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β-Hairpin-Mediated Nucleation of Polyglutamine Amyloid Formation

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Abstract

The conformational preferences of polyglutamine (polyQ) sequences are of major interest because of their central importance in the expanded CAG repeat diseases that include Huntington's disease. Here, we explore the response of various biophysical parameters to the introduction of β-hairpin motifs within polyQ sequences. These motifs (tryptophan zipper, disulfide, D-Pro-Gly, Coulombic attraction, L-Pro-Gly) enhance formation rates and stabilities of amyloid fibrils with degrees of effectiveness well correlated with their known abilities to enhance β-hairpin formation in other peptides. These changes led to decreases in the critical nucleus for amyloid formation from a value of $n^* = 4$ for a simple, unbroken Q_{23} sequence to approximate unitary n^* values for similar length polyQs containing β-hairpin motifs. At the same time, the morphologies, secondary structures, and bioactivities of the resulting fibrils were essentially unchanged from simple polyQ aggregates. In particular, the signature pattern of solid-state NMR ^{13}C Gln resonances that appears to be unique to polyQ amyloid is replicated exactly in fibrils from a β-hairpin polyQ. Importantly, while β-hairpin motifs do produce enhancements in the equilibrium constant for nucleation in aggregation reactions, these K_{n^*} values remain quite low ($\sim 10^{-10}$) and there is no evidence for significant enhancement of β-structure within the monomer ensemble. The results indicate an important role for β-turns in the nucleation mechanism and structure of polyQ amyloid and have implications for the nature of the toxic species in expanded CAG repeat diseases.

Introduction

In Huntington's disease and nine other expanded polyglutamine (polyQ) diseases, a genetic expansion of the polyQ sequence in a disease protein into a pathological repeat length range typically above 35 residues increases disease risk and decreases age of onset.[1] One hypothesized biophysical explanation for this dramatic repeat length effect is that expanded polyQ sequences populate an alternative monomer conformation that triggers a dysfunctional and/or toxic response in the cell.[2] However, the observations of polyQ aggregates in disease brain tissue[3] and of a repeat length dependence of aggregation both *in vitro*[4,5] and in cell and animal models[6] suggest an alternative hypothesis featuring a strong role for polyQ aggregation.[7] For this reason, particular emphasis has been placed on understanding the mechanisms of polyQ amyloid nucleation and how this might be affected by repeat length, sequence context, and cellular environment.[8] Although flanking sequences can clearly have a major impact on aggregation rates and mechanisms,[8–10] it is likely that some fundamental aspects of polyQ amyloid formation deduced from studies on simple polyQ sequences will apply to polyQ behavior in disease proteins.

Mature aggregates of polyQ disease proteins and model peptides exhibit many features of amyloid structure, including a filamentous architecture in electron micrographs[4] and β-rich secondary structure by CD,[11] Fourier transform infrared (FTIR),[10] X-ray diffraction,[12–14] and solid-state NMR (ssNMR).[15,16] In contrast to a number of relatively well-characterized polypeptide amyloids,

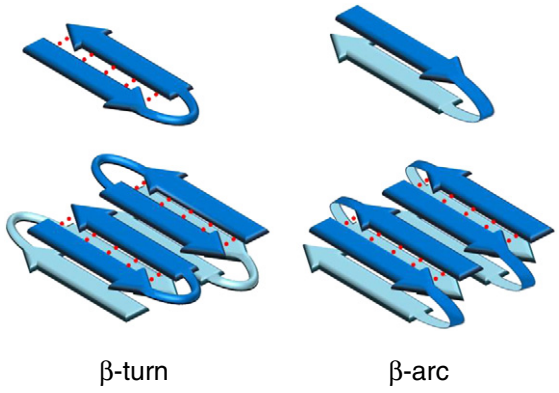

Fig. 1. Reverse-turn models of polyQ peptides and their amyloid fibrils. Schematic antiparallel β-sheet models for a segment of amyloid fibril showing two fundamental ways that fibrils might accommodate longer peptides requiring reverse turns for optimal involvement in fibril structure. In the left panel, monomers are folded into β-hairpins mediated by β-turn chain reversals. In the right panel, monomers are folded into amyloid structure via β-arc chain reversals. Red dotted lines denote backbone intrastrand H-bonding.

which exhibit secondary structures dominated by in-register, parallel β-sheet,[17–19] polyQ amyloid appears more likely to possess an antiparallel β-sheet architecture.[13–16,20,21] Two basic models for how polypeptide sequences might be accommodated into such antiparallel β-sheet structures have been delineated by Kajava et al.[22] In the first, the chain remains within a single sheet by undergoing a series of intramolecularly H-bonded β-turn/β-hairpin chain reversals (Fig. 1). In the second, the chain undergoes reverse turns ("β-arcs") that connect adjacent β-sheets in a conformation that has been referred to as a β-arch[22] (Fig. 1). Such chain reversals are reminiscent of polypeptide conformations found in a number of parallel, in-register β-sheet amyloids, including fibrils of Aβ.[22]

Previously, our laboratory found that all simple polyQ peptides tested with the sequence format $K_2Q_NK_2$ spontaneously form amyloid *via* a classical nucleated growth polymerization mechanism without forming any required, on-pathway non-amyloid intermediates.[11,23] At the same time, nucleation efficiencies varied considerably within this series, such that peptides with polyQ repeat lengths of 23 or lower exhibited a critical nucleus (n^*) of 4, while those with repeat lengths of 26 or above had $n^* = 1$.[23] We interpreted these data to be consistent with β-turn formation playing a critical role in nucleus structure, based on the hypothesis that longer polyQ sequences can form more stable β-hairpins.[23] It seems also possible, however, that the chain reversal required for enhancing polyQ amyloid nucleation is the β-arc[22] in which the reverse-turn conformation is stabilized by side-chain interactions rather than main-chain H-bonding (Fig. 1). Indeed, some have argued that the antiparallel β-sheet architecture of polyQ amyloid may feature such β-arc connectivity.[16]

A number of recent studies have interpreted a variety of biochemical and biophysical data to

Table 1. Structures of peptides

Name	Sequence
$K_2Q_{23}K_2$	KKQQQQQQQQ QQQQQQQQQQ QQQQQKK
$K_2Q_{10}PGQ_{11}K_2$	KKQQQQQQQQ QQPGQQQQQQ QQQQQKK
$K_2Q_{10}pGQ_{11}K_2$	KKQQQQQQQQ QQpGQQQQQQ QQQQQKK
$D_2Q_{23}K_2$	DDQQQQQQQQ QQQQQQQQQQ QQQQQKK
$K_2CQ_{22}CK_2$ (ox.)	KKCQQQQQQQ QQQQQQQQQQ QQQQQCKK
$K_2Q_{11}PGQ_{11}D_2$	KKQQQQQQQQ QQQPGQQQQQ QQQQQQDD
$AcWQ_{22}WTGK_2$	AcWQQQQQQQ QQQQQQQQQQ QQQQQWTGKK
$AcWQ_{11}pGQ_{11}WTGK_2$	AcWQQQQQQQ QQQQpGQQQQ QQQQQQQWTG KK
$K_2Q_{25}K_2$	KKQQQQQQQQ QQQQQQQQQQ QQQQQQQKK
$K_2Q_{41}K_2$	KKQQQQQQQQ QQQQQQQQQQ QQQQQQQQQQ QQQQQQQQQQ QQQKK
Biotinyl-$K_2Q_{30}K_2$	B*KKQQQQQQ QQQQQQQQQQ QQQQQQQQQQ QQQQKK
NLS-$GGQ_{11}PGQ_{12}CK_2$	PKKKRKVGGQ QQQQQQQQQQ PGQQQQQQQQ QQQQCKK
NLS-GGQ25CK_2	PKKKRKVGGQ QQQQQQQQQQ QQQQQQQQQQ QQQQCKK

Lowercase p is the D-enantiomer of Pro. In $K_2CQ_{22}CK_2$ (ox.), the Cys residues are intramolecularly disulfide bonded. "Ac" represents an N^{α}-acetyl group. "*NLS*" is the nuclear localization sequence PKKKRKV. B* is Glu(biotinyl-PEG) (see Materials and Methods).