Neuropeptides 57 (2016) 71–83

Contents lists available at ScienceDirect

Neuropeptides

journal homepage: www.elsevier.com/locate/npep

Self-assembly and sequence length dependence on nanofibrils of polyglutamine peptides

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ARTICLE INFO

Article history:
Received 11 November 2015
Received in revised form 11 January 2016
Accepted 31 January 2016
Available online 3 February 2016

Keywords:
Huntington's disease
Polyglutamine disease
Misfolded polyglutamine
Nanofibrils
PolyQ peptides
Neurodegenerative disease

ABSTRACT

Huntington's disease (HD) is recognized as a currently incurable, inherited neurodegenerative disorder caused by the accumulation of misfolded polyglutamine (polyQ) peptide aggregates in neuronal cells. Yet, the mechanism by which newly formed polyQ chains interact and assemble into toxic oligomeric structures remains a critical, unresolved issue. In order to shed further light on the matter, our group elected to investigate the folding of polyQ peptides – examining glutamine repeat lengths ranging from 3 to 44 residues. To characterize these aggregates we employed a diverse array of technologies, including: nuclear magnetic resonance; circular dichroism; Fourier transform infrared spectroscopy; fluorescence resonance energy transfer (FRET), and atomic force microscopy. The data we obtained suggest that an increase in the number of glutamine repeats above 14 residues results in disordered loop structures, with different repeat lengths demonstrating unique folding characteristics. This differential folding manifests in the formation of distinct nano-sized fibrils, and on this basis, we postulate the idea of 14 polyQ repeats representing a critical loop length for neurotoxicity – a property that we hope may prove amenable to future therapeutic intervention. Furthermore, FRET measurements on aged assemblages indicate an increase in the end-to-end distance of the peptide with time, most probably due to the intermixing of individual peptide strands within the nanofibril. Further insight into this apparent time-dependent reorganization of aggregated polyQ peptides may influence future disease modeling of polyQ-related proteinopathies, in addition to directing novel clinical innovations.

1. Introduction

1.1. Molecular pathophysiology of HD

HD is a neurodegenerative disease characterized by fragmentation and intraneuronal aggregation of the mutant huntingtin protein (Htt) – an aberrant polypeptide consisting of misfolded polyQ segments (Katsuno et al., 2008). The mechanistic hypothesis that links CAG (the three-letter genetic code for the amino acid glutamine) repeat expansion to neurotoxicity involves the accumulation of polyQ sequences in the Htt protein. Expansion of polyQ repeats above a certain critical length induces proteolytic cleavage of the mutant polypeptide – with the corollary N-terminal fragments forming insoluble aggregates (Bates, 2003; Orr, 2012; Wetzel, 2012). These self-assembled aggregates are associated

Abbreviations: HD, Huntington's disease; PolyQ, polyglutamine; FRET, fluorescence resonance energy transfer; Htt, huntingtin protein; CD, circular dichroism; FTIR, Fourier transform infrared spectroscopy; XRD, X-ray diffraction; NMR, nuclear magnetic resonance; AFM, atomic force microscopy; TOCSY, total correlation spectroscopy; HSQC, heteronuclear single-quantum coherence; NOESY, nuclear overhauser effect spectroscopy; BPTI, bovine pancreatic trypsin inhibitor.

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http://dx.doi.org/10.1016/j.npep.2016.01.011
0143-4179/

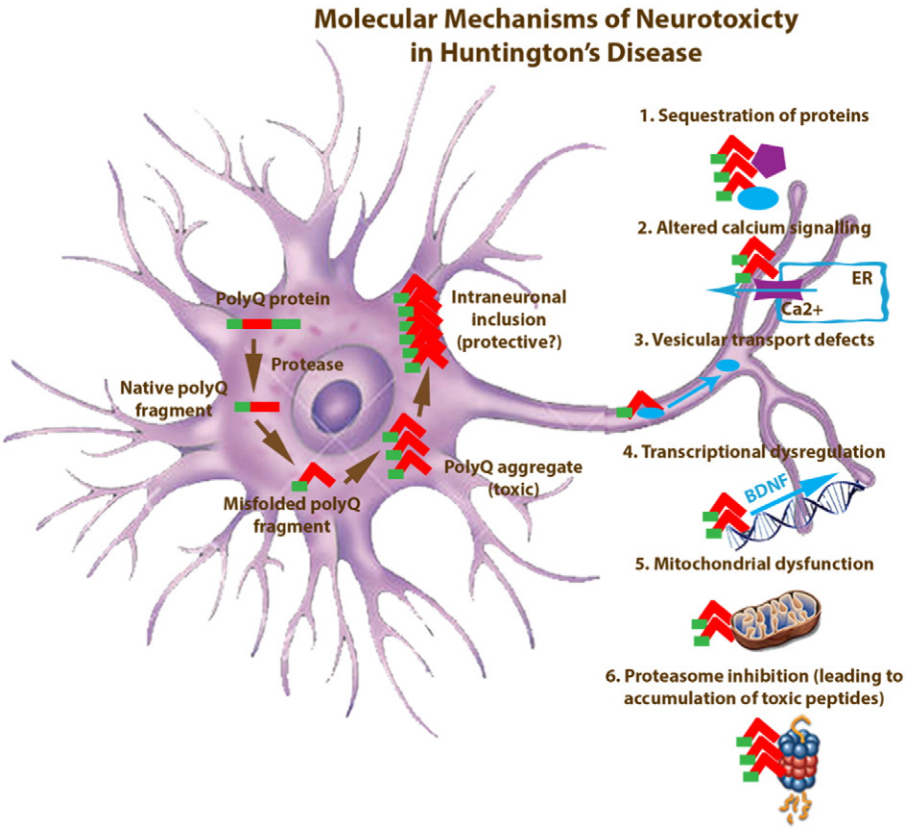

Fig. 1. Mutant Htt is cleaved enzymatically into peptide fragments containing *expanded* polyQ tracts. It is thought that over a critical length, these fragments assume a misfolded configuration, which promotes self-aggregation into toxic oligomers that disrupt various cellular functions.

with cellular dysfunction and consequent neurotoxicity (Fig. 1) (Blum et al., 2013; Cisbani and Cicchetti, 2012; Zheng and Diamond, 2012) and ultimately, are thought to give rise to the neurological sequelae of HD.

Oligomerization of polyQ and other amyloid-forming proteins has long been thought of as a crucial determinant in the development of cytotoxicity (Hands and Wyttenbach, 2010; Hatters, 2012; Janciauskiene et al., 1999; Legleiter et al., 2010; Ono et al., 2009; Shao and Diamond, 2007). Yet, perhaps less well-recognized is that this conversion of polyQ peptides into insoluble aggregates appears to be driven by more than a simple propensity for self-association – with current evidence indicating that a more complex, highly regulated process may be responsible (Bates, 2003; La Spada et al., 2011). In spite of accumulating data linking polyQ length to toxicity, the structural basis for the influence of polyQ repeat length on the folding and self-assembly mechanism has yet to be fully elucidated. In fact, it has been demonstrated that Htt fragments with expanded polyQ segments can sequester transcription factors with polyQ domains as small as 18 residues, suggesting that even smaller polyQ repeat lengths can adopt aberrant structures (Kar et al., 2011; Robertson et al., 2010) and highlighting key gaps in our understanding of the misfolding process.

1.2. Uncertainties in current understanding

One of the major bottlenecks in our understanding of polyQ toxicity is the lack of structural details on these assemblages. The disordered nature of polyQ peptides and their tendency to aggregate make spectroscopic interpretations of structure difficult (Napoletano et al., 2011). Nonetheless, various biophysical methods have met with some success in investigating the gross structure of polyQ peptides; prominent examples include: (1) circular dichroism (CD), which has linked β-sheet formation to aggregation; (2) Fourier-transform infrared (FTIR) spectroscopy, which has linked hydrogen bond formation to aggregation; and (3) X-ray diffraction (XRD) measurements, which have yielded inter-strand distances (Davranche et al., 2011; Ortega et al., 2010).

At present, there is little consensus on the precise conformation adopted by polyQ peptides. Atom energy minimization studies of polyQ with implicit solvation showed that CHARMM parameters produce a β-hairpin structure, whereas AMBER parameters produce a random coil structure (Finke et al., 2004; Tsukamoto et al., 2006; Vanschouwen et al., 2011; Vitalis et al., 2007; Zanuy et al., 2006). In contrast, homology modeling predicted the polyQ region of ataxin-3 to be an α-helix while in vitro coherent anti-stokes Raman microscopy revealed that polyQ fibers assemble into highly rigid β-sheet structures (Perney et al., 2012).

Current literature is also equivocal about whether the length-dependence of toxicity is related to a conformational change in the monomeric state of expanded polyQ peptides. For instance, the Wetzel group (Thakur and Wetzel, 2002; Wetzel, 2012) reported that polyQ peptides with repeat lengths of 5, 15, 28, and 44 residues all adopt random coil structures in solution, whereas a Flory-Huggins mean-field lattice model postulated that polyQ increasingly prefers a β-hairpin state in a length-dependent manner (Crick et al., 2006; Masino et al., 2004). Notably, however, host-guest studies where increasing lengths of a polyQ "guest" were inserted into "host" chymotrypsin inhibitor 2 (CI 2) mutants demonstrated increasing destabilization of the host CI 2 protein. This length-dependent destabilization of monomeric polyQ is consistent with the observed increase in kinetic and thermodynamic stability of ordered, amyloid-like aggregates for polyQ peptides with more than 37 residues (Bhattacharyya et al., 2006; Chen et al., 2001; Chen et al., 2002b; Crick et al., 2006; Thakur and Wetzel, 2002). Overall, these studies suggest that repeat length is likely to be a crucial factor in the oligomerization – and hence neurotoxicity – of destabilized polyQ peptides, although the structural mechanism of this has yet to be clarified.