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Disease-Associated Polyglutamine Stretches in Monomeric Huntingtin Adopt a Compact Structure

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Abnormal polyglutamine (polyQ) tracts are the only common feature in nine proteins that each cause a dominant neurodegenerative disorder. In Huntington's disease, tracts longer than 36 glutamines in the protein huntingtin (htt) cause degeneration. *In situ*, monoclonal antibody 3B5H10 binds to different htt fragments in neurons in proportion to their toxicity. Here, we determined the structure of 3B5H10 Fab to 1.9 Å resolution by X-ray crystallography. Modeling demonstrates that the paratope forms a groove suitable for binding two β-rich polyQ strands. Using small-angle X-ray scattering, we confirmed that the polyQ epitope recognized by 3B5H10 is a compact two-stranded hairpin within monomeric htt and is abundant in htt fragments unbound to antibody. Thus, disease-associated polyQ stretches preferentially adopt compact conformations. Since 3B5H10 binding predicts degeneration, this compact polyQ structure may be neurotoxic.

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Abbreviations used: polyQ, polyglutamine; htt, huntingtin; wt, wild type; HA, hemagglutinin; IP, immunoprecipitation; PDB, Protein Data Bank; CDR, complementarity-determining region; SAXS, small-angle X-ray scattering; Cer, Cerulean fluorescent protein; MBP, maltose binding protein.

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Introduction

An abnormal expansion of the polyglutamine (polyQ) tract in huntingtin (htt) results in a self-aggregating protein and neurodegeneration. Understanding which structures of polyQ in mutant htt and other polyQ-expanded proteins are most closely linked to pathogenesis has important implications for mechanisms of neurotoxicity. For example, unaggregated expanded polyQ has been suggested to mediate toxicity through aberrant recruitment of cellular proteins.[1] If such "recruitment-competent" polyQ is structured, then mutant htt may act as a structural mimic/competitor for the recruited protein's normal binding partners. Alternatively, if "recruitment-competent" polyQ is unstructured, then expanded polyQ may simply provide a longer, more accessible recruitment site than wild-type (wt) stretches of polyQ.

These alternate pathogenic scenarios lead to potentially divergent therapeutic strategies. In the case of a structured polyQ epitope, screens for therapeutics that disrupt toxic structure formation may be warranted. In the case of an unstructured polyQ epitope, therapeutic strategies may instead focus on covalently linking together multiple copies of a molecule that recognizes a short linear array of polyQ.[2–5]

While the structure of aggregated polyQ peptides takes on the classical cross-β-strand structure of amyloid fibrils,[6] the exact structures and pathogenic significance of a range of putative monomeric species and very small oligomeric species of mutant htt are unknown. The first exon of wt htt was recently crystallized, revealing multiple conformations of the polyQ stretch.[7] However, studies on expanded (mutant) polyQ suggest that the unaggregated forms are largely unordered, adopting secondary structure only upon aggregation.[5,8,9] These data led to the concept of "beads on a string," a linear sequence of unaggregated glutamines without secondary structure. In this "linear lattice" model, the toxicity of unaggregated expanded polyQ is caused by an increased accessibility of the polyQ region to cellular ligands.[2,5] Furthermore, if a particular cellular ligand has two polyQ binding sites, then the ligand will exhibit a strong preference for expanded over wt stretches of polyQ driven by increased avidity. Indeed, at least two IgG antibodies (MW1 and 1C2), which inherently have two identical epitope binding sites, have been thought to preferentially bind expanded polyQ, at least in part, by the avidity mechanism implied in the "linear lattice" model.[2,10]

In contrast to the "linear lattice" model, some pieces of *in vitro* experimental evidence indicate that expanded polyQ induces a global change in conformation in unaggregated htt.[1,11,12] However, direct experimental evidence indicating that disease-associated polyQ stretches adopt an emergent conformation *in situ* is lacking. Furthermore, whether any particular emergent conformation formed *in situ* is toxic is also unknown.

We identified a monoclonal antibody, 3B5H10, that recognizes a species of htt in neurons that predicts neurodegeneration better than all other α-htt antibodies tested, including the "linear-lattice"-recognizing antibody MW1.[13] Here, we show that 3B5H10 recognizes a compact two-stranded conformation of polyQ in monomeric htt that emerges when the polyQ stretch expands. Our data show that a specific compact conformation of expanded polyQ forms in unaggregated htt *in situ* and that this compact conformation has particular pathological significance.

Results

Monoclonal 3B5H10 recognizes a structured polyQ epitope in a fragment of htt

Since the epitope recognized by monoclonal antibody 3B5H10 predicted neurodegeneration better than the epitope recognized by MW1,[13] we considered the possibility that 3B5H10 recognizes an epitope formed preferentially by mutant htt rather than a repeated epitope envisioned by the linear lattice model. We reasoned that a conformation that preferentially forms in mutant htt should be stable at disease-associated polyQ lengths, unstable at near-threshold lengths, and relatively unformed at short lengths.

To test this putative difference in stability, we probed the effects of the denaturant SDS on 3B5H10 binding to mutant, threshold, and wt versions of htt. Specifically, we chose to test three different polyQ stretches (Q_{17}, Q_{25}, and Q_{40}) based on the frequency with which the corresponding CAG codon stretches are found in the htt gene within humans. A stretch of Q_{17} is among the most common alleles found in the normal population,[14–17] whereas a stretch of Q_{40} is relatively common among Huntington's disease patients and is fully penetrant.[15,17] Htt alleles with $Q_{23–34}$ are relatively rare but correspond to a transition zone between the most common normal alleles and disease-associated alleles and, therefore, may have particularly interesting biochemical properties.[15,17]

Cells were transfected with N-terminal 171-amino-acid fragments of htt containing 17, 25, or 40 polyQ stretches, as well as hemagglutinin (HA) and FLAG epitope tags fused, respectively, to the N-terminus and C-terminus of htt. Cells were lysed under native conditions 48 h after transfection, and lysates were immunoprecipitated with α-HA