions in proteins, and reduced glutathione (GSH) delivers electrons directly to form reduced PDI (Chakravarthi and Bulleid, 2004). Reduced forms of