Several neurodegenerative diseases are linked to expanded repeats of glutamine residues, which lead to the formation of amyloid fibrils and neuronal death. The length of the repeats correlates with the onset of Huntington’s disease, such that healthy individuals have <38 residues and individuals with >38 repeats exhibit symptoms. Because it is difficult to obtain atomic-resolution structural information for poly(L-glutamine) (polyQ) in aqueous solution experimentally, we performed molecular dynamics simulations to investigate the conformational behavior of this homopolymer. In simulations of 20-, 40-, and 80-mer polyQ, we observed the formation of the “α-extended chain” conformation, which is characterized by alternating residues in the αL and αR conformations to yield a sheet. The structural transition from disordered random-coil conformations to the α-extended chain conformation exhibits modest length and temperature dependence, in agreement with the experimental observation that aggregation depends on length and temperature. We propose that fibril formation in polyQ may occur through an α-sheet structure, which was proposed by Pauling and Corey [Pauling, L. & Corey, R. B. (1951) Proc. Natl. Acad. Sci. USA 37, 251–256]. Also, we propose an atomic-resolution model of how the inhibitory peptide QBP1 (polyQ-binding peptide 1) may bind to polyQ in an α-extended chain conformation to inhibit fibril formation.

α-sheet | amyloidosis | misfolding disease | molecular dynamics | poly(L-glutamine)

Several hereditary neurodegenerative diseases have been linked to expanded glutamine repeats, or poly(L-glutamine) (polyQ), including Huntington’s disease, Kennedy disease, dentatorubral–pallidoluysian atrophy, and Machado–Joseph Disease (1). All of these diseases are more severe and begin earlier in life when the polyQ is >37 residues (1). These diseases are accompanied by the progressive death of neurons, with insoluble, granular, and fibrous deposits being found in the cell nuclei of the affected neurons. Formation of the deposits precedes the appearance of symptoms in transgenic mice, apparently linking onset of symptoms to the formation of amyloid fibrils in vivo (2).

The CAG repeat that encodes the polyQ region is part of exon 1 in the 3,140-residue huntingtin protein (3). The polyQ repeat varies between 16 and 37 residues in healthy individuals, and individuals who are affected by disease have repeats of >38 residues. Kennedy disease (also known as spinocerebellar ataxia type 1) is linked to the expansion of a Gln-rich segment in the androgen receptor (4); healthy individuals have a 15- to 31-residue polyQ segment, and individuals who are affected with the disease have 40–62 Gln residues.

It has not been possible to obtain atomic-resolution structural information for polyQ in aqueous solution by using experimental means. polyQ is insoluble in water, and even short polyQ chains aggregate in solution to form viscous gels. Perutz et al. (5) synthesized a soluble peptide, D$_2$Q$_{15}$K$_2$, and investigated it by means of CD. At acidic pH, at which the Asp residues are uncharged, the peptide was soluble in solution and exhibited a CD spectrum that is characteristic of β-structure. The molar residue ellipticity at 197 nm was independent of peptide concentration, suggesting that the monomers formed hydrogen-bonded hairpins. At neutral pH, fibrillar aggregates formed, giving an x-ray diffraction pattern that is diagnostic of cross-β structure. Modeling suggested that the β-sheets are stabilized by both main-chain–main-chain and side-chain–side-chain hydrogen bonding, forming a “polar zipper” (5).

In recent simulations of other amyloidogenic proteins at low pH, we observed the formation of the α-sheet, which was proposed by Pauling and Corey (6). The α-sheet is formed by hydrogen bonding between adjacent strands in the “α-extended chain” conformation, which is defined by an alternation of residues in the helical αL and αR conformations (Fig. 1). In the β-sheet protein transthyretin (TTR), the α-sheet formed over the strands that are highly protected in hydrogen-exchange experiments probing amyloidogenic conditions (7). In lysozyme and the prion protein, α-sheets formed in the specific regions of the protein that are implicated in the amyloidogenic conversion (8). Given that amyloidogenic proteins of diverse sequences can form amyloid fibrils of similar architecture, there may be some common structural elements of the prefibrillar amyloidogenic intermediate. We proposed that the formation of the α-sheet may be a common conformational transition in the fibrillation of amyloidogenic proteins. Perutz and coworkers (9) hypothesized that expanded polyQ repeats induce the oligomerization of the affected protein. To test this hypothesis, they engineered a GQ$_{16}$G peptide into the inhibitory loop of chymotrypsin inhibitor 2 (CI2), which is a small nonamyloidogenic protein (9). By using analytical ultracentrifugation, they observed that the engineered CI2 formed monomers, dimers, and trimers, whereas the WT protein formed only monomers. Consequently, we used CI2 as a control nonamyloidogenic protein.

Based on his models, Perutz (1) also discussed two possible therapeutic strategies for the prevention of aggregation in glutamine-repeat neurological diseases: stabilization of the native protein with an active-site mimetic and inhibition of aggregation directly by disrupting the polar zipper. The first approach appears to be effective in TTR amyloid diseases; aggregation is minimized upon addition of the native ligand thyroxine or other small molecules that stabilize the native tetrameric structure (10–12). Similarly, administration of androgen to a patient with Kennedy disease slows the progression of the disease (1), and small molecules bind to superoxide dismutase and deter aggregation, providing the possibility of a treatment for amyotrophic lateral
Molecular dynamics (MD) simulations of 20-, 40-, and 80-mer atomic-resolution model of how this inhibitory peptide may bind to each system, and simulations were performed at 37°C for 10 ns each.

MD simulations were also performed for models of polyQ 16-mer built by using five different β-hairpin turn conformations, which were taken from a library of β-hairpins (18), to compare their relative stabilities at 37°C. These five hairpins (2:2 type I, 2:2 type I’, 2:2 type II, 3:5 F, and 4:4 F) are highly populated in the Protein Data Bank (19) and in NMR structures of well ordered β-hairpin peptides (20–26). For each turn, a polyQ model was built from multiple peptide backbones and the lowest-energy structure after 1,000 steps of minimization was used for MD simulation. The following structures were used to build each conformation (residue numbering as in ref. 20, PDB ID codes given in parentheses): 2:2 type I turn (1CYO, 2GCH, 2PAB, INXB, 9PAB, 1EST, and 4FDI), 2:2 type I’ turn (2ACT, 2SOD, 1EST, 2NSN, 2APP, 2GCH, 1BP2, 2SN5, 2SDV, 2CCP, 3GRS, 1SN3, 1UBQ, and 111B), 2:2 type II’ turn (1EST, 1GD1, 1REI, 2FB4, 1REI, 2APP, and 2ALP), 3:5 F turn (3FAB, 2APP, 2GCH, 2PAB, 2IDM, 3CIA, 3TLN, 1OVO, 2LYZ, and 1RNS), and 4:4 F turn (1GB1, 3FAB, 2APP, 4PTI, and 1PSG) to yield the following average turn conformations: 2:2 type I, [αR = (−58, −28), γR = (−118, 0)], 2:2 type I’, [αR = (−54, 41), γR = (−96, −10)], 3:5 F, [αR = (−83, 171), γR = (−61, −26), γR = (−89, 7), γR = (90, 0)], and 4:4 F, [αR = (−68, −22), γR = (−71, −36), γR = (−104, −9), αR = (−73, 38)]. We added ~1,500 water molecules to each simulation box. A simulation of each polyQ 16-mer β-hairpin conformation was performed at 37°C for 10 ns each. As a control, we compared these polyQ hairpin simulations with a stable, experimentally characterized 16-residue β-hairpin, known as the β peptide (26). The structure of this β-hairpin peptide was built with a type I’ turn. The simulation was performed at the experimental NMR temperature of 25°C, in a box with ~1,500 water molecules.

Two MD simulations were also performed for the QBP1 peptide (SNWKKWPQIFDD) at 37°C for 50 ns each, as well as for the noninhibitory WLWPGFD peptide. The first simulation was started from the ideal antiparallel β-sheet conformation defined by (ϕ, ψ) = (−139°, +135°), and the second simulation was started from the ideal PII conformation, (ϕ, ψ) = (−79°, +149°). We included ~2,500 water molecules. Five representative models of the QBP1 peptide in regular α-extended chain conformations were identified from conformational analysis of the two simulations. These structures were used to build models of the QBP1 peptide binding to a polyQ 40-mer chain, derived from the 50°C simulation, in an intermolecular α-sheet conformation. Each complex was minimized 500–1,000 steps before MD simulations. Simulations of the QBP1-40-mer polyQ complexes were performed at 37°C with ~8,300 water molecules. All simulations were performed with the program ENCAD (27) or ILMM (28, 29) by using an 8-Å force-shifted cutoff (updated every two steps) with the protein and water force-field parameters described in refs. 30–32. The polyQ 20-, 40-, and 80-mer and CI2 simulations were performed by using our standard procedures, which neglect repulsive interactions between atoms in neutral groups separated by three or fewer bonds, and with explicit repulsion between all Cα–Cα and neighboring carbonyl groups (and NH groups on the other face) was done to ensure that we were not biased toward α-sheet structure and that we still observed this structure when the repulsive interactions were explicitly included. Here, we present the results for the simulations including the repulsion, representing the more conservative estimates of α-sheet content.

sclerosis (13). A peptide inhibitor of aggregation has been reported for polyQ. QBP1 (polyQ-binding peptide 1) fused to the TAT protein (14) inhibits polyQ aggregation and cell death in mammalian COS-7 cells (15). Coexpression of (QBP1)2 has a dramatic effect in Drosophila expressing the expanded Q92 peptide in the nervous system, resulting in an increase of the median life span from 5.5 to 52 days (16). Here, we propose an atomic-resolution model of how this inhibitory peptide may bind to polyQ.

**Methods**

Molecular dynamics (MD) simulations of 20-, 40-, and 80-mer polyQ were performed at 3°C and 50°C, for 20 ns each. The N terminus was acetylated, and the C terminus was amidated. Similar simulations were also performed for the D2Q15K2 peptide. In the simulations of the D2Q15K2 peptide, Asp residues were charged, corresponding to the more amyloidogenic neutral pH conditions (5). All of these simulations were started from the ideal poly(Pro) II (PII) conformation defined by (ϕ, ψ) = (−79°, +149°), because it has been proposed recently to be a “killer conformation” (17). The starting structures for all simulations were minimized 500–1,000 steps before dynamics. Water molecules were added to make a box that extended ~10 Å from any protein atom, resulting in the addition of ~2,500, 5,700, and 18,800 water molecules for the 20-, 40-, and 80-mer, respectively, and ~3,600 water molecules for the D2Q15K2 peptide. As a nonamyloidogenic control, two unfolding simulations of CI2 were performed at 225°C for 10 ns each.

Because Perutz and coworkers concluded that polyQ segments most likely form a type I β-turn (5, 9), we compared the stability of polyQ in β- and α-hairpin conformations with a type I turn at physiological temperature (37°C). A polyQ 23-mer was built in a type I β-hairpin turn conformation by using a hairpin of TTR as a template (residues 102–124) (18). A similar-length polyQ 22-mer was built in an α-hairpin conformation with a type I turn from MD-generated α-sheet structures of the same GH hairpin of TTR (7). Both hairpin models have 10 regular main-chain hydrogen bonds. We added ~2,000 water molecules.

![Fig. 1. Four random-coil conformations. The simulations of polyQ 20-, 40-, and 80-mer were started from the idealized PII, and the most prevalent sampled conformations are shown.](Image)
Results and Discussion

Conformational Behavior of polyQ Homopolymers. Simulation of 20-, 40-, and 80-mer polyQ began from an extended ideal PII conformation. In each case, the conformation relaxed into a more disordered random-coil state in which the \( \alpha \)-extended chain conformation was populated. The \( \alpha \)-extended chain conformation (Fig. 1) is defined by an alternation of residues in the \( \alpha \) and the \( \beta \) conformations \( \alpha, \beta \), \( \alpha, \beta \), rather than being formed by repeating \( \alpha \)-helices and \( \beta \)-sheets (8). The \( \alpha \)-extended chain formation exhibits modest temperature dependence; the 40- and 80-mer formed more \( \alpha \)-extended conformation at 50°C than at 3°C (Fig. 2A). This finding agrees with the experimental observation that aggregation and amyloid formation are temperature dependent (33). There was also a modest increase in \( \alpha \)-extended chain structure with increasing chain length (Fig. 2B–C). The maximum \( \alpha \)-extended chain segment length at 3°C contained four or five residues, and at 50°C, there was an increase in the number of four-residue segments as well as longer \( \alpha \)-strands (Fig. 2B and C). Isolated segments of alternating \( \alpha \)-structure were observed for all three polyQ peptides, but the longer chains were able to form more extensive sheet-like structures. The polyQ 80-mer at 50°C formed an \( \alpha \)-hairpin and then later collapsed to form a loosely structured sheet (Fig. 2D). Tight hydrogen-bonded sheets did not result at this time scale, but there was a definite tendency toward \( \alpha \)-extended chain structures in polyQ peptides, and the experimentally and clinically observed length cutoff of \( \approx 40 \) residues may be related to the number of residues required to form a stable sheet nucleus.

The formation of fibrillar aggregates precludes the use of most homopolymers as nonamyloidogenic controls (34). Instead, we examined thermal unfolding simulations of CI2. The maximum length of an \( \alpha \)-extended chain structure in unfolded states of CI2 was four residues, with a low population, much lower than one would expect for a 64-residue polyQ chain at elevated temperature (7 vs. \( \approx 30,000 \) counts; Fig. 2A). Overall, although we observed \( \alpha \)-extended chain formation in polyQ and other amyloidogenic proteins, this is not the case for nonamyloidogenic proteins or amyloidogenic proteins under native, nonamyloidogenic conditions (8).
In 20-, 40-, and 80-mer simulations at 3°C, regions of the chain that did not form α-extended chain exhibited a complex equilibrium among many conformations, including repeating PII, type II turns, and repeats of the backbone conformations [αL, βR, β], [αR, PII, αR, PII], and [αL, PII, αL, PII] (Fig. 1). We propose that an equilibrium among these conformations represents the nonamyloidogenic random-coil state of polyQ at low temperature, whereas the α-extended chain conformation [αR, αL, αR, αL] represents an amyloidogenic random-coil state. Also, the peptides formed both type I and II β-turns (35) (data not shown).

**D2Q15K2 Peptide Under Amyloidogenic Neutral pH Conditions.** In the simulations of the D2Q15K2, peptide, beginning from a PII extended structure, Asp residues were charged, corresponding to neutral pH at which the peptide is insoluble, forming both disordered aggregates and amyloid protofibrils (5). Temperature had less influence on the α-extended chain population for D2Q15K2 than for polyQ. In the D2Q15K2 simulation at 3°C, predominantly type II β-turns were observed, and a mixture of type I and II β-turns were populated at 50°C. In the D2Q15K2 simulation at 50°C, residues 8–19 formed an α-hairpin, with a turn over residues 12–16, alternating between [αL, PII, αR, αR, αL] and [αL, PII, αR, αR, PII] (Fig. 3A). The α-hairpin turn was stabilized by Gln side-chain hydrogen bonds and main-chain groups in the turn. Because the α-hairpin turn conformation [αL, αR, αR, αR, αL] is similar to the conformation of a 4:4 F β-hairpin [β, αR, αR, γR, αL, β], we refer to it as a 4:4 α-hairpin (Fig. 3B).

**Comparison of Different polyQ Hairpin Turn Conformations.** In the D2Q15K2 simulation at 50°C, the peptide backbone of residues 8–19 formed an α-hairpin conformation from a type I turn intermediate. To investigate this structure further, we compared the stability of polyQ in β- and α-hairpin conformations with a type I turn at 37°C. The hydrogen bonding network and the turn conformation were much more stable for the α-hairpin than for the β-hairpin during MD. The α-hairpin maintained most of its α-sheet hydrogen bonds, and the β-hairpin partially converted into an α-hairpin. An interesting similarity between the two simulations is that, although they both started with a type I β-turn [β, αR, γR, β], they rearranged to a similar turn [αL, αR, αR, γR, β] and [β, αR, αR, γR, β] for the α- and β-hairpin, respectively. This turn is similar to the 4:4 α-hairpin described above.

This unexpected similarity in α-hairpin turn conformations led us to investigate the relative kinetic stability of five different turns (Fig. 3B). As a control, we compared polyQ with a very stable experimentally characterized 16-residue β-hairpin, the so-called β4 peptide (26). At the experimental NMR temperature of 25°C, the β4 peptide was stable in the simulation, and the β-sheet hydrogen bonding network was maintained (i.e., there were no conversions to α-sheet).

We built the starting structure of β4 from the β-hairpin peptide library, and we have since received the structure and NMR data to better assess our simulation. The ensemble from 1–10 ns satisfied all but 17 of the 173 experimental nuclear Overhauser effects (NOEs) (26); the NMR ensemble of 28 models violated an average of 18 NOEs. Our minimized starting model violated 29 NOEs, all involving at least one side-chain atom. Many of the violations were corrected during the simulation, and of the 17 remaining errant NOEs, 16 were violated in one or more models in the NMR ensemble. Overall, the β4 peptide simulation is in reasonable agreement with the NMR data and, in fact, is comparable to the NMR models. Therefore, our force field and methods do not appear to have an obvious bias toward the α-sheet.

At 37°C, the polyQ β-hairpin conformations were not stable; all conformations showed disrupted β-sheet hydrogen bonding and rearranged turns. After 1,000 steps of minimization, the 4:4 F and the type I turn had the lowest potential energies and were expected to be the most stable. Of the five polyQ β-hairpin conformations, the 4:4 F hairpin was the most stable during MD, but it converted into an α-hairpin. In the simulation of the type I turn, the β-sheet hydrogen bonding network was disrupted by 2 ns, and the turn structure converted into a [αL, αR, αR, αR, αL] conformation, similar to the simulations of the D2Q15K2 peptide and the type I turns built from the TTR GH hairpin. The other turns exhibited similar behavior.
For the five turns, each segment rearranged into related turn conformations with three \( \alpha \)- or \( \beta \)-residues in a row, \([\alpha_3, \alpha_6, \alpha_9]\) or \([\alpha_4, \alpha_7, \alpha_10]\). Hydrogen bonds formed periodically between the Glu side chains of residues 2 and 5, and residues 3 and 4 of the turn. These turns are comparable with the \( \alpha \)-hairpin turns in the D2Q15K2 peptide and in the polyQ type I hairpin turns built from the TTR GH hairpin. This convergence to a common turn structure suggests that the most stable polyQ \( \alpha \)-hairpin turn conformation would be \([\alpha_4, \alpha_7, \alpha_9, \alpha_10, \alpha_3, \alpha_6, \alpha_9]\). The most stable \( \beta \)-hairpin conformation, the 4:4 F turn, has a related conformation \([\beta, \alpha_6, \alpha_9, \gamma_9, \alpha_10]\). Consequently, we propose that polyQ homopolymers form type I turns from random-coil structure and then convert into a 4:4 \( \alpha \)-hairpin.

**Self-Assembly Through an \( \alpha \)-Sheet Intermediate.** The most noticeable characteristic of \( \alpha \)-sheet structure (6) is the alignment of the carbonyl and amide groups participating in hydrogen bonds between the strands forming a “polar-pleated sheet.” This feature of \( \alpha \)-sheet suggests a mechanism for self-assembly into amyloid fibrils. The partial charges from the peptide backbone create two complementary charged interfaces of opposite charge. Arnsdorf and coworkers (36) proposed a model in which proteins build up a molecular dipole under amyloidogenic conditions, which could be explained with our structures.

In a recent study of polyQ aggregation kinetics at 37°C, Wetzel and coworkers (37) described aggregation as a nucleated growth polymerization, but in contrast to conventional models, they found that the aggregation nucleus is a monomer, rather than an oligomer. This finding suggests that the slow nucleation step is the intramolecular transition from a disordered random coil to some other species (37). Their CD data indicate that the formation of \( \beta \)-structure occurs late in the aggregation process, demonstrating that the dominant conformation in the lag phase is not a \( \beta \)-sheet. If the monomeric intermediate nucleus were a \( \beta \)-hairpin, the fast elongation phase would be expected to proceed through random-coil chains in the “\( \beta \)-strand” conformation, docked upon the \( \beta \)-sheet nucleus. It also follows that the peptide would populate random-coil conformations rich in \( \beta \)-strands and \( \beta \)-hairs, conformations to account for rapid aggregation in the elongation phase. In our simulations, polyQ \( \beta \)-hairpins were not stable at 37°C, nor was there a large population of \( \beta \)-strand or \( \beta \)-hairs.

Alternatively, we propose that the monomeric nucleus is an \( \alpha \)-sheet. In this scenario, the elongation phase would occur through a sufficient population of random coil in the \( \alpha \)-extended chain conformation, or \( \alpha \)-hairpins, to form a stable, intramolecular \( \alpha \)-sheet. Based on our simulations, we propose that type I turns form as a component of random-coil structure and convert into a 4:4 \( \alpha \)-hairpin. Also, the 4:4 \( \alpha \)-hairpin was more stable than the investigated \( \beta \)-hairpin conformations, suggesting that it is a more likely structure for the aggregation nucleus at 37°C. A nucleus with \( \alpha \)-sheet structure is also consistent with a greater population of \( \alpha \)-extended chain than \( \beta \)-strand in random-coil states of polyQ and the lack of \( \beta \)-structure in the lag phase by CD (37). As the protofibril matures, the reverse transition from \( \alpha \)-to \( \beta \)-sheet may become more favorable during the progression from the soluble phase to the ordered insoluble phase. This type of structural transition in a protein fiber is analogous to structural transitions in silk, in both the soluble and solid phases (38). On the basis of several recent solid-state NMR studies, it has been suggested that highly ordered amyloid fibrils are composed of \( \beta \)-sheet (39, 40), but \( \alpha \)-sheet structure is also compatible with the solid-state NMR observables for fibrils (7).

Nevertheless, we favor the idea of the \( \alpha \)-sheet in the soluble oligomers and conversion to \( \beta \)-sheet in mature fibrils, particularly given the fact that there is an antibody that binds soluble oligomers with various sequences but does not crossreact with the fibrils or the native state (41), suggesting that the epitope that the soluble oligomers present is a unique backbone conformation.

Another interesting, but very speculative, finding from our simulations is the similarity between the topology of the compact, messy, sheet-like structure formed by the polyQ 80-mer and the converted, scrapie-like form of the prion protein (PrPSc) obtained in MD simulations at low pH (42, 43) (Fig. 2D Right). Although the Q80 structure is loose and the chain segments do not form extensive hydrogen bonds (as expected for a sheet), the chain topology is remarkably similar between the two putative amyloidogenic conformers, aside from the change in direction of the lone strand on the right. However, the threading between the strands is quite different. Also, in both structures the strand farthest to the left is more variable. It was quite surprising to see polyQ adopt this topology because the arrangement of the prion protein is presumably influenced by its context (that is, the other parts of the protein), whereas polyQ is an isolated peptide. The PrPSc-like structure shown in Fig. 2D is our building block for the formation of protofibrils. For example, the sheet of a monomer binds to the handle (protrusion on the right), and they form a spiraling sheet (43). Whether polyQ might oligomerize in a similar fashion remains to be determined.

**Binding of the Inhibitory QBP1 Peptide.** Using a phage display library, Burke and coworkers (15) found six Trp-rich peptides that preferentially bind to pathogenic-length polyQ-domain proteins. The most potent of these peptides, QBP1, binds to long, pathogenic polyQ and inhibits cell death in mammalian COS-7 cells (15). The Trp-rich motif (KWKK) was identified as one of the most important determinants of binding, and replacement of Lys with Leu substantially reduced the inhibitory effect (42). A spacer residue was required (most often Pro), and then either GFF or another repeat of KWKK. It has been suggested (44) that the KWKK motif binds to the polyQ domain in a rare aggregation-prone conformation and that the adjacent Pro interferes with \( \beta \)-sheet formation. QBP1 inhibits early steps in

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**Fig. 4.** Model of the QBP1 peptide–40-mer polyQ complex. Shown are the initial minimized conformation of the docked complex (upper) and the conformation after 10 ns (lower). The most populated hydrogen bonds during the simulation are shown in magenta, and less populated hydrogen bonds are shown in light blue. Near each hydrogen bond, the percentage of the time that the hydrogen bond was intact at 6–10 ns is given. Steric clashes with the side chain of the Pro residue in the QBP1 peptide (red dashed lines) discourage another polyQ chain from hydrogen bonding to the carbonyl face of the QBP1–polyQ complex, directly inhibiting elongation of the \( \alpha \)-sheet.
aggregation, and addition of QBP1 after aggregation does not reverse the process (44). The QBP1 peptide appears to inhibit aggregation by binding to either the monomeric nucleus or the amyloidogenic random-coil conformations to prevent formation of the monomeric nucleus, both of which would prevent the elongation phase.

We performed 50-ns MD simulations of the QBP1 peptide, starting from two different conformations. The α-extended chain conformations formed with higher populations in the WKWW motif than the GIFD portion of the peptide. Because the α-extended chain was the most populated regular secondary structure in the dynamic ensemble, a conformation that is not sampled in two separate 50-ns simulations of the noninhibitory W2WWPGIFD point mutant, we reasoned that the QBP1 peptide may bind to polyQ repeats in this way.

Five different α-extended conformations of the QBP1 peptide were docked to a 40-mer polyQ in an intermolecular −extended conformations of the QBP1 peptide

The QBP1 peptide could inhibit aggregation by binding to the aggregation nucleus (α-hairpin) and/or dominant random-coil conformation in the lag phase (α-extended chain), thus preventing the elongation phase. Although the QBP1 peptide does not have any effect on mature amyloid fibrils, it is possible that QBP1 could bind to soluble oligomeric intermediates, which have been shown to be cytotoxic (45).

Conclusions

MD simulations of polyQ 20-, 40-, and 80-mer show relaxation from the ideal PPII conformation into a more disordered random-coil state in which the α-extended chain conformation was populated. The α-extended chain conformation exhibited clear length and temperature dependence, in accord with the experimental observation that polyQ aggregation and amyloid formation depend on length and temperature. We observe α-extended chain formation in polyQ and regions of other amyloidogenic proteins (8), but we did not observe significant α-extended chain in nonamyloidogenic proteins.

In a simulation of the D0,15K2 peptide corresponding to amyloidogenic conditions, the peptide formed type I turns from random-coil structure and converted into a 4.4 α-hairpin. We propose that an α-sheet is the likely structure for the aggregation nucleus at 37°C. An α-sheet nucleus is consistent with a greater propensity of α-extended chain than β-strand in random-coil states of polyQ and the lack of β-sheet structure in the lag phase according to CD (37). The QBP1 peptide, a potent aggregation inhibitor, may bind to expanded polyQ repeats in an α-sheet conformation. In this way, the QBP1 peptide may inhibit aggregation by binding to an α-sheet nucleus and/or its precursor in the random-coil pool, thus preventing the elongation phase.

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